Public Comment
Selenium TMDL North San Francisco Bay
Deadline: 2/12/16 by 12:00 noon

SAN FRANCISCO BAYKEEPER®

February 12, 2016

Ms. Jeanine Townsend, Clerk to the Board
State Water Resources Control Board
1001 I Street, 24th Floor, Sacramento, CA 95814
sent via electronic mail: commentletters@waterboards.ca.gov

Re: San Francisco Baykeeper comments on the proposed approval of an amendment to the Water Quality Control Plan for the San Francisco Bay Basin to Establish a Total Maximum Daily Load and Implementation Plan for Selenium in North San Francisco Bay

Dear Ms. Townsend and Members of the Board,

On behalf of San Francisco Baykeeper and our over 3,000 members, we respectfully submit these comments on the proposed approval of an amendment to the Water Quality Control Plan for the San Francisco Bay Basin ("Basin Plan") to Establish a Total Maximum Daily Load and Implementation Plan for Selenium in North San Francisco Bay ("Proposed TMDL").

As a long-time water quality advocate for the Bay, Baykeeper recognizes the difficulties of addressing selenium (Se) enrichment from diverse sources, as well as the confounding effects of biomagnification in the benthic macro-vertebrate community. Recent research indicates current conditions are resulting in significant impacts to resident white sturgeon (*Acipenser trasnmontanus*), as well as the more Se-sensitive and federally-listed green sturgeon (*Acipenser medirostris*). However, the Regional Board's decision to maintain the existing selenium load though the Proposed TMDL process ignores volumes of peer reviewed literature and government reports, is unwarranted, and fails to ensure protection of the Bay's beneficial uses, including Estuarine Habitat (EST) and Preservation of Rare and Endangered Species (RARE).

Given the documented impairment to the federally-listed green sturgeon, and numerous other species, due in part to existing Se contamination, we request re-analysis of the Proposed TMDL to ensure adequate protection of beneficial uses and to facilitate recovery of this species. Comments contained within this letter reflect consideration of prior response to comments and communications with Regional Board staff, though additional comments herein discuss issues of monitoring and Clean Water Act consistency.

Baykeeper is also concerned that stakeholder engagement on this Proposed TMDL over the past 10+ years has largely been limited to discussions between the Regional Board and oil refinery representatives. We can find no evidence to suggest Regional Board staff sought the input of readily available international experts on selenium contamination, located in local USGS offices, UC Davis and elsewhere, to support this Proposed TMDL. Nor were the recommendations of these experts, as found in peer-reviewed literature and available technical reports, taken into consideration in the monitoring and modeling components on this Proposed TMDL. Further, it appears all technical reports in support of this Proposed TMDL were prepared by consultants of Western States Petroleum Association, with no apparent third party review of these particular documents, despite the availability of leading experts within the region, such as Dr. Samuel Luoma

and Dr. Theresa Presser. Peer review of the Proposed TMDL itself was limited to scientists located out of state and it is not clear whether they were asked to review supporting technical reports or supporting data.

Status quo approach proposed in the Proposed TMDL is insufficient to ensure species protection

The Proposed TMDL assumes a 'hold the line approach', partly due to comparison of a small dataset of Se fish tissue concentrations, which the Regional Board felt did not significantly violate the U.S. Environmental Protection Agency's ("EPA") 2015 *Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater* ("Draft Criteria"). The proposed fish tissue target of 8.1 µg/g whole-body dry weight ("dw") and 11.8 µg/g muscle tissue dw is approximately equivalent to, though slightly higher than the Draft Criteria.[1] EPA's review found the white sturgeon to be most sensitive among the species considered. Hence, the Draft Criteria is based on the concentration expected to impose deleterious effects on 10% of the population (EC10) for white sturgeon. The value of 8.1 µg/g for whole-body dw in the Proposed TMDL appears in U.S. Geological Survey (USGS) and USFWS reports as the EC10 value for adult female white sturgeon and appears to have been drawn from a PhD dissertation which found the effect on white sturgeon to include larval edema and skeletal defects.[2,3,4]

This value, however, is noticeably higher than the EC10 value considered protective of all fish, including green and white sturgeon under low flow conditions, which is considered to be 5.0 µg/g.[5,6,7] Further, the Draft Criteria falls short of considering site-specific data and literature indicating reproductive impairment of white sturgeon (and by proxy, green sturgeon) is already occurring. [8,9,10] Impacts from selenium in North San Francisco Bay have been well documented over the last 30 years – with expert findings indicating significant

1 U.S. Environmental Protection Agency, Office of Water. 2015. Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2015. Available at http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/selenium/loader.cfm?csModule=security/getfile&PageID=71800

2 Presser TS and Luoma SM. 2010. Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California. U.S. Geological Survey, Menlo Park, California. Available at http://www.epa.gov/region9/water/ctr/selenium-modeling_admin-report.pdf

3 U.S. Fish and Wildlife Service, 2009, Selenium effect levels for selected representative/surrogate species: U.S. Fish and Wildlife Service, Sacramento, California, 22 p.

4 Linville RG. 2006. Effects of excess selenium on the health and reproduction of white sturgeon (Acipenser transmontanus): implications for San Francisco Bay-Delta: Davis, University of California at Davis, Ph.D. dissertation, 232 p.

5 Presser TS and Luoma SM. 2010. Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California. U.S. Geological Survey, Menlo Park, California. Available at http://www.epa.gov/region9/water/ctr/selenium-modeling_admin-report.pdf

6 U.S. Fish and Wildlife Service, 2005, Public comment package in response to U.S. Environmental Protection Agency's Draft Aquatic Life Criteria Document for Selenium (Federal Register 69:75541-75546: December 17, 2004): U.S. Fish and Wildlife Service, Washington, DC, 23 p and attachments.

7 Skorupa, JP, Presser TS, Hamilton SJ, Lemly AD, and Sample BE. 2004. EPA's draft tissue based criterion: a technical review: U.S. Fish and Wildlife Report presented to U.S. Environmental Protection Agency, June 16, 2004, 35 p. (collaborative report) Available at http://wwwrcamnl.wr.usgs.gov/Selenium/Library_articles/skorupa_et_al_2004.pdf

8 U.S. Fish and Wildlife Service. 2008. Species at risk from selenium exposure in the San Francisco estuary. Sacramento (CA): U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office. 81 p.

9 Presser TS and Luoma SM. 2013. Ecosystem-scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan. *San Francisco Estuary and Watershed Science*, 11(1). http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

10 Presser TS and Luoma SM. 2013. Appendix A: Ecosystem-Scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation. *San Francisco Estuary and Watershed Science*, 11(1). Available at http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

risk to wildlife. A 2013 paper describing the model developed in the USGS Menlo Park office by international experts on selenium toxicity summarizes the situation as follows[11]:

> *Historic and more recent data show that certain predator species are considered most at risk from Se in the Bay-Delta (e.g., white and green sturgeon, scoter, scaup) because of high exposures obtained when they consume the estuary's dominant bivalve, Corbula amurensis, an efficient bioaccumulator of this metalloid (Stewart and others 2004; Presser and Luoma 2006). The latest available surveys of Se concentrations in C. amurensis and white sturgeon (Acipenser transmontanus) that were feeding (based upon isotopic evidence) in Carquinez Strait, Suisun Bay, and San Pablo Bay (Stewart and others 2004; Linares and others 2004; Kleckner and others 2010; Presser and Luoma 2010b; SFEI 2009) continue to show concentrations exceeding U.S. Fish and Wildlife Service (USFWS) dietary and tissue toxicity guidelines (Skorupa and others 2004; Presser and Luoma 2010b). Sturgeon contain higher concentrations of Se than any other fish species, reflecting their position as a top benthic predator (Stewart and others 2004).*
>
> *Surveys of surf scoter (Melanitta perspicillata) and greater scaup (Aythya marila) that feed voraciously on C. amurensis as they overwinter in Suisun Bay (SFEI 2005; De La Cruz and others 2008; De La Cruz 2010; Presser and Luoma 2010b) show Se has bioaccumulated to levels in muscle and liver tissue that may affect their ability to successfully migrate and breed (Heinz 1996; USDOI 1998; Ohlendorf and Heinz 2011).*
>
> *Endangered Species Act requirements led to a number of species being determined as jeopardized by Se in the Bay-Delta under a proposed chronic aquatic life Se criterion of 5 µg L-1 (USFWS and NOAA Fisheries 2000), including delta smelt (Hypomesus transpacificus); longfin smelt (Spirinchus thaleichthys); Sacramento splittail (Pogonichthys macrolepidotus); Sacramento perch (Archoplites interruptus); tidewater goby (Eucyclogobius newberryi); green sturgeon (Acipenser medirostris) and its surrogate white sturgeon (Acipenser transmontanus); steelhead trout (Oncorhynchus mykiss); Chinook salmon (Oncorhynchus tshawytscha); California clapper rail (Rallus longirostris obsoletus); California least tern (Sterna antillarum browni); bald eagle (Haliaeetus leucocephalus); California brown pelican (Pelecanus occidentalis californicus); marbled murrelet (Brachyramphus marmoratus); and giant garter snake (Thamnophis gigas). Recent analysis by the USFWS (2008a) of 45 species assumed the species most at risk depended on benthic food webs: greater scaup; lesser scaup (Aythya affinis); white-winged scoter (Melanitta fusca); surf scoter; black scoter (Melanitta nigra); California clapper rail; Sacramento splittail; green sturgeon; and white sturgeon. Not enough species-specific information is currently available for consideration of Se exposures for the giant garter snake, an endangered aquatic predator (USFWS 2006,*

11 Presser TS and Luoma SM. 2013. Ecosystem-scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan. San Francisco Estuary and Watershed Science, 11(1). http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

> *2009); the Dungeness crab (Cancer magister), an invertebrate that consumes C. amurensis (Stewart and others 2004); or for species that are within the Dungeness-crab food webs.*

In light of findings expressed in readily available literature and data, maintenance of current Se loads is not protective of existing beneficial uses. Moreover, the Draft Criteria, which Regional Board staff has used to support the rationale for the fish-tissue target in the Proposed TMDL, has not undergone public or inter-agency review, is clearly marked with the disclaimer "Do not distribute, quote or cite", and appears to have not fully characterized the results of recent studies regarding green sturgeon impacts. In addition, the Draft Criteria does not consider the basic question of whether the selection of an EC10 value is actually protective of sensitive and listed species.

When EPA last requested formal comment on aquatic life criteria for selenium from the U.S. Fish and Wildlife Service (USFWS) in 2005, which are generally consistent with the 2015 Draft Criteria, comments included:[12]

> *Selenium is a particularly potent environmental stressor for fish and wildlife, and USFWS scientists (often in collaboration with the U.S. Geological Survey's Biological Resources Division (BRD), EPA's Office of Research and Development (ORD), and university researchers), have produced a substantive portion of the scientific record documenting the ecotoxicology of selenium through a combination of field and laboratory research.*

USFWS concluded:

> *…the proposed tissue value of 7.91 µg/g selenium (parts per million; EPA 2004) is not protective of fish or aquatic-dependent wildlife. In the study cited in the Draft Criteria Document (EPA 2004) as the basis for the 7.91 µg/g proposal (i.e., Lemly 1993), the lowest observed adverse effects (tissue) concentration (LOAEL) was <5.85 µg/g, and this value appears to be an LC-40 (see Attachments 1 and 2). Based on linear extrapolation, an underestimate of effects levels as these curves are exponential, the USFWS has concluded the 7.91 µg/g was greater than an LC-50 for the Lemly (1993) experiment because response curves for selenium are typically very steep (i.e., Lemly 2002; Holm et al. 2003)… Based on this data and other data presented later in this review the USFWS believes that a tissue concentration less than 5 µg/g would provide an appropriate level of protection, not only for aquatic organisms but also for wildlife.*

And just prior to release of these comments, USFWS presented a technical review of EPA's Draft Tissue-Based Selenium Criterion, including a pointed critique of California's draft tissue-based criterion - strongly suggestive that regulators were influenced by Central Valley water contractors to rely on EPA's draft document and the associated fish-tissue criteria:

> *In California, water users within the federal Central Valley Project are citing the draft 7.9 µg/g tissue-based criterion as scientific support for seeking relaxed environmental terms and*

12 69 Fed. Reg. 75541-46 (Dec. 17, 2004)

> *conditions on long-term water contract renewals that, once negotiated, would not be renewed again for at least 25 years (56-57). Decisions that may be irreversible for decades to come are being proposed based on the presumed scientific soundness of EPA's draft tissue-based chronic criterion for selenium.*[13]

Although a copy of the Proposed TMDL was circulated to USFWS and USGS, no formal consultation or personal request to comment was solicited to ensure the TMDL reflects site-specific conditions or appropriate species protections. As a result, no comments on the Proposed TMDL were received from any agency other than EPA. Baykeeper recognizes the Proposed TMDL fish tissue targets are roughly consistent with EPA's Draft Criteria of 8.0 µg/g dry weight. However, since this criteria was only released in July 2015 and EPA never appeared to respond to USFWS critiques, revisions of the Draft Criteria are quite possible.

Further, the Draft Criteria does not reflect the presence of sensitive or listed species and bases the measurement endpoint on the EC10 of fish that do not include the most sensitive fish species in the Bay-Delta, such as the green sturgeon. This issue was partially targeted for critique by peer-reviewers of the 2015 Draft Criteria. For instance, when asked to "comment on EPA's use of the effects concentration 10th percentile (EC10) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element", Dr. Kevin Brix states:

> *It is unclear to me why EPA has selected the EC10 as a measurement endpoint for these studies… It seems to me that the ECx selected should be based on the level of protection EPA intends to provide and this is independent of variability in exposure… Given the above, I do not believe EPA has provided a scientific rationale for use of the EC10 in a tissue-based criterion as providing an equivalent level of protection as an EC20 in a water-based criterion.*[14]

In the Regional Board's response to comments on the Proposed TMDL, personal communications with Dr. Brix confirmed this statement did not imply that the EC10 was under-protective and that he did not consider the USEPA draft criteria as being under-protective for the purposes of setting national criteria. Dr. Brix was not asked, however, to weigh in on whether the standard was appropriate for site-specific conditions - where listed species maintain critical habitat and where selenium-related impacts have been documented. EPA still has not provided rationale as to the use of the EC10 value and the Regional Board did not evaluate whether managing North San Francisco Bay in a manner that places approximately 10% of listed sturgeon species at significant risk is appropriate.

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13 Skorupa JP, Presser TS, Hamilton SJ, Lemly AD, and Sample BE. 2004. EPA's draft tissue based criterion: a technical review: U.S. Fish and Wildlife Report presented to U.S. Environmental Protection Agency, June 16, 2004, 35 p. (collaborative report) Available at http://wwwrcamnl.wr.usgs.gov/Selenium/Library_articles/skorupa_et_al_2004.pdf

14 External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014. Submitted to Joseph Beaman Health and Ecological Criteria Division 4304T Office of Science and Technology U.S. Environmental Protection Agency. Available at http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/selenium/upload/selenium-peer-review-report.pdf

When asked the same question regarding the use of the EC10 standard, Dr. Nicholas S. Fisher (Distinguished Professor & Director, Consortium for Inter-Disciplinary Environmental Research, Stony Brook University) simply replied: "Strikes me as rather arbitrary".

While EPA did consider some of the recent research on green sturgeon, the conclusions derived in the Draft Criteria do not seem consistent with those made by the authors of the cited study. For instance, EPA's Draft Criteria claims:

> *The De Riu et al. (2014) study suggests that green sturgeon may be more sensitive to selenium than white sturgeon and also that the draft EPA whole body concentration of 8.0 mg/kg dw will be protective, based on the survival and growth data and the observation that the control whole body tissue concentrations are similar to the proposed criterion.*

It is true that whole body concentrations in the control group were similar to the proposed criterion. After 8-weeks of dietary exposure at levels present in North San Francisco Bay, Se concentration in green sturgeon was 7.1 µg/g and those in white sturgeon were 5.6 µg/g, versus a Draft Criteria of 8.0 µg/g. However, green sturgeon fed a diet maintaining Se concentrations within the range currently found in the North Bay had a 60% reduction in growth rates after 8 weeks of exposure. In contrast, growth rates in white sturgeon were unaffected, leading researchers to conclude:[15]

> *Our results showed that a dietary Se concentration at 19.7 ± 0.6 mg Se/kg, which is in range with the reported Se concentrations of the benthic macro-vertebrate community of the San Francisco Bay, had adverse effects on both sturgeon species. However, the exposure had a more severe pathological effect on green sturgeon, suggesting that when implementing conservation measures, this federally listed threatened species should be monitored and managed independently from white sturgeon when developing conservation measures to protect this threatened SFBD population segment from Se exposure.*

In the Regional Board's response to this comment, this finding was dismissed by staff on the grounds that since "the high spatial and seasonal variability in density and abundance of *C. amurensis* in the North Bay, as well as the change in concentrations with time, the potential of dietary selenium levels in excess of 10 µg/g at any given time is low'.[16] USGS monitoring, however, indicates dietary selenium concentrations are nearly always in excess of 10 µg/g at some monitoring stations. And at the Carquinez Strait (station 8.1), downstream of several major oil refineries, Se concentrations in *C. amurensis* approximates the 19.7 ± 0.6 mg Se/kg concentrations of concern for several consecutive months, particularly during low flow

15 Riu ND, Lee JW, Huang SSY, Moniello G, and Hung SSO. 2014 Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon. Aquatic Toxicology. 148: 65-73.

16 CA Regional Water Quality Control Board San Francisco Bay Region Resolution No. R2-2015-0048. Amending the Water Quality Control Plan for the San Francisco Bay Basin to Establish a Total Maximum Daily Load and Implementation Plan for Selenium in North San Francisco Bay, Appendix D, Response to comments. Available at http://www.waterboards.ca.gov/sanfranciscobay/board_info/agendas/2015/November/6_appendix_d.pdf

conditions.[17] Such observations are in conflict with statements made in the Proposed TMDL, including "Because selenium bioaccumulation is a long-term process, there is no evidence that selenium bioaccumulation is notably higher at any particular time of year, despite the strong seasonal variability in loads reaching the North Bay."[18]

The De Riu et al. (2014) study did not evaluate whether the proposed Draft Criteria was appropriate but did find that current conditions are insufficient to ensure protection for the green sturgeon. Accordingly, the Proposed TMDL is arbitrary and capricious on the basis it relies primarily on Draft Criteria, rather than site-specific data and local expertise, and it fails to incorporate a margin of safety necessary for protection of beneficial uses. To provide an appropriate level of protection, the Regional Board should consider establishing a more conservative level of protection for sensitive species and/or utilizing Se concentrations in bivalves as an indicator species, as well as a robust monitoring program, as articulated by USGS experts,[19] to assess selenium exposure and risk in North San Francisco Bay.

TMDL fails to consider best available science regarding selenium exposure and risk

EPA has stated that agencies preparing a TMDL should base their decisions on the "best available science and data." U.S. EPA, Draft Guidance for Water Quality-based Decisions: The TMDL Process (2nd Edition), EPA 841-D-99-001 (August 1999) ("1999 TMDL Guidance")[20] at 3-20. The Regional Board cannot show that it established the Proposed TMDL based on the best available science and cannot claim the introduction of an adequate margin of safety, because it failed to consider a wealth of data and literature regarding selenium exposure and risk in the San Francisco Estuary, including data which indicates white sturgeon populations already exceed fish tissue criteria on a seasonal or inter-annual basis.[21] Reports from the USFWS, for example, states "white sturgeon in the San Francisco Bay estuary are producing eggs with as much as 35-times normal selenium content" and "it is highly probable that these fish are reproductively impaired due to selenium exposure".[22]

While Se impacts to white sturgeon have been documented on an on-going basis for some time in North San Francisco Bay, recent research has found the green sturgeon to be even more sensitive to Se exposure. The Proposed TMDL relies on the assumption that white sturgeon can serve as a surrogate for green sturgeon with respect to selenium exposure. In a statement from the Proposed TMDL, which is essentially unchanged

17 Kleckner AE, Stewart AR, Elrick K, and Luoma SN. 2010. Selenium concentrations and stable isotopic compositions of carbon and nitrogen in the benthic clam Corbula amurensis from Northern San Francisco Bay, California: May 1995–February 2010: U.S. Geological Survey Open-File Report 2010-1252, 34 p.

18 Proposed TMDL at p. 2

19 Presser TS and Luoma SM. 2013. Ecosystem-scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan. San Francisco Estuary and Watershed Science, 11(1). http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

20 Available at http://nepis.epa.gov/Exe/ZyPDF.cgi/P1007N47.PDF?Dockey=P1007N47.PDF

21 USGS scientists in Bay Area offices maintain a library of articles and reports related to selenium exposure, many of which are applicable to, or directly the subject of, selenium exposure in the San Francisco Estuary. Available at http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

22 U.S. Fish and Wildlife Service. 2008. *Species at risk from selenium exposure in the San Francisco estuary*. Sacramento (CA): U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office. 81 p.

from the 2011 Preliminary Project Report for the selenium TMDL[23], staff concluded "… white sturgeon is generally considered to be a representative surrogate species for the green sturgeon". This statement is taken out of context from an unpublished 2008 report from USFWS staff, who made a coarse generality regarding the absence of selenium data for green sturgeon at the time.[24] Since that time, several studies have been carried out in the Bay-Delta, leading to presentation of research by one of the same biologists who made the 2008 statement calling for a revisionist stance on selenium and sturgeon:

> *…This analysis indicates that white and green sturgeon are among the most sensitive of fish to adverse effects of selenium, with the listed green sturgeon being the more sensitive of these two species. These levels of sensitivity evidently put sturgeon at substantial risk at current levels of exposure in the San Francisco Bay area. Selenium concentrations in food items of sturgeon in the San Francisco Bay area are almost always high enough that they may cause at least 10 percent mortality in hatchling green sturgeon (≥3.58 µg/g), and they are frequently high enough that they may cause at least 10 percent mortality among hatchling white sturgeon (≥10.8 µg/g) as well.*[25]

National Marine Fisheries Service (NMFS) also recognizes "Recent studies have shown that green sturgeon are more sensitive to selenium than white sturgeon".[26] And the 2014 paper from UC Davis researchers and others, cited above, "…showed that a dietary Se concentration at 19.7 ± 0.6 mg Se/kg, which is in range with the reported Se concentrations of the benthic macro-vertebrate community of the San Francisco Bay, had adverse effects on both sturgeon species" and that the green sturgeon should be "monitored and managed independently from white sturgeon".[27] Other UC Davis researchers have made public statements that green sturgeon are more sensitive to selenium and that "white sturgeon are not an appropriate surrogate for green sturgeon in determining the effects of [methylmercury and selenium] on sturgeon bioenergetics".[28]

In light of the fact that recent research indicates the federally-listed green sturgeon is likely experiencing significant impacts associated with selenium at concentrations found in their existing diet, we respectfully request the State Board to reject the status quo approach, in which the proposed TMDL is equivalent to the

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23 California Regional Water Quality Control Board, San Francisco Bay Region. 2011. Total Maximum Daily Load, Selenium in North San Francisco Bay. Preliminary Project Report. Available at http://www.waterboards.ca.gov/rwqcb2/water_issues/programs/TMDLs/northsfbayselenium/SeTMDL_PreliminaryReport_01-11.pdf

24 Beckon WN and TC Maurer. 2008. Unpublished Report: Potential Effects Of Selenium Contamination On Federally-Listed Species Resulting From Delivery of Federal Water to The San Luis Unit. U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office. Available at wwwrcamnl.wr.usgs.gov/Selenium/Library_articles/Beckon_and_Maurer_Effects_of_Se_on_Listed_Species_SLD_2008.pdf

25 Presentation Abstract from the 2012 Norcal Setac Annual Meeting. Toxicity of Selenium to White and Green Sturgeon. W. N. Beckon, U.S. Fish and Wildlife Service, Sacramento, CA. Available at: https://norcalsetac.files.wordpress.com/2015/02/2012_norcal_setac_annual_meeting_agenda_final_28apr12-1.pdf

26 National Oceanic and Atmospheric Administration, National Marine Fisheries Service. 2010. 75 CFR 30714.

27 Riu ND, Lee JW, Huang SSY, Moniello G, and Hung SSO. 2014 Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and whilte sturgeon. Aquatic Toxicology. 148: 65-73.

28 Presentation from RC Kaufman, AG Houck, JJ Cech on the Effects of Dietary Selenium and Methylmercury on Green and White Sturgeon Bioenergetics in Response to Changed Environmental Conditions Available at http://wwwrcamnl.wr.usgs.gov/Selenium/Library_articles/san_luis_articles/Kaufman_et_al_Effects_of_Dietary_Se_and_Hg_2008.pdf

existing load to the Bay. Through failure to reflect best available science in this Proposed TMDL, as the Regional Board has done here, the decision approving the Proposed TMDL was arbitrary and capricious, an abuse of discretion, and/or otherwise not in accordance with law. *See, e.g., Northwest Environmental Advocates v. U.S. E.P.A*., 855 F. Supp. 2d 1199, 1217-18 (D. Or. 2012) (finding EPA approval of TMDL to be arbitrary and capricious where it failed to "use the best scientific data available" and ignored "historical changes to salmonid populations and river conditions")..

Failure to introduce margins of safety _necessary to achieve TMDL objectives

Under the CWA, either the State or EPA is required to establish a TMDL for impaired waters "at a level necessary to implement the applicable water quality standards with seasonal variations and a margin of safety which takes into account any lack of knowledge concerning the relationship between effluent limitations and water quality." 33 U.S.C. § 1313(d)(1)(C). Here, the Regional Board has not built into the Proposed TMDL a "margin of safety" because it has not taken into account the fact drought conditions in California are the "new normal", resulting in increased selenium concentrations in the water column, thus facilitating greater rates of bioaccumulation.[29,30] Additionally, as discussed above, the Regional Board did not introduce an adequate margin of safety to account for heightened Se sensitivity in green sturgeon, compared to white sturgeon.

Implementation of the California Water Fix, or 'twin tunnels' project would exacerbate this issue further. Researchers have estimated that increased diversion of the Sacramento River (low Se concentrations) accompanied by greater inflows from the San Joaquin River (high Se concentration) to the Delta and the Bay could result in a doubling of particulate Se concentrations in the Bay.[31] To account for observed shifts in Se exposure under variable flow conditions, researchers have recommended that protective Se concentrations in bivalves and fish should be based upon the most sensitive species (green and white sturgeon) at the most sensitive times (low flow dry years).[32] Such analysis was conducted by leading experts, indicating the level of protection for sturgeon would equate to a fish tissue concentration of 5 µg/g Se, dw fish whole-body.[33]

Moreover, in approving the Proposed TMDL, the Regional Board ignored policies that require adequate reasonable assurances that nonpoint sources of pollution will be reduced in impaired waters polluted by both point sources and nonpoint sources of pollution. See Guidance for Water Quality-Based Decisions: the TMDL Process, EPA440/4-91-001 (April 1991) ("1991 TMDL Guidance") ("In order to allocate loads among both point and nonpoint sources, there must be reasonable assurances that nonpoint source loads will in

29 Diffenbaugh NS, Swain DL, and Touma D. 2015. Anthropogenic warming has increased drought risk in California. PNAS 112(13):3931-3936

30 Presser TS and Luoma SM. 2013. Ecosystem-scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan. San Francisco Estuary and Watershed Science, 11(1). http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

31 Meseck SL, Cutter GA. 2006. Evaluating the biogeochemical cycle of selenium in San Francisco Bay through modeling. Limnology and Oceanography 51(5):2018–2032.

32 Stewart AR, Luoma SN, Schlekat CE, Doblin MA, Hieb KA. 2004. Food web pathway determines how selenium affects ecosystems: a San Francisco Bay case study. Environmental Science and Technology 38(17):4519–4526.

33 Presser TS and Luoma SM. 2013. Ecosystem-scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan. San Francisco Estuary and Watershed Science, 11(1). http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

fact be achieved."). "Reasonable assurance means a high degree of confidence that wasteload allocations and /or load allocations in TMDLs will be implemented by Federal, State or local authorities and /or voluntary action." 1999 TMDL Guidance at 3-23 (emphasis added). Based on the Proposed TMDL, it is not clear whether Se TMDLs in the Central Valley are on track for attainment of 2019 load allocations. Nor is it articulated in the Proposed TMDL how Region 2 will accomplish the stated intention to "work with the State and the Central Valley Water Boards to ensure the current load allocation for the Central Valley watershed in the TMDL is attained."[34]

Because the Regional Board ignored CWA requirements to establish an adequate margin of safety in the Proposed TMDL and to have adequate assurances that nonpoint sources will meet load reductions, the decision approving the Proposed TMDL was arbitrary and capricious, an abuse of discretion, and/or otherwise not in accordance with law.

Monitoring is insufficient to determine protection of sensitive species

"[I]mplementation and follow-up monitoring of TMDLs is crucial to the success of any State water quality program."[35] Any TMDL submittal in California must include an implementation plan as well as a monitoring plan.[36] A monitoring plan must be "designed to determine the effectiveness of the implementation actions and to help determine whether allocations are met. The monitoring or modeling plan must be designed to describe whether allocations are sufficient to attain water quality standards and how it will be determined whether implementation actions, including interim milestones, are occurring as planned. The monitoring approach must also contain an approach for assessing the effectiveness of best management practices and control actions for nonpoint sources."[37]

The Proposed TMDL fails to specify any monitoring requirements for fish tissue and receiving water analysis. The only requirement is for continuation of "discharger-funded RMP monitoring of selenium in fish and water at a spatial scale and frequency to determine whether concentrations in fish, specifically sturgeon, remain low and water column and fish tissue targets are met".[38] Fish tissue monitoring for Se in sturgeon has been carried out at the sole discretion of the Regional Monitoring Program's ("RMP") Steering Committee. To date, green sturgeon have not been sampled and monitored for Se, though white sturgeon have been routinely sampled (in 1997, 2000, 2003, 2006, 2009, and 2014) as part of the RMP Status and Trends sport fish monitoring program. However, the number of fish collected in each round of sampling has been small (~12 fish per round) and out of cost considerations, the sampling frequency has recently been reduced to a once in five year cycle going forward. No statistical analysis has been performed to determine the appropriateness of the current monitoring program, though it is unlikely the current program satisfies TMDL requirements to determine the effectiveness of the implementation actions.

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34 Proposed TMDL at p. 6
35 1999 TMDL Guidance at 3-22
36 See Water Code §§ 13050(j), 13242; Memorandum from William R. Attwater, Chief Counsel, State Water Resources Control Board, to Gerard J. Thibeault, Executive Officer, Santa Ana Regional Water Quality Control Board (Mar. 1, 1999); *see also* 1999 TMDL Guidance at 3-23
37 Ibid at 3-23
38 Proposed TMDL at p. 7

Moreover, the Proposed TMDL fails to consider recommendations from experts on monitoring programs suitable to determine compliance with TMDLs or fish tissue guidelines. For example, Luoma and Presser (2013) recognize Se concentrations in fish or bird tissues appear to be good indicators of ecological risks from Se. They state that key invertebrates, such as *C. amurensis*, may, however, be a more pragmatic indictor for frequent biological monitoring and provide the following:

> *Given that (1) suspended particulate material Se concentrations are key to accurate prediction of prey and predator Se concentrations; and (2) dissolved Se concentrations are constrained to a narrow dynamic range within the estuary, a suspended particulate material Se concentration also may be a sensitive parameter on which to assess change. Dissolved Se concentrations appear to be the variable of choice for regulatory agencies, however, because of links to total maximum daily loads.*[39]

These same researchers estimate that under existing low flow conditions, 23 to 66% of dissolved Se measurements in the Bay exceeded the value predicted necessary to meet a fish tissue Se concentrations roughly equivalent to the Draft Criteria. And under guidelines they felt were appropriate to protect endangered species, 100% exceedance occurs at low flow conditions.[40] This finding is startling and deserves to be confirmed through robust monitoring, the standards for which must be established in this TMDL.

The Proposed TMDL fails to provide any level of monitoring specificity and fails to recognize the fact monitoring frequencies, program designs and partner agencies are placed at the discretion of RMP management. Because the Regional Board ignored CWA requirements to establish a monitoring program, the decision approving the Proposed TMDL was arbitrary and capricious, an abuse of discretion, and/or otherwise not in accordance with law.

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39 Presser TS and Luoma SM. 2013. Ecosystem-scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan. San Francisco Estuary and Watershed Science, 11(1). http://wwwrcamnl.wr.usgs.gov/Selenium/library.htm

40 Ibid

Selenium bioaccumulation is complicated by multiple factors and risks vary by sub-region within the San Francisco Estuary. Scientists have the ability, however, to quantify these risks and assess load reduction scenarios, if needed. Given that a fundamental objective of the TMDL is to protect beneficial uses from selenium contamination, greater consideration must be granted to impacts on the federally-listed green sturgeon and other sensitive species. Recent research indicates existing conditions pose a significant risk, which does not comport with a TMDL that requires no reduction in loads and maintenance of the status quo – a concept that is fundamentally at odds with the objectives of TMDL development and implementation.

Sincerely,

Ian Wren
Staff Scientist, San Francisco Baykeeper

Erica A. Maharg
Staff Attorney, San Francisco Baykeeper

Appendix A: Copies of Cited Reports and Scientific Literature

II. 26 NOTICES OF COMMENCEMENT FROM: 11/10/04 TO 11/30/04—Continued

Case No.	Received Date	Commencement Notice End Date	Chemical
P–04–0648	11/10/04	10/22/04	(G) Amine functional epoxy resin salted with organic acid
P–04–0672	11/15/04	11/05/04	(G) Isocyanate functional polyester urethane polymer
P–04–0691	11/15/04	11/05/04	(G) Urethane acrylic hybrid polymer
P–04–0712	11/23/04	11/04/04	(G) Azole polymer
P–04–0722	11/18/04	10/19/04	(G) Acrylic polymer
P–04–0723	11/18/04	10/19/04	(G) Acrylic polymer
P–04–0743	11/17/04	11/08/04	(G) Substituted phosphonic acid compounded with substituted urea
P–04–0759	11/24/04	10/25/04	(G) Aliphatic polyamine
P–04–0766	11/23/04	11/01/04	(G) Mineral/vegetable oil based alkyd
P–04–0769	11/18/04	11/08/04	(G) Substituted methyl ester of octadecanoic acid
P–04–0801	11/23/04	11/16/04	(G) Aluminum alkoxide complex

List of Subjects

Environmental protection, Chemicals, Premanufacturer notices.

Dated: December 7, 2004.

Vicki Simons,

Acting Director, Information Management Division, Office of Pollution Prevention and Toxics.

[FR Doc. 04–27672 Filed 12–16–04; 8:45 am]

BILLING CODE 6560–50–S

ENVIRONMENTAL PROTECTION AGENCY

[FRL–7850–1]

Notice of Availability of Draft National Pollution Discharge Elimination System (NPDES) General Permits MAG910000 and NHG910000 for Discharges From Groundwater Remediation and Miscellaneous Surface Water Discharge Activities in the States of Massachusetts and New Hampshire and Indian Country Lands in the State of Massachusetts

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of Availability of Draft NPDES General Permits MAG910000 and NHG910000: Extension of Comment Period.

SUMMARY: On Friday, November 2, 2004, the Environmental Protection Agency's New England Regional Office (EPA–NE) published a Notice of Availability for the Draft National Pollutant Discharge Elimination System (NPDES) General Permits MAG910000 and NHG910000 for Discharges from Groundwater Remediation and Miscellaneous Surface Water Discharge Activities in the States of Massachusetts and New Hampshire and Indian Country Lands in the State of Massachusetts in the **Federal Register** (69 FR 63531). In response to requests from sources that may be eligible for coverage under these general permits, EPA–NE is extending the comment period for these permits.

DATES: The comment period is being extended from December 17, 2004, to January 18, 2005. Comments must be received or postmarked by midnight on January 18, 2004. Interested persons may submit comments on the draft general permit as part of the administrative record to the EPA–NE at the address given below. Within the comment period, interested persons may also request in writing a public hearing pursuant to 40 CFR 124.12 concerning the draft general permit. Such requests shall state the nature of the issues proposed to be raised at the hearing. A public hearing may be held at least thirty days after public notice whenever the Regional Administrator finds that response to this notice indicates significant public interest. In reaching a final decision on the draft permits, the Regional Administrator will respond to all significant comments and make responses available to the public at EPA–NE's Boston office. All public comments or requests for a public hearing must be submitted to the address below.

ADDRESSES: Written comments may be hand delivered or mailed to: Roger A. Janson, Director, Municipal Permits Branch (CMP), EPA–NE, 1 Congress Street, Suite 1100, Boston, Massachusetts 02114–2023.

EPA also requests that comments be sent via e-mail to *Rapp.Steve@EPA.GOV*. However, no facsimiles (faxes) will be accepted. A copy of all comments and supporting materials should also be submitted to:

In MA: Mr. Paul Hogan, NPDES Permit Unit, MA Dept. of Env. Protection, 627 Main Street, Worcester, MA 01608.

In NH: Mr. George Berlandi, NH Dept. of Env. Services, Wastewater Engineering Bureau, 29 Hazen Drive, P.O. Box 95, Concord, NH 03302–0095.

The draft permit is based on an administrative record available for public review at the EPA address listed above. Copies of information in the record are available upon request. A reasonable fee may be charged for copying.

FOR FURTHER INFORMATION CONTACT: Additional information concerning the draft permit may be obtained between the hours of 8 a.m. and 4 p.m., Monday through Friday excluding holidays from: Steven Rapp, Office of Ecosystem Protection, Environmental Protection Agency, 1 Congress Street, Suite 1100 (CPE), Boston, MA 02114–2023, telephone: (617) 918–1551, e-mail: *Rapp.Steve@EPA.GOV*.

SUPPLEMENTARY INFORMATION: The draft general permits may be viewed over the Internet via the EPA–Region 1 Web site. For dischargers in Massachusetts, *see http://www.epa.gov/ne/npdes/mass.html#dgp*. For dischargers in New Hampshire, *see http://www.epa.gov/ne/npdes/newhampshire.html#dgp*.

Dated: December 8, 2004.

Robert W. Varney,

Regional Administrator, Region 1.

[FR Doc. 04–27666 Filed 12–16–04; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[OW–FRL–7849–4]

Notice of Draft Aquatic Life Criteria for Selenium and Request for Scientific Information, Data, and Views

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of Availability of Draft Aquatic Life Criteria Document for Selenium, and Request for Scientific Information, Data, and Views Pertaining to the Criteria.

SUMMARY: The Environmental Protection Agency announces the availability of a

draft aquatic life criteria document for selenium and requests scientific information, data, and views. The document contains draft water quality criteria recommendations for the protection of freshwater and saltwater aquatic life. EPA is soliciting information, data, and views on issues of science pertaining to the information the Agency used to derive the draft criteria. When completed and published in final form, the revised criteria will replace EPA's current recommended aquatic life criteria for selenium. EPA's recommended water quality criteria provide technical information for states and authorized tribes in adopting water quality standards, but themselves have no binding legal effect.

DATES: Scientific views, data, and information should be submitted by April 18, 2005.

ADDRESSES: Scientific information, data, and views may be submitted electronically, by mail, or through hand-delivery/courier. Follow detailed instructions provided in section C of the **SUPPLEMENTARY INFORMATION** section.

FOR FURTHER INFORMATION CONTACT: Charles Delos, e-mail *delos.charles@epa.gov* or postal address, Mail Code 4304T, U.S. EPA, 1200 Pennsylvania Avenue NW., Washington, DC 20460 at (202) 566–1097.

SUPPLEMENTARY INFORMATION:

A. Which Entities Might Be Interested?

Entities potentially interested in today's notice are those that discharge or release selenium to surface waters, and federal, state, tribal, and local authorities that regulate selenium levels in surface water. Categories and entities interested in today's notice include but are not limited to:

Category	Examples of interested entities
State/Local/Tribal Government.	States, municipalities, tribes.
Industry	Mining, coal-fired power generation.
Agriculture	Irrigated agriculture.

This table is not intended to be exhaustive. Other types of entities not listed in the table may also be interested.

B. How Can I Get Copies of the Draft Document and Related Information?

1. *Docket.* EPA has established an official public docket for this action under Docket ID No. OW–2004–0019. The official public docket is the collection of materials that are available for public viewing at the Water Docket in the EPA Docket Center, EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the Water Docket is (202) 566–2426. Alternatively, copies of the draft may be obtained from EPA's Water Resource Center by phone at (202) 566–2426, or by e-mail to *center.water.resource@epa.gov* or by conventional mail to: EPA Water Resource Center, 4101T, 1200 Pennsylvania Avenue NW., Washington, DC 20460.

2. *Electronic Access.* Use *http://www.epa.gov/waterscience/criteria/aqlife.html* to obtain the draft document. Use *http://www.epa.gov/fedrgstr/* to obtain this **Federal Register** document electronically.

An electronic version of the public docket is available through EPA's electronic public docket and comment system, EPA Dockets. You may use EPA Dockets at *http://www.epa.gov/edocket/* to access the index listing of the contents of the official public docket and to access those documents in the public docket that are available electronically. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in section B.1. Once in the system, select ''search,'' then key in the appropriate docket identification number.

Certain types of information will not be placed in the EPA Dockets. Information claimed as Confidential Business Information (CBI) and other information whose disclosure is restricted by statute, which is not included in the official public docket, will not be available for public viewing in EPA's electronic public docket. EPA's policy is that copyrighted material will not be placed in EPA's electronic public docket but will be available only in printed, paper form in the official public docket. To the extent feasible, publicly available docket materials will be made available in EPA's electronic public docket. When a document is selected from the index list in EPA Dockets, the system will identify whether the document is available for viewing in EPA's electronic public docket. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in section B.1.

It is important to note that EPA's policy is that data, information, and views, whether submitted electronically or in paper, will be made available for public viewing in EPA's electronic public docket as EPA receives them and without change, unless the data or information contains copyrighted material, CBI, or other information whose disclosure is restricted by statute. When EPA identifies copyrighted material, EPA will provide a reference to that material in the version of the document that is placed in EPA's electronic public docket. The entire printed document, including the copyrighted material, will be available in the public docket.

Data, information, and views submitted on computer disks that are mailed or delivered to the docket will be transferred to EPA's electronic public docket. Data, information, and views that are mailed or delivered to the Docket will be scanned and placed in EPA's electronic public docket. Where practical, physical objects will be photographed, and the photograph will be placed in EPA's electronic public docket along with a brief description written by the docket staff.

C. How Do I Submit Scientific Information, Data, or Views?

You may submit scientific information, data, or views electronically, by mail, or through hand delivery/courier. To ensure proper receipt by EPA, identify the appropriate docket identification number in the subject line on the first page.

1. *Electronically.* EPA recommends that you include your name and mailing address, or e-mail address or other contact information, particularly if you submit data in tables or figures. Also include this contact information on the outside of any disk or CD ROM you submit, and in any cover letter accompanying the disk or CD ROM. This ensures that you can be identified as the submitter and allows EPA to contact you in case EPA has technical difficulties reading your submission or needs further information on the substance of your submission. EPA's policy is that EPA will not edit your submission, and any identifying or contact information provided in the body of the submission will be included in the official public docket, and made available in EPA's electronic public docket. If EPA cannot read your submission due to technical difficulties and cannot contact you for clarification, EPA may not be able to consider it.

i. *EPA Dockets.* Your use of EPA's electronic public docket to submit data, information, and views to EPA electronically is EPA's preferred method for receiving submissions. Go directly to

EPA Dockets at *http://www.epa.gov/edocket* and follow the online instructions. Once in the system, select ''search,'' and then key in Docket ID No. OW–2004–0019. The system is an ''anonymous access'' system, which means EPA will not know your identity, e-mail address, or other contact information unless you provide it.

ii. *E-mail.* Submissions may be sent by electronic mail (e-mail) to *ow-docket@epa.gov* attention Docket ID No. OW–2004–0019. In contrast to EPA's electronic public docket, EPA's e-mail system is not an ''anonymous access'' system. If you send an e-mail directly to the Docket without going through EPA's electronic public docket, EPA's e-mail system automatically captures your e-mail address. E-mail addresses that are automatically captured by EPA's e-mail system are included as part of the submission that is placed in the official public docket, and made available in EPA's electronic public docket.

iii. *Disk or CD ROM.* You may send your submission on a disk or CD ROM to the mailing address identified in section B.1. These electronic submissions will be accepted in WordPerfect or ASCII file format. Avoid the use of special characters and any form of encryption.

2. *By Mail.* Send an original and three copies of your submission to: Water Docket, Environmental Protection Agency, Mailcode: 4101T, 1200 Pennsylvania Ave., NW., Washington, DC 20460, Attention Docket ID No. OW–2004–0019.

3. *By Hand Delivery or Courier.* Deliver your submission to: EPA Docket Center (EPA/DC), EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC 20460, Attention Docket ID No. OW–2004–0019. Such deliveries are only accepted during the Docket's normal hours of operation as identified in section B.1.

D. What Are EPA Recommended Water Quality Criteria?

An EPA recommended water quality criterion is a level of a pollutant or other measurable substance in water that, when met, will protect aquatic life and/or human health. Section 304(a) of the Clean Water Act (CWA) requires EPA to develop and publish and, from time to time, revise, recommended water quality criteria to accurately reflect the latest scientific knowledge. Water quality criteria developed under section 304(a) provide guidance to states and tribes in adopting water quality criteria into their water quality standards under section 303(c). Once adopted by a state or tribe, the water quality standards then are a basis for developing regulatory controls on the discharge or release of pollutants and other alterations of water quality. EPA's section 304(a) criteria also provide a scientific basis for EPA to develop any necessary federal water quality regulations under section 303(c) of the CWA.

The draft criteria in today's notice are based on the factors specified in section 304(a) of the Clean Water Act, including the kind and extent of effects of the pollutant on human health and aquatic organisms. Under the Clean Water Act, the EPA can not consider the economic and technical feasibility of meeting the draft criteria in their development. Economic and technical feasibility factors are considered by states and tribes when they adopt water quality criteria into their water quality standards under section 303(c) of the Act and when states, tribes, and EPA consider variance requests for regulatory controls. Moreover, states and tribes may also consider alternative scientifically-defensible approaches to adopting criteria into their water quality standards that may be different from approaches presented by EPA in final water quality criteria published under section 304(a).

E. What Is Selenium and Why Are We Concerned About It?

Selenium is a naturally-occurring element that is nutritionally essential. However, it has been toxic to aquatic life and terrestrial wildlife where concentrations were excessive. Under real-world field conditions, aquatic life is exposed to selenium primarily through the diet. When the input of a toxic substance to an organism is greater than the rate at which the substance is lost, the organism is said to bioaccumulate that substance. Although selenium bioaccumulates in aquatic organisms, it is not significantly biomagnified. That is, concentrations do not increase significantly in aquatic organisms at each successive level of the food chain. For aquatic life, the lowest toxic thresholds (the smallest levels at which toxic effects are noticeable) are generally associated with effects on larval offspring of the adult fish that were exposed to excessive selenium or with effects on juvenile fish.

Being a natural element, selenium is everywhere in the environment. Concerns about too much selenium in water have most often been associated with irrigation return flows from soils that are naturally high in selenium, ash pond discharges from coal-fired power plants (due to the selenium content of coal), and certain mining activities (due to exposure of selenium-bearing soil or rock to weathering).

F. What Has EPA Done in the Past on the Aquatic Life Criteria for Selenium?

EPA's currently-recommended aquatic life water quality criteria for selenium were published in 1987. EPA made minor adjustments in the criteria concentrations when it converted the selenium criteria from a total recoverable (dissolved plus particulate) measurement basis to a dissolved measurement basis in 1995 and 1999 as follows: (a) In 60 FR 15366, March 23, 1995, only for the Great Lakes Initiative; (b) in 60 FR 22228, May 4, 1995, only for the saltwater criteria; and (c) in 64 FR 19781, April 22, 1999, optionally for freshwater nationwide.

In 1996, EPA proposed but did not complete an additional change in the freshwater acute criterion for the Great Lakes system (61 FR 58444, November 14, 1996). In 2000, EPA revoked the existing acute criterion for the Great Lakes system (65 FR 35283, June 2, 2000) in response to a lawsuit challenging the use of a single acute criterion applicable to selenite and selenate, the two common chemical forms of selenium (see *AISI* v. *EPA*, 115 F. 3d 979 (D.C. Cir. 1997)).

EPA's most recent compilation of criteria presents (a) the above-mentioned 1996 GLI proposed freshwater acute criteria, (b) the 1987 freshwater chronic criterion, and (c) the 1987 saltwater acute and chronic criteria as converted to dissolved in 1995. You can find the compilation at *www.epa.gov/waterscience/standards/wqcriteria.html*.

In 1998 EPA held a peer consultation workshop to evaluate possible courses of action regarding the selenium aquatic life criterion and notified the public of our intent to review the selenium criteria. In 1999, EPA announced its intention to revise its national aquatic life criterion for selenium and requested data (64 FR 58409, October 29, 1999).

In 2002, EPA prepared an early draft revision of its aquatic life criteria document and submitted it to peer review (Versar 2002, Lemly 2004). EPA considered the comments and suggestions submitted by the peer reviewers (U.S. EPA 2004b) and made many technical and scientific changes in response (U.S. EPA 2004a). In the future, EPA will review any scientific information, data, and views submitted in response to today's notice. The Agency will also continue to work closely with the U.S. Fish and Wildlife Service and other key federal agencies to arrive at final water quality criteria

for selenium which are protective of aquatic life.

Today's announcement of the draft aquatic life criteria document for selenium has no effect on EPA's human health criteria recommendation for selenium published in 2002 (see *http://epa.gov/waterscience/standards/wqcriteria.html*).

G. What Are the Draft Aquatic Life Criteria Values?

The draft selenium criteria recommendations state that freshwater aquatic life should be protected under the following conditions:

A. The concentration of selenium in whole-body fish tissue is not more than 7.91 µg/g (micrograms per gram) dw (dry weight). This is the chronic exposure criterion. In addition, if whole-body fish tissue concentrations exceed 5.85 µg/g dw during summer or fall, fish tissue should be monitored during the winter to determine whether the selenium concentration exceeds 7.91 µg/g dw.

B. The 24-hour average concentration of total recoverable (dissolved and particulate) selenium in water seldom (*e.g.*, not more than once in three years) exceeds 258 µg/L for selenite, and likewise seldom exceeds the numerical value given by exp(0.5812[ln(sulfate)]+3.357) for selenate. These are the acute exposure criteria. At an example sulfate concentration of 100 mg/L, the 24-hour average selenate concentration should not exceed 417 µg/L. Sulfate is a commonly measured water quality parameter that has been found to have a mitigating influence on the acute toxicity of the selenate form of selenium.

Likewise, the draft selenium criteria recommendations state that saltwater aquatic life should be protected from acute effects of selenium if the 24-hour average concentration of selenite seldom exceeds 127 µg/L. Because selenium might be as chronically toxic to saltwater fishes as it is to freshwater fishes, the fish community should be monitored if selenium exceeds 5.85 µg/g dw in summer or fall or 7.91 µg/g dw during any season in the whole-body tissue of saltwater fishes.

H. What Would the Draft Aquatic Life Criteria Recommendations Protect?

The draft selenium criteria recommendations were derived from data on aquatic life and are intended to protect aquatic life. Specifically, the draft chronic exposure recommendation is designed to protect against mortality, reproductive interferences, and growth abnormalities in fish and other aquatic organisms due to long-term excessive exposure to selenium in the aquatic food chain. The draft acute exposure recommendations are designed to protect against lethality or immobilization of aquatic organisms due to brief elevated exposure to selenium in water.

Although the draft recommendation took into account dietary exposure for aquatic life, no nationally-applicable scientific methodology yet exists to derive national water quality criteria to protect birds or terrestrial wildlife that consume fish, water, or aquatic plants and organisms that contain selenium. Therefore, this draft selenium recommendation is not designed to protect birds or terrestrial wildlife. (Similarly, EPA's existing 1987 water quality criteria for selenium were not designed to protect birds or wildlife.) However, EPA is working with the U.S. Fish and Wildlife Service and other interested federal agencies to develop selenium criteria protective of wildlife within the State of California. The California-specific wildlife criteria effort is separate from the national-scale draft aquatic life criteria announced in today's notice. Its development is on a different time track; it involves analysis of toxicity data for aquatic-dependent wildlife (not aquatic life); and it is intended to apply only to California.

I. How Do the Draft Aquatic Life Criteria Recommendations Differ From Previous Criteria Recommendations?

In contrast to the existing 1987 freshwater chronic criterion, which was expressed as a conventional water concentration, the draft freshwater chronic criterion sent to peer review in 2002 and the draft criterion announced in today's notice are each expressed as a whole-body fish tissue concentration (µg selenium per gram of fish tissue on a dry weight basis). At a given location or for a given water body, a fish tissue level of selenium can be used with a site-specific bioaccumulation factor to estimate the concentration of selenium in the water. A bioaccumulation factor is a measured or predicted ratio between the tissue concentration and the water concentration of a chemical, in this case, selenium.

Early in the process of developing these draft criteria, EPA concluded, and the peer reviewers agreed that a fish-tissue approach is better than a conventional water concentration approach to protect aquatic life from the chronic adverse effects of selenium. Because fish and aquatic invertebrates are exposed to selenium primarily through their diet rather than directly through water, the fish-tissue concentration better reflects site-specific exposure and risk than does the water concentration. Therefore, using the fish-tissue approach allows users to consider site-specific factors in translating to a water concentration.

However, consistent with the type of toxicity tests used for their derivation, the draft aquatic life criteria to protect against the acute effects of selenium in fresh water and salt water are expressed as traditional water concentrations (total recoverable selenium). Expanding the toxicity database with a substantial number of more recent acute toxicity tests yielded relatively little change in the freshwater selenite criterion, but yielded a substantial increase in the selenate criterion due to repeated retesting of an amphipod that formerly appeared to have an anomalously low LC50, and due to normalization of the acute data for sulfate concentration. Normalization of all acute test results for sulfate concentration reveals that some species formerly thought to be highly sensitive were actually tested at low sulfate. Including sulfate in the draft criteria formula assures their protection at low sulfate concentrations. Expansion of the database caused the saltwater selenite criterion to decrease because a scallop, formerly untested, was found to be highly sensitive. A saltwater chronic criterion is not presented in the draft announced today, because EPA lacks sufficient and appropriate data to derive one.

J. Are There Particular Issues on Which EPA is Requesting Scientific Information, Data, and Views?

EPA is requesting information, data, and views on all facets of the science supporting the draft criteria recommendations for selenium, but it is particularly interested in the following topics:

1. The Appropriateness of Basing the Freshwater Chronic Criterion on a Tissue Concentration

Because the same water concentration may yield different amounts of bioaccumulation and therefore different levels of risk at different sites, EPA developed this draft criterion as a fish tissue concentration to reduce the need for resetting the criterion on a site-by-site basis. Where translation from the tissue benchmark to a water concentration is needed, a bioaccumulation factor (BAF), which may vary substantially from site to site, would need to be established.

Participants in the 1998 Peer Consultation Workshop suggested that a tissue-based approach for a selenium aquatic life criterion would be feasible

(U.S. EPA 1998). The underlying concept is different from that used historically for developing aquatic life criteria that are applied to the water column, the surrounding environment shared by a range of aquatic species. Nevertheless, this tissue-based approach appears to be appropriate because, at concentrations not far above the draft criterion, selenium is toxic to the offspring (embryos, larvae, or juveniles) of sensitive species, but not to the adult fish that might be present and from which an environmental sample could be taken.

EPA is requesting scientific information, data, and views on (a) the concept of protecting aquatic life by applying a criterion to whole-body fish tissue concentrations of selenium, (b) the appropriateness of applying a fish tissue-based water quality criterion uniformly across waterbodies to protect sensitive species, and (c) the possibility of applying the same criterion to invertebrate tissue where invertebrate samples are obtained with or in place of fish tissue samples.

Because EPA has not yet made decisions on the form or values of its final water quality criteria for selenium, EPA has not yet developed implementation procedures. Therefore, EPA is also interested in scientific information, data, and views on (d) approaches for sampling tissues, and (e) available data for deriving localized BAF values for translating the tissue concentrations to water concentrations, where needed for pollution control decisions.

2. Studies of Freshwater Aquatic Life Effects and Chronic Effect Concentrations

Based on studies involving exposure through a contaminated diet, the genus mean chronic EC20 (concentration effecting 20% of test organisms) for effects on larval or juvenile common sunfish (Lepomis) was found to be 9.5 µg/g dry weight whole body concentration of selenium in the adult parental fish or in the juveniles (depending on the study). This genus mean value is based on four studies. No data indicated that other genera were more sensitive than Lepomis. Useful chronic toxicity data were available for a rotifer (a small invertebrate), chinook salmon, rainbow trout, cutthroat trout, fathead minnow, flannelmouth sucker, razorback sucker, stripped bass, and a mixture of sunfish.

One of the above studies was by Lemly (1993), who investigated overwinter survival of juvenile bluegill in the laboratory. This study consisted of a control (only background selenium exposure) and one elevated selenium exposure level, both subjected either to (a) a temperature regime of 20 °C for 180 days, or (b) a temperature regime changing from 20 °C to 4 °C over the course of 60 days, and remaining at 4 °C for the remaining 120 days of the study. He observed substantially less survival when elevated selenium was combined with low temperature. The whole body concentration associated with mortality was 5.85 µg/g at Day 60 just prior to a significant increase in mortality, and 7.91 µg/g later in the study during and subsequent to the death of 40% of the organisms. For the same selenium exposure at 20 °C, mortality was 6% and whole body concentrations were 5.74 µg/g. Little mortality was observed at either temperature regime for unexposed organisms, but since there was only one selenium treatment, no concentration-response curve can be constructed.

One possible implication of the Lemly (1993) study might be that effects on overwinter survival of juveniles occur at lower concentrations than do effects on reproduction or early life stages. In the Monticello macrocosm study, at 4 to 5°C overwinter conditions, reproductive success and adult bluegill overwinter survival were unaffected at concentrations higher than those of the Lemly (1993) study (Hermanutz *et al.* 1996, corrected by Tao *et al.* 1999, and peer reviewed in Versar 2000).

Based on the Lemly (1993) results, to protect sensitive fish species under winter conditions, EPA has set the draft criterion at 7.91 µg/g, the concentration measured during the period of reduced survival, with the provision that winter monitoring should be performed if summer or fall tissue levels exceed 5.85 µg/g, the concentration occurring prior to the period of reduced survival. Three of five peer reviewers of the 2002 draft questioned whether the results from only one study should be used as the basis for lowering the nationally recommended criteria from 9.5 µg/g to 7.91 µg/g as EPA has done in this document. On the other hand, U.S. Fish and Wildlife Service (White 2002) has questioned whether 7.91 µg/g is sufficiently protective, citing the high mortality observed at that tissue concentration during the study.

EPA is requesting scientific information, data, and views on (a) the most appropriate interpretation and use of the Lemly (1993) results, and its applicability to a range of climatic regimes and fisheries types and (b) other data that may be relevant to the winter exposure issue. Because EPA expects it has seen all the available laboratory studies relevant to the issue, it is particularly interested in field observations (such as age structure or species occurrence) that may be relevant to the selenium winter exposure issue under various climatic conditions. EPA is also requesting scientific information, data, and views on (c) approaches for accounting for different climatic conditions.

3. Alternative Values for the Freshwater Chronic Criterion

The current draft criteria document has set the aquatic life criterion for selenium at a whole body fish tissue concentration of 7.91 µg/g, with the provision that winter monitoring should be performed if summer or fall tissue levels exceed 5.85 µg/g. EPA is requesting information and analyses relevant to alternative fish tissue benchmarks. EPA will only consider analyses that have a formal, fully transparent, and reproducible derivation from laboratory or field data, where all the supporting information quantifies a toxic effect metric and an exposure metric.

EPA is also receptive to formally-derived benchmarks applicable to other aquatic media, such as water, sediment, or prey tissue. Again, the derivations should be transparent and fully reproducible from laboratory or field data.

4. Site-Specific Factors Affecting the Freshwater Chronic Criterion

Expressing the chronic criterion as a tissue concentration rests on the assumption that there is reasonable geographic uniformity in the tissue threshold, while the BAF, and therefore the water concentration threshold, may vary considerably across sites. EPA believes that the route of exposure affects the tissue threshold. The same tissue concentration, if accumulated through water-only exposure, appears to be more toxic than if accumulated via diet. Fish provided with an uncontaminated diet and exposed to very high water concentrations of selenium (for example, 300 µg/L in the Cleveland *et al.* (1993) study) may show effects when whole body concentrations exceed only 4 µg/g. When exposed through a contaminated diet but essentially uncontaminated water in the same study, effects were not observed until tissue concentrations exceeded around 13 µg/g.

Because EPA did not use studies involving uncontaminated diets coupled with high water exposures, the criterion assumes that the dominant environmental exposure route for the target species is dietary. Consistent with the views of the EPA peer consultation

workshop in 1998, EPA believes that this assumption corresponds to the real-world problems of selenium contamination.

While recognizing that the BAF can vary from site to site, EPA is requesting scientific information, data, and views on the general approach of using a uniform tissue benchmark (expressed as total selenium concentration in whole body) without regard to site differences that might include:

- The species to be protected,
- The type of water body,
- The character of the food web, for example, autochthonous versus nonseleniferous allochthonous,
- The form and concentration of selenium in the water or diet,
- The form of selenium in the sampled tissue,
- The nature of the selenium release,
- Interactions with other trace elements,
- Acclimation or adaptation,
- Hormesis,
- Climatic conditions, and
- Any other relevant site factors.

EPA is also requesting scientific information, data, and views relevant to the need for and appropriate basis for adjusting the tissue benchmark to account for site-specific factors.

5. Saltwater Chronic Criterion

For chronic exposure, we found no data that were useful for deriving a saltwater aquatic life criterion. However, selenium might be as toxic in the tissues of saltwater organisms as it is in the tissues of freshwater organisms. Therefore, the draft contains the cautionary recommendation that the status of the saltwater fish community be monitored if selenium exceeds 5.85 µg/g dw in summer or fall or 7.91 dw during any season (same as the freshwater benchmarks) in the whole-body tissue of saltwater fishes.

EPA is requesting scientific information, data, or views on (a) toxicity thresholds applicable to protecting saltwater organisms exposed to selenium through the food chain, or (b) the appropriateness of extending to saltwater what is known about freshwater toxicity thresholds.

6. Acute Criteria Concentrations

As discussed above, selenium toxicity problems have generally involved contamination of the food web. If the diet of the target species is not contaminated, very high water-column concentrations are needed to bring out effects, particularly when exposure is brief. As with bioaccumulative pollutants in general, acute toxicity (that is, toxicity from a brief sharp increase in the water concentration) is of less concern than chronic exposure through the food chain.

Nevertheless, a large body of toxicity test data are available for brief water-only exposure. Therefore, EPA was able to derive acute criteria to protect aquatic life against the toxic effects of that type of exposure to selenium. For ambient freshwater, the draft selenite or Se (IV) acute criterion is 258 µg/L, and the draft sulfate-dependent selenate or Se (VI) criterion ranges from 109 to 1590 µg/L at sulfate concentrations from 10 to 1000 mg/L. For ambient saltwater the draft selenite acute criterion is 127 µg/L.

EPA is requesting scientific information, data, and views on the appropriateness of the draft values for the acute exposure criteria.

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Dated: December 9, 2004.

Geoffrey H. Grubbs,

Director, Office of Science and Technology.

[FR Doc. 04–27665 Filed 12–16–04; 8:45 am]

BILLING CODE 6560–50–P

FEDERAL RESERVE SYSTEM

Agency Information Collection Activities: Proposed Collection; Comment Request

AGENCY: Board of Governors of the Federal Reserve System

SUMMARY: On June 15, 1984, the Office of Management and Budget (OMB) delegated to the Board of Governors of the Federal Reserve System (Board) its approval authority under the Paperwork Reduction Act, as per 5 CFR 1320.16, to approve of and assign OMB control numbers to collection of information requests and requirements conducted or sponsored by the Board under conditions set forth in 5 CFR 1320 Appendix A.1. Board–approved collections of information are incorporated into the official OMB inventory of currently approved collections of information. Copies of the OMB 83–Is and supporting statements and approved collection of information instruments are placed into OMB's public docket files. The Federal Reserve may not conduct or sponsor, and the respondent is not required to respond to, an information collection that has been extended, revised, or implemented on or after October 1, 1995, unless it displays a currently valid OMB control number.

Request for comment on information collection proposal

The following information collection, which is being handled under this delegated authority, has received initial Board approval and is hereby published for comment. At the end of the comment period, the proposed information collection, along with an analysis of comments and recommendations received, will be submitted to the Board for final approval under OMB delegated authority. Comments are invited on the following:

a. whether the proposed collection of information is necessary for the proper performance of the Federal Reserve's functions; including whether the information has practical utility;

b. the accuracy of the Federal Reserve's estimate of the burden of the proposed information collection, including the validity of the methodology and assumptions used;

c. ways to enhance the quality, utility, and clarity of the information to be collected; and

d. ways to minimize the burden of information collection on respondents, including through the use of automated collection techniques or other forms of information technology.

22nd Annual Meeting of the Northern California Regional Chapter of the

Society of
Environmental
Toxicology
And
Chemistry

Seeing the Forest Through the Trees: Integrated Science, Informed Policy

May 2-3, 2012
University of California, Berkeley
Clark Kerr Campus

Northern California Regional Chapter

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AQUA Science

SCHEDULE

22nd Annual Meeting of the Northern California Regional Chapter of the Society of Environmental Toxicology and Chemistry

May 2-3, 2012
University of California, Berkeley
Clark Kerr Campus

Wednesday, May 2, 2012
Day One – Short Course

Time	Description	Instructor	Location: Room
8:00-8:30	Registration		Krutch Theatre Entry
8:30-12:00	Ecological Risk Assessment and Management – Process and Applications, Module 1	Kimberley Walsh, ARCADIS; Mala Pattanayek, ARCADIS	Room 102

- **Box lunches will be provided to meeting attendees each day.**
- **Parking is available for purchase on-site each day for $12 per day.**

SCHEDULE

Thursday, May 3, 2012
Day Two – Conference

Time	Description	Speaker(s)	Location: Room
8:00-12:00	Registration	NA	Krutch Theatre Entry
9:00-9:10	Welcoming Address	Alex Francisco, NorCal SETAC Past President	Krutch Theatre
9:10-9:20	SETAC N.A. Address	Mary Reiley and Bridgette DeShields	Krutch Theatre
	Introduction of Plenary Speakers	Alex Francisco, NorCal SETAC	Krutch Theatre
9:20-9:45	Plenary Speech: Valuing Nature's Benefits to Society	Guy Ziv, Ph.D., Stanford University	Krutch Theatre
9:50-10:15	Plenary Speech: Talk to the People Who Live There: Using the Dynamics of Environmental Discrimination to Assess Cumulative Impacts	Rachel Morello-Frosch, Ph.D., University of California, Berkeley	Krutch Theatre
10:20-10:45	Plenary Speech: An Overview of the Natural Resource Damage Assessment Process and the Role of Large Integrated Datasets	Jennifer Holder, Ph.D., ERM	Krutch Theatre
10:45-11:00	Panel Discussion, Audience Q&A	Moderator: Alex Francisco	Krutch Theatre
11:00-12:00	**POSTER SESSION AND BREAK**		Garden Room
11:30-13:00	Registration, Box Lunch Pick-up		Krutch Theatre Entry; Building 14 Hallway
12:00-13:00	Student -Mentor Lunch	Students, Sustaining Members, Speakers and NorCal SETAC BOD	Garden Room
13:00-14:40	Session 1: Toxicity Testing and Modeling	Session Chair: David Ostrach	Krutch Theatre
13:00-14:40	Session 2: Contaminant Fate and Transport	Session Chair: Katie Henry	Room 102
13:00-14:40	Session 3: Advances in Toxicogenomics	Session Chair: Eugenia McNaughton	Room 104
14:40-15:00	**BREAK**		
15:00-16:40	Session 4: Issues in Human Health Risk	Session Chair: David Ostrach	Krutch Theatre
15:00-16:40	Session 5: Monitoring Contaminants in the Environment	Session Chair: Charlie Huang	Room 102
16:40-17:00	**POSTER SESSION AND BREAK**		Garden Room
17:00-17:15	Members Meeting	All	Garden Room
17:15-18:00	Social Reception and Student Awards	All	Garden Room

SCHEDULE

PLATFORM SESSIONS

Sessions 1, 2 and 3 (13:00 – 14:40)

Time	Session 1: Toxicity Testing and Modeling Chair: David Ostrach Room: Krutch Theatre	Session 2: Contaminant Fate and Transport Chair: Katie Henry Room: 102	Session 3: Advances in Toxicogenomics Chair: Eugenia McNaughton Room: 104
13:00-13:20	**Beckon W, U.S. Fish and Wildlife Service, Sacramento, CA:** *Toxicity of Selenium to White and Green Sturgeon*	**Jones R,** Bayer CropScience**, Stilwell, KS:** *Important Pathways for Residential Runoff Transport of Pyrethroids*	**Page K, UC Berkeley, Berkeley, CA:** *Metallostasis Genes Regulate in vivo Aluminium Levels and Sensitivity to Aluminium Exposure in Caenorhabditis elegans*
13:20-13:40	**Clark S, Pacific EcoRisk, Fairfield, CA**: *Reproduction Toxicity with Ceriodaphnia dubia: "False Positives" Due to Epibionts*	**deBerry B, URS, Oakland, CA:** *Mercury Erosion Control and TMDL Implementation at Former Mercury Mine*	***Gaytan B, UC Berkeley, Berkeley, CA:.***Using Yeast Functional Toxicogenomics to Decipher the Toxicity of Organochlorinated Pesticides*
13:40-14:00	***Callinan K, University of California Davis, CA:** *The Toxicity and Interactions among Common Aquatic Contaminants in Binary Mixtures*	**Phillips B, University of California, Davis, CA:** *Optimization of an Integrated Vegetated Treatment System and Evaluation of Landguard A900 Enzyme: Reduction of Water Toxicity Caused by Organophosphate and Pyrethroid Pesticides*	***Hasenbein M, Technische Universität München, Freising, Germany:** *Genomic Assessments in Delta Smelt (Hypomesus Transpacificus) Exposed to River Water Downstream of the Sacramento Regional Waste Water Treatment Plant*
14:00-14:20	***Hasenbein S, University of California Davis, CA:** *Effect Assessment of Tertiary Pesticide Mixtures on the Amphipod Hyalella Azteca and the Midge Chironomus Tentans*	***Jasper J, University of California, Berkeley, CA:** *Fate of Trace Organic Contaminants in Unit Process Treatment Wetlands*	***Scanlan L, UC Berkeley, Berkeley, CA:**. *Toxicity of silver nanowires on Daphnia magna*
14:20-14:40	**Panel Q & A**	**Panel Q & A**	**Panel Q & A**

SCHEDULE

PLATFORM SESSIONS

Sessions 4, 5 and 6 (15:00 – 16:40)

Time	Session 4: Issues in Human Health Risk Chair: David Ostrach Room: Krutch Theatre	Session 5: Monitoring Contaminants in the Environment Chair: Charlie Huang Room: 102
15:00-15:20	**Brown F, Department of Toxic Substances Control, Berkeley, CA:** *Levels of Halogenated Flame Retardants (HFRs) in House Dust from Northern California Homes*	**Siegler K, University of California Davis, CA:** *The Stream Pollution Trends (SPoT) Program: Evaluating Trends in Stream Contaminants and Toxicity in California*
15:20-15:40	***Li X, University of California, Berkeley, CA:** *Pulmonary Toxicity and Biodistribution of Therapeutic Nanomachines*	**Mckenzie E, University of California Davis, CA**: *A powerful technique for the analysis of metal complexation by macro-molecules – a case study of storm event distributions*
15:40-16:00	***Roegner A, University of California, Davis, CA:** *Microscale Hepatocyte Aggregate Culture (MHAC) and Microcystins (MCs): A potential novel in vitro tool for evaluating congener hepatotoxicity*	***Houtz E, University of California, Berkeley, CA:** *Oxidative Detection of Precursors of Perfluorinated Acids in Aqueous Film Forming Foams (AFFF) and AFFF-impacted groundwater*
16:00-16:20	**Panel Q & A**	**Clark S, Pacific EcoRisk, Fairfield, CA**: *A comprehensive study of pyrethroids in the American River: Information Learned to Date*
16:20-16:40	**Panel Q & A**	**Panel Q & A**

*** Student presentation – please remember to fill out an evaluation if you view this presentation**

Members Meeting - Students and Non-Members Welcome in Garden Room

Social Reception and Student Awards in Garden Room

Plenary Speakers

Guy Ziv , Ph.D., Scientific Development Lead, Natural Capital Project, Stanford University (guyziv@stanford.edu)

"Valuing Nature's Benefits to Society"

Ecosystems provide numerous benefits to society, including water, food, and climate regulation. While we usually account for expected gains due to land management decisions, more often than not we ignore the detrimental impacts of our actions on other aspects. Getting qualitative and quantitative about those trade-offs is the goal of the Natural Capital Project, and the toolset we produce, InVEST - Integrated Valuation of Ecosystem Services and Trade-Offs. In this talk I will present InVEST, and demonstrate how this approach has been successfully applied in multiple locations, with varying policy-contexts including land management decisions, optimal conservation planning and marine spatial planning.

Guy Ziv is leading the development of terrestrial and freshwater environmental services within InVEST. He is a physicist experienced in modeling natural and artificial complex systems. His past projects include analyzing trade-offs between hydropower dams construction and fish biodiversity and productivity in the Mekong River Basin, and quantifying bird communities resilience to agricultural intensification in Costa-Rica. His research interest is the interplay between policy, land management decisions and land use change impacts on Environmental Services. He holds a Ph.D. in Physics from the Weizmann Institute of Science, and was a Research Associate at Princeton University before joining the Natural Capital Project.

Rachel Morello-Frosch , Ph.D., Associate Professor of Environmental Science, Policy and Management and the School of Public Health, University of California, Berkeley (rmf@berkeley.edu).

"Talk to the People Who Live There: Using the Dynamics of Environmental Discrimination to Assess Cumulative Impacts"

Although research has generally demonstrated a pattern of disproportionate exposures to toxics among communities of color and the poor, with racial differences often persisting across economic strata, most previous analyses are limited to illustrating how inequities in hazard exposures are spread across the landscape, shedding little light on their origins, the reasons for their persistence, and the cumulative impacts of environmental and psycho-social stressors. Environmental justice advocates have pushed researchers and policy makers to “move upstream”

to address and prevent the cumulative impacts of chemical and non-chemical stressors on disadvantaged communities. A new environmental justice screening method (EJSM) can inform regulatory decision-making and environmental health policy. The method assumes that community engagement in research on causes and development of new screening approaches is essential to ensuring the rigor, relevance and reach of the emerging science on cumulative impacts.

Dr. Morello-Frosch examines race and class determinants of environmental health among diverse communities in the United States. Along with academic and regulatory colleagues, she has developed scientifically valid and transparent tools for assessing the cumulative impacts of chemical and non-chemical stressors to inform regulatory decision-making and environmental policy, advancing environmental justice goals and addressing the disparate impacts of chemical and non-chemical stressors in vulnerable communities.

Jennifer Holder, Ph.D., Lead of the Sediment and Watershed Integrated Management (SWiM) practice at ERM, (jennifer.holder@erm.com).

"An Overview of the Natural Resource Damage Assessment Process and the Role of Large Integrated Datasets"

Natural resource damage assessments (NRDA) focus on the restoration of natural resource services lost to the public (ecological as well as recreational) as a result of hazardous substance or oil releases. NRDAs encompass the evaluation of small spills in a limited area, through complex river systems, to large regions such as the Gulf of Mexico. Historical data sets, as well as data collected specifically for the NRDA, are integral to the process of estimating the size of the injury and defining the amount of restoration necessary to offset the losses. This presentation will provide an overview of the NRDA process, discuss the types of datasets generally used, and discuss challenges with the use and management of disparate datasets.

Jennifer Holder, PhD is a partner and lead of the Sediment and Watershed Integrated Management (SWiM) practice at ERM. Dr. Holder has over 20 years of environmental industry experience and has conducted ecological assessments in aquatic, sediment, and terrestrial habitats, including National Priority List, RCRA and NRD sites. Her strong experience in evaluating the impacts of contaminants on the environment has resulted in her key role in assessing injuries and supporting damage assessments for a number of Natural Resource Damage Assessments (NRDAs). Her background in ecology also adds to her ability to evaluate and/or implement potential restoration alternatives, an important component of the NRDA process. Jennifer was awarded a B.A. from the University of California, Santa Cruz in Biology and a Ph.D. in Zoology from the University of California, Berkeley. She has numerous publications and has presented at scientific conferences and technical workshops in the United States, South America and Europe.

Platform Presentation Abstracts

Please note: Abstract titles followed by an "*" indicate student presenters. Student presenters will also be indentified at the beginning of their talks by the Session Chair. Please remember to fill out an evaluation if you view this presentation.

Session 1: Toxicity Testing and Modeling

Toxicity of Selenium to White and Green Sturgeon. W. N. Beckon, U.S. Fish and Wildlife Service, Sacramento, CA.

Fish of the genus *Acipenser* (sturgeon) are likely to be among the most vulnerable to selenium exposure in the San Francisco Estuary because these fish feed predominantly on benthic invertebrates, including the Asian clam, *Corbula amurensis*. This clam is an efficient bioaccumulator of selenium. The best data available for the most sensitive endpoint for sturgeon come from studies in which the survival of larvae was monitored following micro-injection of organic selenium (L-selenomethionine) into the yolk sacs of newly hatched larvae. Benchmark larval selenium concentrations from these studies were translated, by means of regressions, to selenium concentrations in the tissue and diet of adult white and green sturgeon. This analysis indicates that white and green sturgeon are among the most sensitive of fish to adverse effects of selenium, with the listed green sturgeon being the more sensitive of these two species. These levels of sensitivity evidently put sturgeon at substantial risk at current levels of exposure in the San Francisco Bay area. Selenium concentrations in food items of sturgeon in the San Francisco Bay area are almost always high enough that they may cause at least 10 percent mortality in hatchling green sturgeon (≥3.58 µg/g), and they are frequently high enough that they may cause at least 10 percent mortality among hatchling white sturgeon (≥10.8 µg/g) as well.

Reproduction Toxicity with *Ceriodaphnia dubia*: "False Positives" Due to Epibionts. S.L. Clark, R. S. Ogle, Pacific EcoRisk, CA, D. Schwartz M. Maidrand, and A. Johnson, Sacramento Regional County Sanitation District.

Numerous factors can affect a toxicity test, including the presence of non-target organisms (e.g., pathogens). In the mid-1990's, testing labs began reporting the presence of pathogen-related mortalities (PRM) in the chronic fathead minnow test, which resulted in the EPA's revision of the 2002 chronic testing manual to recognize and address PRM. However, potential pathogens are not limited to the fathead minnows. Recent microscopic examination of *Ceriodaphnia dubia* (exhibiting reduced reproduction) revealed the presence of epibionts (i.e., organisms living on the surface of another organism), which were determined to be stalked ciliates. Food, detritus, and solids readily adhered to the epibionts' sticky stalks. The extremely rapid proliferation of the epibionts and the accumulation of particulates to the ebiponts' sticky stalks resulted in the *Ceriodaphnia* becoming covered such that feeding and molting appeared to be inhibited. The source of the epibionts is unknown, but the test interference occurred in fall/winter; the epibiont has not been previously identified in the discharger's effluent. Without microscopic identification of the epibiont interference in the testing, routine analysis of the test data would have given a

"false positive" for the reproduction test endpoint. Regulatory implications of the epibionts, and possible laboratory procedures/treatments to reduce epibionts will be discussed.

***The Toxicity and Interactions among Common Aquatic Contaminants in Binary Mixtures.** K. Callinan, University of California, Davis, CA, L. Deanovic, University of California, Davis, CA, I. Werner, Eawag, Dübendorf, Switzerland, S. Fong, Central Valley Regional Water Quality Control Board, Rancho Cordova, CA, S. Teh, University of California, Davis, CA.

Mixtures of pesticides and contaminants are ubiquitous in the aquatic environment, yet their toxic interactions are not well characterized. Mixtures containing pyrethroid pesticides are particularly important due to their high toxicity and environmental prevalence. In this study, multiple binary mixtures were tested for toxic effects and interactions on *Hyalella azteca*, including four pyrethroid pesticides in all binary combinations, as well as mixtures of the pyrethroid, bifenthrin, with chlorpyrifos, copper or ammonia. Five replicates of ten amphipods were exposed to variable concentrations of contaminants, both individually and in mixtures. Mortality, swimming velocity and growth were measured upon test termination after 10 days of exposure. Data were analyzed for mixture interactions using Generalized Linear Model statistics and mortality data were compared against the additive models of Concentration Addition (CA) and Independent Action (IA). Results indicate that mixtures of the neurotoxic pesticides, bifenthrin, permethrin, cyfluthrin, lambda-cyhalothrin and chlorpyrifos most commonly followed the model of CA, while mixtures of bifenthrin with either copper or ammonia followed IA or resulted in less than additive toxicity. With the exception of ammonia, most exposures affected swimming performance and growth in a concentration-responsive manner and the binary mixtures of all chemicals were additive.

***Effect assessment of tertiary pesticide mixtures on the amphipod *Hyalella azteca* and the midge *Chironomus tentans*.** S. Hasenbein, Department of Anatomy, Physiology and Cell Biology, University of California, Davis, CA, S.P. Lawler, Department of Entomology, University of California, Davis, CA, J.P. Geist, Chair of Aquatic Systems Biology, Technische Universitaet Muenchen, Germany, R.E. Connon, Department of Anatomy, Physiology and Cell Biology, University of California, Davis, CA.

The aim of the study was to address mixture effects of pyrethroid pesticides permethrin and lambda-cyhalothrin along with the organophosphate, chlorpyrifos, upon two aquatic invertebrates, *Hyalella azteca* and *Chironomus tentans*, following 10 day exposure tests. Exposure of *C. tentans* to chlorpyrifos alone did not cause significant decrease in growth, whereas exposure to the other pesticides and the mixtures did. At lower concentrations swimming behavior in the single-exposures had a greater response than the mixture. Sublethal concentrations of lambda-cyhalothrin used for *H. azteca* resulted in a decrease in weight. Swimming performance was affected at low concentrations of lambda-cyhalothrin and chlorpyrifos, and at higher concentrations in the mixture exposures. The conducted tests highlight the importance of using a number of different endpoints to adequately assess the effects of both single and mixed compounds.

Session 2: Contaminant Fate and Transport

Important Pathways for Residential Runoff Transport of Pyrethroids. R.L. Jones, Bayer CropScience, Stilwell KS, P.C. Davidson, Waterborne Environmental, Champaign, IL, C.M. Harbourt, Waterborne Environmental, Champaign, IL P. Hendley, Syngenta Crop Protection, Greensboro, NC.

Replicated runoff studies to determine the major pathways for transport of pyrethroids applied to suburban residences were conducted at a full scale test facility near Porterville, California. Tests plots mimicked sloping front lawns and house fronts of California residential developments and included stucco walls, garage doors, driveways, sloping lawns, and residential sprinkler systems. Each of the six lots also included a rainfall simulator to generate artificial rainfall events. In the tests conducted to date, transport occurred in runoff from lawn irrigation (mostly from water landing on hard surfaces) and natural and simulated rainfall events. Under typical application practices the washoff from the driveway and garage door and wall directly above the driveway resulted in the largest masses of pyrethroids leaving the plot, with the losses from applications to vertical wall above grass, the grass next to the wall, and the lawn being an order of magnitude less. With recently adopted label practices, the washoff from the driveway decreased by more than a factor of ten and the washoff from the garage door and the walls above the driveway were reduced by about a factor of five.

Mercury Erosion Control and TMDL Implementation at Former Mercury Mine. B. de Berry, T. Cooke, URS, Oakland, CA, M. A. Assaf, Santa Clara County, Los Gatos, CA.

In 2000, pursuant to a Remedial Action Order from DTSC, Santa Clara County removed mercury mining wastes exceeding the human health action level of 400 mg/kg from the Senador Mine area. In 2010, the EPA established fish-tissue water quality objectives and a TMDL for mercury in the Guadalupe River Watershed. Although storm water sampling confirms a significant drop in mercury loads from the Senador Mine watershed post-remediation, the area continues to generate particulate mercury during storms which may contribute to methylmercury formation in downstream reservoirs. URS is leading the study which combines sampling of soils for THg with an erosion potential analysis to prioritize areas for remedial action. Review of the laboratory results confirms that earlier remedial actions were overall successful in achieving the human health action level; only 1.7% of the soil samples had THg concentrations exceeding 400 mg/kg. Analysis of potentially leachable Hg (0.5N HCl extraction) indicated a small percentage of the THg is soluble. TMDL implementation measures will likely consist of channel realignment around contaminated zones to reduce erosion.

Optimization of an Integrated Vegetated Treatment System and Evaluation of Landguard A900 Enzyme: Reduction of Water Toxicity Caused by Organophosphate and Pyrethroid Pesticides. B.M. Phillips, B.S Anderson, K. Siegler, J.P. Voorhees, R.S. Tjeerdema, University of California Davis, Environmental Toxicology, P. Robins, R. Shihadeh, Monterey County Resource Conservation District, R. Budd, Department of Pesticide Regulation.

Runoff from irrigated agriculture in Monterey County contributes a significant amount of water to local stream flow, and several studies have measured toxic pesticide concentrations and biological impacts in receiving systems. On-farm practices such as vegetated treatment systems (VTS) and enzyme application can reduce concentrations of pesticides in runoff. A redesigned integrated VTS was evaluated with a series of field experiments. The VTS was constructed in a ditch that included a 40m section for sedimentation, a 170m section of vegetation, and included a flashboard riser to control the volume of water in the vegetated section. Laboratory experiments were conducted to determine the optimal dose and mixing time of Landguard A900 enzyme to reduce concentrations of organophosphate pesticides. A series of trials were conducted on a larger, unvegetated drainage ditch to determine the efficacy of the enzyme in a setting with up to twenty times the discharge volume. Field trials included measurements of water toxicity and chemistry at the input and output of each system. These trials were conducted during actual irrigation events that varied in runoff magnitude. The VTS reduced concentrations of pyrethroids, organochlorines and total suspended solids by 97-100%. Landguard application in the larger drainage completely removed chlorpyrifos and diazinon.

***Fate of Trace Organic Contaminants in Unit Process Treatment Wetlands.** J.T. Jasper, D.L. Sedlak, University of California, Berkeley, Berkeley, CA.

Trace organic contaminants, such as pharmaceuticals and personal care products, are commonly measured in wastewater effluent at environmentally significant concentrations. While technologies such as ozonation and reverse osmosis have been shown to be capable of removing many of these contaminants from wastewater, they are too expensive to be employed by most municipalities. Engineered treatment wetlands offer a cost-effective, low-energy alternative. In order to design treatment wetlands that efficiently remove trace organic contaminants from wastewater, a greater understanding of the removal mechanisms operating in wetlands is necessary. To address this issue, we have studied the fate of a suite of commonly occurring wastewater-derived trace organic contaminants in a pilot-scale unit process wetland receiving secondary-treated wastewater effluent in the town of Discovery Bay, CA. Monitoring studies have shown efficient removal of all the compounds studied, with the exception of carbamazepine, in both periphyton-dominated and bulrush-dominated unit process wetlands. Laboratory experiments suggest that sorption and biotransformation are important in both the bulrush and periphyton wetlands, while photolysis is also important for certain compounds in the shallow periphyton wetland.

Session 3: Advances in Toxicogenomics

Metallostasis Genes Regulate *in vivo* Aluminium Levels and Sensitivity to Aluminium Exposure in *Caenorhabditis elegans.* K.E Page, UC Berkeley, Berkeley, CA, D.W. Killilea, Children's Hospital Oakland Research Institute, Oakland, CA, K.N. White, University of Manchester, Manchester, United Kingdom, C.R. McCrohan, University of Manchester, Manchester, United Kingdom, G.J. Lithgow, Buck Institute For Research on Aging, Novato, CA.

Aluminium is a highly abundant toxic metal previously shown to alter metal homeostasis (*metallostasis*). Here we show that reducing the expression of genes predicted to encode metal transport or binding proteins in *C. elegans* not only alters susceptibility to Al toxicity, but also alters the *in vivo* levels of Al in unexposed worms. A set of *C. elegans* genes was selected for their predicted roles in metal regulation, based on amino acid sequence similarity to genes in other species. The effect of gene knockdown on the changes to Al levels present in unexposed worms (via ICP-AES), and tolerance/susceptibility to Al exposure were tested using RNA interference (RNAi). Genes were analyzed for significant difference from the control for both assays, and eleven genes (from 55 tested) were found to change both Al abundance and sensitivity to Al exposure. A gene encoding the stress response transcription factor DAF-16 (a FOXO-like protein) was prominent amongst these eleven genes, implicating it as a major regulator of survival in response to Al toxicity.

***Using Yeast Functional Toxicogenomics to Decipher the Toxicity of Organochlorinated Pesticides.** B. Gaytan, UC Berkeley, Berkeley, CA, A. Loguinov, UC Berkeley, Berkeley, CA, N. Denslow, University of Florida, Gainesville, FL, C. Vulpe, UC Berkeley, Berkeley, CA.

Exposure to organochlorinated pesticides (OCPs) has been linked to neurotoxicity, endocrine disruption, and cancer, but the cellular mechanisms of toxicity remain largely unknown. It was hypothesized that a chemical genomics approach using a *Saccharomyces cerevisiae* gene deletion library could help elucidate the cellular mechanisms by which various OCPs induce toxicity. Pools of deletion strains were exposed in triplicate for five and fifteen generations to the IC20, 50% IC20, and 25% IC20 OCP concentrations. The oligo sequences unique to each deletion strain were PCR-amplified and hybridized to TAG4 arrays to identify sensitive, unaffected, and resistant strains. The overrepresented biological terms within the data assisted in the selection of individual deletion strains for growth curve experiments. It is demonstrated here that genes involved in transcriptional elongation, nitrogen utilization, and amino acid sensing are necessary for resistance to the toxaphene OCP. Analyses for the dieldrin OCP indicate that amino acid sensing and components of the pyruvate dehydrogenase complex are critical for cell survival under dieldrin exposure and that leucine rescues its toxicity. Future investigations will refine the mechanism(s) in yeast and perhaps examine how the knockout or knockdown of orthologs in higher organisms, such as *C. elegans* or human cell lines, affects OCP toxicity.

***Genomic Assessments in Delta Smelt (*Hypomesus Transpacificus*) Exposed to River Water Downstream of the Sacramento Regional Waste Water Treatment Plant.** M. Hasenbein, Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management, Technische Universität München, Freising, Germany, J.P. Geist, Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management, Technische Universität München, Freising, Germany, Richard Connon, School of Veterinary Medicine, University of California.

The delta smelt (*Hypomesus transpacificus*) is an endangered pelagic fish species, endemic to the Sacramento-San Joaquin Estuary, California. Multiple factors, including contaminants, are postulated to contribute to their population decline. Impacts of contaminants on aquatic

organisms are often subtle and difficult to determine. We utilize microarray technology to assess the sublethal responses of delta smelt larvae following a 7-d exposure to ambient water collected at the California Department of Water Resources field station at Hood on the Sacramento River. We identified 103 genes responding significantly to exposure (cut-off $p<0.05$). A total of 94 genes were assigned a function/pathway, whereas 9 genes remained unknown. Significant differences in transcriptional responses were confirmed by qPCR assessments for Atrogin-MAFbx32 (+2.48-fold change), Tropomyosin (-1.80-fold change), Alpha Actin (-1.33-fold change), Collagen XI (-4.06-fold change), Tubulin Cofactor beta (+1.84-fold change), relative to GAPDH. These and other transcriptional differences identified by microarray assessments, indicate impacts on molecular pathways involving energy metabolism, DNA and RNA processing, development of bone and muscle and on the immune system. Results indicate that contaminants originating from sites upstream of Hood are a potential cause for delta smelt growth and development abnormalities, significantly impacting on their immune system.

Toxicity of silver nanowires on *Daphnia magna. L.D. Scanlan, University of California Berkeley, Berkeley, CA, B. Gilbert, Earth Sciences Division LBNL, Berkeley, CA, C, Tran, University of California, Los Angeles, Los Angeles, CA, P, Luong, University of California Berkeley, Berkeley, CA, D. Nowinski, University of California Berkeley, Berkeley, CA, C.D. Vulpe, University of California Berkeley, Berkeley, CA.

Nanowires (NWs) are nanoparticles (NPs) with a high aspect ratio; the length of the particle is much longer than the width. Their shape and physiochemical properties make them ideal for use as building blocks in nano-scale devices and their use is expected to increase. In this study, we investigated the physical characteristics and toxicity of four silver nanowires (AgNWs). Because they are made of silver, AgNWs have an inherent potential for toxicity to aquatic organisms such as *Daphnia magna*. We therefore determined the acute LC_{50} for all four AgNWs and performed microarray gene expression assays to investigate each wire's mode of toxicity. We found that none of the AgNWs are as toxic to *Daphnia magna* as ionic silver ($AgNO_3$). Smaller wires were usually but not always more toxic to *Daphnia*. Speciation studies indicate that ionic silver released from the NWs is not responsible for all of the observed AgNW toxicity. AgNWs were observed in the hemolymph of the daphnids after exposure and the AgNW coatings were altered *in vivo*. Gene expression data suggest that modes of toxicity of AgNWs are different from ionic silver.

Session 4: Issues in Human Health Risk

Levels of Halogenated Flame Retardants (HFRs) in House Dust from Northern California Homes. F.R. Brown, Environmental Chemistry Laboratory, Department Toxic Substances Control, Berkeley, CA (1), T.P. Whitehead, University of California, Berkeley, CA, M. Petreas, J.S. Park (1).

As the use of various PBDEs is decreased or eliminated, industry is substituting other brominated and/or chlorinated FRs, i.e. Halogenated Flame Retardants (HFRs). This raises the question about

people's potential exposure to these HFRs. We developed high resolution GC/MS methodology to measure PBDEs, PCBs and new HFRs in house dust samples collected from vacuum cleaner bags in 2010. Thirteen HFRs were measured and, in these preliminary results, the most abundant HFRs measured were TBB, BTBPE, TBPH, and DBDPE, with PBEB and HBB being detected at lower levels. TDCPP was also detected at high levels in the samples, but in the blank as well, thus rendering the results for TDCPP not useable. Four of these HFRs, BTBPE, DBDPE, TBB, and TBPH were also reported in another study of house dust (Stapleton et al, Environ Sci Tech, 2008) and our preliminary results appear to be comparable. The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

***Pulmonary Toxicity and Biodistribution of Therapeutic Nanomachines.** X.T. Li, UC Berkeley, Berkeley, CA, M. Xue, UCLA, Los Angeles, CA, J. Evans, PNNL, Washington, F. Hayes, UC Davis, Davis, CA, H. Aaron, UC Berkeley, Berkeley, CA, E. A. Eisen, UC Berkeley, Berkeley CA, M.Takeuchi, Kyoto Sangyo University, Kyoto, Japan, C. Vulpe, UC Berkeley, Berkeley, CA, J. Zink, UCLA, Los Angeles, CA, S. Risbud, UC Davis, Davis, CA, K. E. Pinkerton, Center for Health and the Environment, Davis, CA.

The use of nanoparticle carriers is an exciting field to improve drug targeting. Inhalation delivery using nanomachines is becoming more popular in developing delivery methods to efficiently deposit therapeutics into the respiratory and central nervous systems. However, the safety of nanomachines in an inhalation model has not been extensively studied. We aerosolized and delivered functionalized mesoporous silica nanocages in an in vivo model to investigate the effectiveness and toxicity of a model nanomachine taking into account the complex interactions of copolymer functionalization and aerosol optimization. F-MSiN was aerosolized using a miniHEART nebulizer and mice were exposed to the aerosol through a nose-only port system for 5 hours. Aerosol size distribution was sampled using cascade impactors and electrostatic precipitators. Samples were analyzed with confocal microscopy, SEM, EDS, and TEM. Bronchoalveolar lavage fluid (BALF) was collected to assess pulmonary inflammation. Aerosolized F-MSiN ranged from 50nm-2um and localized in alveolar macrophages. Cytotoxicity assays demonstrated a lack of neutrophil or eosinophil influx. We conclude that F-MSiN can be effectively aerosolized as respirable particles that reach the entire respiratory tract with no detected acute toxicity. F-MSiN have the potential to be developed as pulmonary therapeutic nanomachines.

***Microscale Hepatocyte Aggregate Culture (MHAC) and Microcystins (MCs): A potential novel in vitro tool for evaluating congener hepatotoxicity.** A. Roegner and B. Puschner, University of California, Davis**;** A. Khademhosseini, Harvard/MIT Health Sciences, Cambridge.

Globally prevalent in freshwater harmful algael blooms, microcystins (MCs) comprise a family of acutely hepatotoxic cyanotoxins. A tragic acute intoxication of renal dialysis patients in 1996 brought home the importance of developing rapid and accurate assays for toxicity of the over 80 congeners identified in surface waters worldwide. Inhibitors of the ubiquitously expressed

protein phosphatases 1/2A, MCs have resulted in numerous animal intoxications, yet protein phosphatase inhibition poorly predicts congener toxicity in vivo. Organic anion transporter polypeptides (OATPs) expressed on the sinusoidal membrane of liver cells are critical for uptake and hepatotoxicity. We aimed to evaluate whether immortalized liver cells grown in aggregates demonstrate increased expression and functionality of critical transporters normally down regulated in planar culture, thereby providing a potential in vitro tool to rapidly evaluate MC toxicity. Human hepatoma cells HEPG2 grown in MHAC and traditional planar culture were compared for mRNA expression of OATPs and for uptake of fluorescently labeled known substrates. Increased expression of OATPs was documented in aggregates relative to planar culture, along with increased uptake of fluorescently labeled substrates. Inhibition of uptake of the fluorescent compounds by xenobiotics, including microcystins, provides a novel in vitro assay for potential toxicity of surface waters.

Session 5: Monitoring Contaminants in the Environment

The Stream Pollution Trends (SPoT) Program: Evaluating Trends in Stream Contaminants and Toxicity in California K. Siegler, UC Davis, Monterey, CA, B.M. Phillips, UC Davis, Monterey, CA, B.A. Anderson, UC Davis, Monterey, CA, J.P. Voorhees, UC Davis, Monterey, CA, S. Katz, UC Davis, Monterey, CA L. Jennings, UC Davis, Monterey, CA, and R.J. Tjeerdema, UC Davis, Davis, CA.

The Stream Pollution Trends (SPoT) program is a statewide monitoring program under the umbrella of the Surface Water Ambient Monitoring Program (SWAMP). SPoT is designed to detect trends in contamination and toxicity in major watersheds of California. Sites at the base of 100 watersheds were selected for integrative measurements of sediment toxicity and a suite of pesticides, trace metals, and industrial compounds. Toxicity was observed at 20% (2008), 30% (2009), 22% (2010), and 19% (2011) of the sites using the 10d *Hyalella azteca* test. The prevalence of pyrethroid pesticide detections increased from 55% in 2008 to 76% in 2010. Detections of the organophosphate pesticide chlorpyrifos decreased from 11 sites in 2008 to zero in 2010. In 2010 and 2011, a subset of sites was tested for toxicity at 15ºC, as well as the standard test temperature of 23ºC. In 2010, the percent of sites that were toxic increased from 33% (2010) and 33% (2011) when tested at 23ºC to 58% (2010) and 67% (2011) when tested at 15ºC. This suggests pyrethroid pesticides contributed to the observed toxicity. The overall trends suggest that sediment toxicity levels are fairly consistent, pyrethroid detection is increasing, and organophosphate and organochlorine pesticide detections are decreasing.

A powerful technique for the analysis of metal complexation by macro-molecules – a case study of storm event distributions. E.R. McKenzie, University of California Davis, Davis, CA, P.G. Green, University of California Davis, Davis, CA, T.M. Young, University of California Davis, Davis, CA.

High pressure size exclusion chromatography (SEC) coupled with an online inductively coupled plasma mass spectrometer (ICP-MS) is a powerful tool to assess the size dependence of metal

complexation for macro-molecules (<300 kDa) such as natural organic matter (NOM). This system was applied in the assessment of storm event samples from four land uses: highway, urban, agricultural, and natural. Al was associated with large macromolecules. Absorbance (λ=254 nm) was used to detect organic matter (OM), which was primarily detected with molecular weights 3-6 kDa; Cu, Zn, and Ni were also detected in this same size range, indicating that they were likely complexed by the OM. Cr, Mn, Co, Ni, and Pb were commonly detected as dissolved constituents (<100 Da). Only small shift in size associated complexations were observed during the storm. SEC – ICP-MS is a powerful tool for assessing metal complexation; SEC – IPC-MS application to storm event samples revealed both complexed metals (Cu, Zn, and Ni), as well as bioavailable metals (Cr, Mn, Co, Ni, and Pb).

***Oxidative Detection of Precursors of Perfluorinated Acids in Aqueous Film Forming Foams (AFFF) and AFFF-impacted groundwater**. E.F. Houtz, D.L. Sedlak, University of California, Berkeley, Berkeley, CA.

Aqueous Film Forming Foam (AFFF) is a complex mixture of hydrocarbon and fluorocarbon surfactants that is used by the military and municipalities to extinguish liquid hydrocarbon (e.g. fuel) based fires. The use of AFFF above unlined soil has led to high concentrations of AFFF-derived perfluorinated compounds (PFCs), including PFOS and PFOA, in underlying groundwater. The adverse health effects associated with PFOS and PFOA led AFFF manufacturers to discontinue the direct use of these compounds and reformulate their products with different fluorochemicals. Despite reformulations, newly manufactured AFFF contain fluorochemicals that may abiotically or biologically transform to the PFCs, but these PFC precursor compounds are largely proprietary and are difficult to measure directly. To quantify difficult-to-measure precursors, we developed a chemical oxidation method that converts precursors to measurable perfluoroalkyl carboxylic acids. We have discovered through this technique that many major AFFF formulations contain high concentrations of fluorochemicals that may transform to the PFCs in the subsurface. We have used this oxidative method to measure PFC precursors in AFFF-impacted groundwater and sediments. Using oxidative precursor measurements, relative PFC and precursor movement in the subsurface was investigated.

A comprehensive study of pyrethroids in the American River: Information Learned to Date. S.L. Clark & R.S. Ogle, Pacific EcoRisk, CA, T. Albertson, Caltest Analytical, CA, C. Harbourt & G. Hancock, Waterborne Environmental, MI, G. Mitchell, FMC Agricultural Products, NJ, A. Barefoot and D.M. Tessier, DuPont Crop Protection, DE, M. Dobbs, Bayer CropScience, NC, and P. Hendley & K. Henry, Syngenta Crop Protection, LLC, NC.

The American River is considered to be a high quality water source. However, a previous study reported that pyrethroid insecticides were present in water samples collected over a 30 km reach of the American River at concentrations that exhibited toxicity to the amphipod *Hyalella azteca*, based on grab samples collected during 4 storm events and one dry weather event. A follow-up monitoring study is currently underway with the goal of providing a more robust picture of the

condition of the American River. Water samples have been collected during 3 rain ("wet") and 2 dry events along cross-river transects at 7 sites, with 5 stations per transect and 3 depths per station; sediment samples were also collected at the cross-river transect stations during a dry weather event. Two additional events are planned for the future. These samples were analyzed for the same 8 pyrethroid pesticides measured in the previous study. None of the 8 pyrethroids were detected in any of the dry weather event water samples, and sediment samples ranged from ND (not detected) to 5 ng/L. Results for the first wet event are currently undergoing review and will also be discussed.

Poster Presentation Abstracts

(by Poster Number)

Please note: Abstract titles followed by an "*" indicate posters by student presenters. Please remember to fill out an evaluation if you view this presentation.

1. **Degradation Rates of 11 Pyrethroids under Aerobic and Anaerobic Conditions in the Laboratory.** B.N. Meyer, C. Lam, S. Moore, R.L. Jones, Bayer CropScience, Stilwell, KS.

   Registrants of pyrethroids are conducting a number of studies to better understand the transport of pyrethroids from urban and residential applications to surface water, their persistence in water, and their impact on aquatic organisms. In the study described on this poster, degradation of eleven pyrethroids was measured over approximately 100 days in three sediment/water systems under aerobic and anaerobic laboratory conditions at 25ºC in the dark. The three California sediments represented a range of textures and organic matter. Test compounds were bifenthrin, cypermethrin, zeta-cypermethrin, cyfluthrin, beta-cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, gamma-cyhalothrin, lambda-cyhalothrin, and permethrin. The test compounds were applied as two test mixtures (six active ingredients per mixture, with bifenthrin common to both) at approximately 50 µg of test compound per kg of sediment (dry weight). Extracts of sediment/water were cleaned up by SPE, concentrated, and analyzed by GC/MS (except deltamethrin) against matrix-matched standards with cyfluthrin-d_6 as internal standard. Deltamethrin was analyzed by LC/MS/MS using deltamethrin-phenoxy-$^{13}C_6$ as internal standard. The study was fully replicated and, for the same sediments, results from the two test mixtures indicate general agreement between degradation rates measured for bifenthrin and related isomeric products (e.g. cyfluthrin and beta-cyfluthrin). Degradation was generally faster under aerobic conditions compared to anaerobic.

2. **Monitoring for Imidacloprid in California Surface Waters.** E.A. Kanawi, R. Budd, M. Ensminger, K. Starner, S. Gill, K. Goh, California Department of Pesticide Regulation Environmental Monitoring Branch Surface Water Program, Sacramento, CA.

   Imidacloprid is a systemic neonicotinoid insecticide used for crop and seed protection, structure and landscape maintenance, as well as on domestic pets to control a variety of insects. Imidacloprid acts through disruption of nicotinic acetylcholine receptors within the nervous system of insects including non-target arthropods that may be beneficial to pest management. Because of its moderate solubility and persistence in aquatic environment, imidacloprid has the potential to contaminate surface water in regions where it is applied. Currently there is a paucity of monitoring data evaluating offsite transport. Therefore, the California Department of Pesticide Regulation has begun sampling for imidacloprid. Beginning in 2010 surface water samples were collected from agricultural and urban regions throughout California and analyzed for imidacloprid. Samples were collected during dry conditions and during storm events at sites receiving residential runoff, as well as during the irrigated dry-season at sites receiving predominantly agricultural runoff. Imidacloprid was

detected in 67 of 75 agricultural run-off samples (89%); concentrations exceeded the U.S. EPA's chronic invertebrate Aquatic Life Benchmark of 1.05 µg/L in 14 samples (19%). Within urban run-off samples, imidacloprid was detected in 55 of 100 samples (55%) with a single sample exceeding 1.05 µg/L.

3. **Monitoring pollution variability within watersheds: An analysis of the effectiveness of watershed characterization within the Stream Pollution Trends (SPoT) Monitoring Program.** S.B. Katz, UC Davis, Monterey, CA, B.S. Anderson, UC Davis, Monterey, CA, B.M. Phillips, UC Davis, Monterey, CA, K. Siegler, UC Davis, Monterey, CA, J.P. Voorhees, UC Davis, Monterey, CA, L.L. Jennings, UC Davis, Monterey, CA, J.W. Hunt, UC Davis, Monterey, CA and R.S. Tjeerdema, UC Davis, Davis, CA.

   The Stream Pollution Trends (SPoT) program conducts statewide monitoring surveys as part of the Surface Water Ambient Monitoring Program (SWAMP). Sediment samples have been collected annually since 2008 at streams throughout California and analyzed for sediment toxicity and a suite of pesticides, trace metals and trace organic compounds. These data are used to evaluate long term water quality trends statewide. Sampling stations are located at the base of watersheds using a USGS NAWQA integrator site design. In order to investigate how well SPoT base-stations represent spatial and temporal variability in the watersheds, an additional 2-3 stations were sampled and analyzed 3 times per year (summer, fall and winter) throughout 3 different watersheds in both 2010 and 2011. Toxicity and total pyrethroid concentrations (2010) were then analyzed using an Analysis of Variance (ANOVA) to determine statistical differences among the samples. Results were varied and indicated that there were significant spatial, seasonal and yearly differences in 5 of the 6 watersheds where variability studies were conducted. These findings demonstrate the utility of variability studies in future SPoT surveys.

4. *** Effect of Arsenic and Arsenic Metabolites on L-Type Calcium Channel and Large Conductance Calcium-Activated Potassium Channel Expression and Activities in Vascular Smooth Muscle.** K.P. McPherson, R. Khalili, C.E. Pace, J.E. Angermann, School of Community Health Sciences, University of Nevada, Reno., Reno, NV.

   Chronic ingestion of well water contaminated with inorganic arsenic has also been epidemiologically associated with development of hypertension, yet cellular mechanisms by which both inorganic arsenic and methylated arsenic metabolites exert this effect are not well elucidated. Both inorganic arsenite ('iAs^{3+}') and monomethylarsonous acid ('$MMAs^{3+}$') are believed to affect the activity of the 'L-type' calcium ion channel ('LTCC'), which plays a key role in the maintenance of vascular tone and intracellular Ca^{2+} entry. Intracellular Ca^{2+} can regulate the activity of large conductance Ca^{2+}-activated potassium ion channel ('BK_{Ca}'), a known modulator of cellular depolarization that has been recently implicated in the development of hypertension. The present study examined the effects of iAs^{3+} and $MMAs^{3+}$ on expression and activities of LTCC and BK_{Ca} channels in acutely isolated and primary / tissue cultured rat thoracic aorta, and the experimental A7r5 rat thoracic aorta smooth muscle

cell line using whole-cell patch clamp, vascular contractility, and real-time RT-QPCR. Initial results indicate significant alterations in smooth muscle cell morphology, viability, and responsiveness to phenylephrine-induced vasoconstriction upon acute and subchronic exposure to both iAs^{3+} and MMA^{3+}. LTCC activity is also altered following iAs^{3+} exposure. Both iAs^{3+} and MMA^{3+} affect the activities of key ion channels governing the maintenance of vascular smooth muscle tone.

5. *** Exploring the Mechanisms of Toxicity of Polybrominated Diphenyl Ethers in Daphnia Magna.** D.T. Nowinski, L.D. Scanlan, A.A. Arai, C.D. Vulpe University of California, Berkeley, CA.

   Penta and Octa Brominated Diphenyl Ethers (PBDEs) are flame retardants that were incorporated into a wide array of products until their toxic potential lead to a global ban in 2005. Since the chemicals were manufacturing additives, they are not chemically bound to the products, and they leach out into the environment where they have been found to persist and bioaccumulate. *Daphnia magna* were used as a representative aquatic organism for toxicity testing. A 48-hour acute toxicity assay and probit analysis were used to determine the acute LC_{50}. The LC_{50} of PentaBDE and OctaBDE were found to be 0.058mg/L and 5.963 mg/L, respectively. A 48-hour exposure was set up at one-tenth the LC_{50} for microarray analysis. It was discovered that the differential expression caused by each chemical was unique. A Kegg pathway analysis was determined to be insignificant due to the lack of annotated genes in the *Daphnia* genome. qPCR is being performed to validate array results.

6. ***Benthic macroinvertebrate community responses to a diesel oil spill in an urban stream.** M. G. Peterson, University of California, Berkeley, Berkeley, CA, L. Hunt, University of California, Berkeley, Berkeley, CA, V. H. Resh, University of California, Berkeley, Berkeley, CA.

   Urban streams face multiple challenges from human activities, including un-intentional exposure to chemical contaminants, which can cause both short- and long-term impairment to stream biotic communities. We used a Before-After-Control-Impact (BACI) experimental design to assess community-level effects in macroinvertebrate fauna downstream of an un-intentional 700-850 gallon diesel spill in the north fork of Strawberry Creek, an urban Mediterranean-climate stream in Berkeley, California. Benthic macroinvertebrates were sampled monthly at four sites within the two-fork system for one year pre-spill and at 3, 18, 34, and 65 days post-spill. At 3 days post-spill, the impact reach macroinvertebrate abundance was reduced by 65% and percent Ephemeroptera, Plecoptera, and Trichoptera (%EPT) was reduced by 90% compared with pre-spill levels; meanwhile, upstream control sites in both forks remained similar between pre- and post-spill. Abundance and %EPT remained decreased when sampled 18 days and 34 days later. As of 65 days post-spill, macroinvertebrate abundance had not recovered to pre-spill levels; however, %EPT did recover. Re-colonization by EPT taxa within 65 days at the impact site, which lies below the confluence of the two forks, may be due to input from the unaffected fork, suggesting that

multiple-fork complexity may quicken downstream recovery time in Strawberry Creek.

7. *** Evaluation of Drug Toxicity with the Soil Annelid Contact Toxicity Test.** W. Tang and T.J. Smith, University of the Pacific, Stockton, CA.

   In addition to their potential value for *in situ* bioremediation, the earthworm as a laboratory model may offer insight into mechanisms of xenobiotic toxicity. Using the filter paper contact toxicity test, the LD50s of a series of salicylates and phenolics were determined. The rank order in toxicity of these chemicals were compared with mammalian (rat, oral dosing) LD50s and found to be similar. To determine if protein secretion from chemical stress would be a more sensitive toxicity marker for the above xenobiotics, worms were exposed to either sodium salicylate or acetaminophen at a no effect level (NOAEL) and at the LD100 through filter paper contact. After 72 h exposure, the worms were removed and protein remaining on the filter paper was measured using the Bradford method. For both drugs, differences in protein secretion were statistically significant among control (no drug), NOAEL and LD100 groups ($P < 0.05$). These results indicate that lethality and stress-induced protein secretion assessed with the earthworm contact toxicity test may be useful for the evaluation of xenobiotics for both environmental and pharmaceutical toxicity studies.

8. *** Fact or Fiction: Is there a link between drywells and groundwater contamination?** A. Ashoor, N. Pi, & B. Washburn, Office of Environmental Health Hazard Assessment, Cal/EPA, Sacramento, CA.

   Impervious surfaces characteristic of urban areas have resulted in increased stormwater runoff with elevated pollutant levels. In an effort to protect water quality and aquatic habitat, traditional stormwater management systems, which divert stormwater off site, are being replaced with low impact development (LID) practices which infiltrate runoff on site and provide the added benefit of augmenting the aquifer. One challenge to LID practices is poorly-infiltrating soils, common in many parts of California. Drywells can be used to overcome this dilemma. They are typically a 3 foot wide hole in the ground that is filled with rock/gravel which extends down 15-35 feet. Some are concerned that drywells could introduce contaminants into the groundwater and pollute drinking water. To address this issue, OEHHA has reviewed key state and federal reports as well as peer-reviewed literature. There is little data to support this assertion. The data suggests that with proper usage and design, drywells can be used for stormwater management without adverse effects on groundwater quality. Details of the studies and their implications will be presented at the meeting.

9. *** Potential Role of DNA Damage and Repair in Trichloroethylene Renal Toxicity.** Vanessa De La Rosa, Jonathan Asfaha, Chris Vulpe, UC Berkeley, Berkeley, CA

   Trichloroethylene (TCE) is a common drinking water contaminate and human carcinogen.

Previous studies have implicated the TCE metabolite, DCVC, as a renal toxicant, yet the molecular events mediating renal toxicity remain convoluted. Using a functional genomics approach in yeast, we aim to gain a better understanding of the mechanisms involved in TCE mediated renal toxicity. The yeast deletion library, consisting of over 4600 strains, each with a single gene knocked out was treated with DCVC to identify genes required in response to exposure. Enrichment analysis conducted on the resulting gene profile revealed an overrepresentation of genes involved in DNA repair processes. Confirmation of sensitivity using flow cytometry showed translesion synthesis (TLS) and nucleotide excision repair (NER) deficient strains were most sensitive to DCVC exposure. These genes function in concert to repair DNA crosslink damage in yeast and higher organisms. The involvement of the error prone translesion synthesis pathway in repair can increase the rate of mutagenesis and result in genome instability. Western blot analysis of post-translational modifications further supports the presence of DNA damage and TLS activation. These results suggest the metabolite DCVC causes DNA crosslink damage and DNA repair mechanisms play an important role in TCE mediated renal toxicity.

10. *** Polychlorinated biphenyl spatial patterns in San Francisco Bay forage fish.** B.K. Greenfield, University of California, Berkeley, CA, R.M. Allen, San Francisco Estuary Institute, Richmond, CA.

    Industrialized waterways frequently contain nearshore hotspots of legacy polychlorinated biphenyl (PCB) contamination, with uncertain contribution to aquatic food web contamination. We evaluated the utility of estuarine forage fish as biosentinel indicators of local PCB contamination across multiple nearshore sites in San Francisco Bay. Concentrations in topsmelt (*Atherinops affinis*) and Mississippi silverside (*Menidia audens*) were comparable to those of high lipid sport fish in the Bay, and strongly correlated with spatial patterns in sediment contamination. The average sum of 209 PCB congeners in fish from 12 targeted stations (441 ± 432 ng g^{-1} wet weight, mean ± SD) was significantly higher than 17 probabilistic stations (138 ± 94 ng g^{-1}). At probabilistic stations, concentrations in topsmelt (185 ± 82 ng g^{-1}) were higher than silverside (90 ± 82 ng g^{-1}), likely due to habitat differences and elevated lipid content in topsmelt. The highest concentrations were from targeted Central Bay locations, including Hunter's Point Naval Shipyard (1347 ng g^{-1}; topsmelt) and Stege Marsh (1337 ng g^{-1}; silverside). Targeted sites exhibited increased abundance of lower chlorinated congeners, suggesting local source contributions, including Aroclor 1248. These findings indicate that current spatial patterns in PCB bioaccumulation correlate with historical sediment contamination due to industrial activity.

11. **Evaluating the Toxicity of Hypersaline Brine Using Nine California Ocean Plan Toxicity Test Protocols.** L. L.Jennings, UC Davis, Monterey, CA; J. P. Voorhees, UC Davis, Monterey, CA; S.B. Katz, UC Davis, Monterey, CA; K. Siegler, UC Davis, Monterey, CA; B. M. Phillips, UC Davis, Monterey, CA; B. S. Anderson, UC Davis, Monterey, CA;R. S. Tjeerdema, UC Davis, Monterey, CA.

As water needs increase in California, coastal cities are exploring ocean desalination as a freshwater supply alternative. Desalinization results in the discharge of hypersaline brine to the ocean, and there is concern this could impact marine receiving waters. This study determined the salinity tolerance of seven marine organisms using nine California Ocean Plan protocols. Test organisms included: red abalone (*Haliotis rufescens*), giant kelp (*Macrocystis pyrifera*), bay mussel (*Mytilus galloprovincialis*), mysid shrimp (*Americamysis bahia),* topsmelt (*Atherinops affinis*), and purple sea urchin (*Strongylocentrotus purpuratus*). Sand dollars (*Dendraster excentricus*) will be evaluated when spawning organisms are available. Salinity tolerances were determined with an initial range-finder test followed by two definitive tests. Preliminary results showed that salinity tolerance varied by protocol. Euryhaline species were more tolerant to higher salinities than were marine species. The most sensitive organisms and endpoints were sea urchin and abalone development (38‰) > mussel development (43‰) > sea urchin fertilization (44‰) > mysid survival (48‰) > kelp germination and growth (55-58‰) > topsmelt survival and biomass (60‰). Results of these experiments will be used by the State Water Resources Control Board to establish discharge requirements for desalinization facilities.

12. *** Spatial variability of methylmercury in San Francisco Bay sediments.** H. Kaufman, B. Oldham, A. Luengen. University of San Francisco, San Francisco, CA.

    Sediments were collected from San Francisco Bay in October and December, 2011 to analyze the spatial variability of methylmercury (MeHg) concentrations. We hypothesized that concentrations would be higher in South Bay than North Bay. Surface sediments were collected using a benthic grab and subsampled using clean techniques and procedures to avoid oxidation. In the laboratory, samples were digested with a 25% KOH:methanol solution and analyzed using a MERX model III Cold Vapor Atomic Florence Spectrophotometer (CVAFS). Preliminary results showed that MeHg ranged from 0.029 ng g^{-1} to 1.74 ng g^{-1} wet weight. In these preliminary analyses, the lowest MeHg concentrations were found near Honker Bay. The result was consistent with previous studies by the Regional Monitoring Program, which found the lowest MeHg concentrations in sediments in the northern estuary. The highest MeHg concentrations (1.74 ng g^{-1}) were near Candlestick Park, in relatively shallow waters (3.3 m), about 1000 feet from shore. Concentrations south of the Dumbarton Bridge were relatively lower (0.12 ng g^{-1}) than those near Candlestick Park, contrary to previous studies, which have reported high MeHg concentrations in Lower South Bay. Regional variation in methylation rates or proximity to shore may explain our results, but more samples are needed.

13. *** Pesticide Use in the San Francisco Estuary Utilizing updated GC/MS and LC/MS/MS Techniques**. M.M. McWayne, J.L. Orlando, M.L. Hladik, K.L. Smalling, and K.M. Kuivila, USGS Pesticide Fate Research Group, Sacramento, CA.

    Current-use pesticides pose a threat to aquatic organisms in the San Francisco Estuary watershed. Pesticide use is continually changing; therefore, analytical methods must also

evolve. Gas chromatography/mass spectrometry (GC/MS) is routinely used as a robust and effective technique to measure semi-volatile pesticides in water, while liquid chromatography tandem mass spectrometry (LC/MS/MS) can be used to analyze polar, non-volatile pesticides and pesticide degradates in water. Our GC/MS and LC/MS methods were designed and modified to analyze over 100 pesticides and pesticide degradates in water including several rice herbicides, neonicotinoid insecticides, and 34 fungicides, many of which are rarely included in monitoring studies. These methods were used to analyze water samples collected weekly from April through June of 2011 at three sites in the Sacramento/San Joaquin Delta and Grizzly Bay. These sites are designated as areas of critical habitat for the threatened Delta Smelt. Eighteen pesticides, of varying type and use, were detected including diuron and its degradates 3,4-DCA and DCPMU, several fungicides, and the rice herbicide clomazone. This study illustrates the need for sensitive and robust methods capable of analyzing a variety of pesticides with different physical-chemical properties in order to understand the potential effects of mixtures on aquatic organisms.

14. *** Evaluating Microcystins (MCs) as a Potential Neurotoxin in *Caenorhabditis elegans* (*C. elegans*).** C. Moore, B. Puschner, N. E'toile, University of California, Davis.

    Blue-green algae toxins found worldwide, MCs can contribute to multifactorial diseases in mammals through several toxic mechanisms including protein phosphatase (PP) inhibition. While acute hepatotoxic effects have been intensively studied, chronic effects of MCs on the nervous system are unknown. The remarkable genetic and neurobiochemical conservation between *Caenorhabditis elegans* (*C. elegans*) and humans provide an ideal neurotoxicity model. A novel exposure method using *C. elegans* was developed to evaluate the *in vivo* effects of chronic MC exposure on neurodevelopment. Small agar plates seeded with *E. coli* were covered with 100 µl of MCs, from 0-1000 µg/L, and sterile glass beads were used to evenly spread the MCs. MC solutions were allowed to settle and 300 synchronized *C. elegans* eggs were placed on each plate for 3 days at 20°C. Exposed and non-exposed adult *C. elegans* were compared. Chemotaxis indices to the odors benzaldehyde and diacetyl were used to measure behavior patterns. A colorimetric assay using p-nitrophenyl phosphate was developed to study effect of MCs on PP rates of activity in protein extracts from *C. elegans*. To facilitate MC uptake, *C. elegans* strains with weakened cuticles were utilized. PP activity may increase in chronic exposures, leading to altered behavior.

15. **Detection of PBDEs, TBPH and Other New Brominated Flame Retardants in Human Serum** Weihong Guo, Yunzhu Wang, Myrto Petreas, June-Soo Park. Environmental Chemistry Laboratory, California Department of Toxic Substances Control, California Environmental Protection Agency.

    Firemaster 550, a mixture of four flame retardants that are either known to be toxic or lack adequate information, continues to be used as a replacement for polybrominated diphenyl ether (PBDE) flame retardants. Two of the four ingredients, i.e., 2,3,4,5-tetrabromo-ethylhexylbenzoate (TBB) and 2,3,4,5-tetrabromo-bis(2-ethylhexyl) phthalate (TBPH), have

been found in blubber of marine mammals as far as the North Pole and also are detected in house dust, sewage sludge from wastewater treatment plants. Sharing similar properties with PBDEs, these new brominated fire retardants (new BFRs) are likely to bioaccumulate through the food chain and/or via inhalation/ingestion of house dust and, therefore, may pose health risks. We have developed an analytical method that can detect TBB, TBPH, as well as other commonly used new BFRs alternatives (2,4,6,-tribromophenyl allyl (ATE), 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (α,β-TBECH), 2-bromoallyl-2,4,6-tribromophenyl ether (BATE), Pentabromotoluene (PBT), Pentabromoethylbenzene (PBEB), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), Hexabromobenzene (HBB)) simultaneously with PBDEs in human serum. The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

16. **Simultaneous Determination of Bisphenol A, 2.4-Dibromophenol, 2.4.6-Tribromophenol and Tetrabromobisphenol A in human serum samples by LC-MS/MS.** Syrago-Styliani E. Petropoulou, Tan Guo, Weihong Guo, Myrto Petreas,June-Soo Park, Environmental Chemistry Laboratory, Department of Toxic Substances Control, 700 Heinz Av, S 100, Berkeley, CA 94710.

Brominated flame retardants, especially polybrominated phenols (PBPs) in commercial products have raised increasing concerns due to their potential toxicities in humans and wildlife. PBPs are used as additive compounds in polymers such as epoxy and polycarbonated resins. BPA is also reported as an obesogen, causing advanced puberty and increasing body weight in female mice offspring. In the present work, we report a new LC-MS/MS method using isotopic dilution for the determination of PBPs in human serum. BPA is present in serum in its free form, and as a glucuronide adduct that appears to bioaccumulate. The method was validated for the quantitation of the total amount of BPA and the other PBPs in human serum samples. Samples were denatured using formic acid with enzymatic deconjugation of the glucuronides, followed by an off-line solid phase extraction procedure. Based on the accuracy, precision, stability and reproducibility the method can be used for Biomonitoring purposes. The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

17. *** Does the Pesticide Endosulfan affect Disease Susceptibility in Cascades frogs?** D.R. Reagan, San Francisco State University, San Francisco, California, C. Davidson, San Francisco State University, San Francisco, California.

Amphibian populations around the world have experienced sharp declines, the causes of which are still not well understood. Disease caused by a chytrid fungus (*Batrachochytrium dendrobatidis*) is a leading cause of amphibian declines, but it is unclear how disease interacts with environmental factors and frog susceptibility. This study aims to determine if sub-lethal exposure to the pesticide endosulfan affects Cascades frog's susceptibility to

chytrid fungus. We conducted a laboratory experiment in which we exposed juvenile Cascades frogs from two distinct populations to either endosulfan or the chytrid fungus or a combination of the two. We found that exposure to endosulfan did not significantly affect growth or mortality, either directly or in interaction with chytrid.

18. Monitoring of Fipronil and Bifenthrin within Urban Streams of California. E.R. Russell, R. Budd, M. Ensminger, S. Gill, and K. Goh, California Department of Pesticide Regulation, Sacramento, CA, R. Tjeerdema, University of California Davis, Davis, CA.

Runoff from urban landscapes has been linked to pesticide detections in adjacent waterways, where concentrations can reach levels detrimental to aquatic macroinvertebrates. Over 4 million kg a.i. of pesticides are applied annually by professional applicators for landscape maintenance in California, with an additional unreported amount by residential users. The California Department of Pesticide Regulation has begun monitoring urban streams throughout California to determine presence of pesticides originating from urban landscapes. Water samples were collected between December, 2009 and October, 2011 at 34 sites located at residential storm drain outfalls or within receiving waters of adjacent urban creeks. The insecticides bifenthrin and fipronil were two of the most common pesticides detected both temporally and spatially. Statewide, bifenthrin was detected in 157 of 191 samples, with 82% of samples having concentrations greater than the US EPA aquatic life benchmark (0.0013 ug/L). Fipronil was detected in 89 of 159 samples, with 56% of samples greater than the benchmark (0.011 ug/L). Bifenthrin had a higher frequency of detection in northern California (85%), while fipronil was detected at higher frequency in southern California (74%). Both pesticides were detected at higher frequency during storm events.

19. Exposure to different strains of the fungal pathogen *Batrachochytrium dendrobatidis* results in drastically different levels of mortality among Cascades frogs. D. Rejmanek, University of California, Davis, CA, J. Piovia-Scott, University of California, Davis, CA, J.E. Foley, University of California, Davis, CA, S. Lawler, University of California, Davis, CA, C. Davidson, San Francisco State University, San Francisco, CA, K. Pope, United States Forest Service, Arcata, CA, K. Aceituno, U.S. Fish and Wildlife Service, Sacramento, CA, C. Johnson, U.S. Fish and Wildlife Service, Sacramento, CA.

In 2006, *Batrachochytrium dendrobatidis* (*Bd*), an emerging water-borne fungal pathogen was discovered in Cascades frogs (*Rana cascadae*) in California. In the Lassen area, the Cascades frog was once common but is now found in only 10 small populations. In the Trinity Alps the species is still widespread. The timing and speed of the decline coupled with the discovery of *Bd* in the remaining populations place *Bd* as a prime suspect. We exposed juvenile Cascades frogs to one of two different *Bd* strains – either cultured from a frog collected in the Trinity Alps or from a frog collected in Lassen. In two separate trials we exposed frogs to *Bd* zoospores of either the Lassen strain (N=46) or the Trinity Alps strain (N=56). After 15 weeks, 30 of the frogs exposed to the Lassen strain were still alive. In contrast, all but 1 of the frogs exposed to the Trinity Alps strain died within 2 to 4 weeks of

exposure. These findings show drastic differences in virulence between *Bd* strains collected from two separate Cascades frog populations and suggest that, in addition to environmental and chemical stressors, *Bd* strain type likely plays a significant role in frog mortality.

20. Automated Storm Runoff Sampling From Residential Areas. J. Sisneroz, Q. Xiao, L.R. Oki, B.J. Pitton, University of California, Davis, CA, D.L. Haver, T. J. Majcherek, University of California Cooperative Extension Orange County, Irvine, CA, R.L. Mazalewski, Consultant, Davis, CA and M. Ensminger, California Department of Pesticide Regulation, Sacramento, CA.

Since 2006, automated sampling equipment has been used to collect storm runoff samples from residential areas in Sacramento and Orange Counties. The study sites were selected for a University of California study to evaluate pesticide, nutrient, biological, and other constituents in urban runoff. Samples and water measurements are taken at storm drain outfalls to examine runoff at a neighborhood level. Each site utilizes a Hach 950 Flow Meter with bubble depth and a velocity sensor coupled with a Hach 900 MAX Portable Sampler. Rainfall triggers the collection of samples that is based on flow measurements from the flow meter. A sample is collected when a set pacing volume flows through the monitoring point. To collect samples for the duration of a storm, the pacing volume was determined based on forecasted rainfall amounts and a drainshed model that used a surface analysis to estimate the volume of runoff generated by the storm. Flow-weighted sampling allows for a more accurate characterization of pollutant loading in storm runoff due the ability to collect many samples based on runoff volume over the course of the storm.

21. A case study of causal analysis: Stressor Identification. W. Wieland, K. Pulsipher, & B. Washburn. Office of Environmental Health Hazard Assessment, Cal/EPA, Sacramento, CA.

Stressor Identification (SI) is a causal assessment process developed by the US EPA to identify probable causes of impairment in a watershed. We used SI to analyze stressors in the Dry Creek watershed to discover the reason for the decline in the abundance and diversity in aquatic life, in particular anadromous fish. The SI process involves listing candidate causes, analyzing data from the case and from other situations, and characterizing causes based on the weight of evidence. We used five different criteria (e.g., stressor-response relationship, etc.) to characterize cause(s) of impairment. Data was collected from 10 different sites throughout the watershed. Benthic macroinvertebrates (BMIs) were the indicator used to evaluate aquatic health. Relationships between contaminants, physical habitat alterations, land use characteristics, and BMI metrics were compared. Of all of the stressors evaluated, large amounts of silt/sand/fine gravel was found to be the most probable cause of impairment. The surrogate for urbanization, percent impervious cover, was the landscape stressor that was most highly correlated with BMI metrics. Conversely, the percent open space, especially in close proximity to the study sites, was strongly associated with greater abundance and diversity of BMIs. Water quality parameters were weakly correlated with BMI metrics.

U. S. Department of the Interior
Fish and Wildlife Service

Potential Effects Of Selenium Contamination On Federally-Listed Species Resulting From Delivery Of Federal Water To The San Luis Unit

U.S. Fish and Wildlife Service
Sacramento Fish and Wildlife Office
Environmental Contaminants Division

Artwork by Mirriam Morrill

For the U. S. Bureau of Reclamation
Under Agreement # 05AA210003

March 2008

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Introduction

Federal water delivered to the San Luis Unit (the Project) is used principally for irrigated agriculture. Due to a nearly-impervious soil layer, irrigated agriculture in this area is unsustainable without subsurface drainage to keep the water table below the root zone of crops and to ameliorate the accumulation of salts in the soil. Therefore, an analysis of the effects of the delivery of federal water must include the effects of subsurface drainwater that may seep, be conveyed, or be carried by floodwaters downstream into sloughs and rivers and thence into the San Francisco Bay/Delta estuary.

Within the direct footprint of the project, consideration must be given to the effects of conveying and storing drainwater, as well as applying drainwater to irrigate salt-tolerant plants in reuse areas, and evaporating drainwater in evaporation ponds or solar evaporators. These are likely to be components of any long-term continuation of irrigated agriculture in the San Luis Unit. In this area, the subsurface drainage of irrigated lands mobilizes selenium that has been historically sequestered in the soil. Selenium concentrations in agricultural drainwater from this area reach levels that, when bioaccumulated through food chains, cause adverse effects on aquatic and aquatic-dependent wildlife. Where such drainwater is applied to uplands, as in reuse areas, strictly terrestrial wildlife may be impacted as well.

Downstream from the San Luis Unit, any drainwater from the Project area is diluted by relatively low-selenium water from rivers that drain the Sierra Nevada Mountains. However, as the San Joaquin River reaches the San Francisco Bay/Delta estuary, flow velocities decrease and salinity increases. In these slow-moving, saline waters, with abundant introduced filter-feeding invertebrates, ecosystems have developed that evidently are much more effective than riverine

ecosystems at bioconcentrating water-borne selenium. Therefore, potential downstream effects must be considered.

Although selenium is the principle contaminant of concern in drainwater from this area, mercury in the soil may be similarly mobilized and bioconcentrated to toxic concentrations in food chains. However, less is known about mercury contamination in the San Luis Unit, and measures to minimize and mitigate selenium contamination could ameliorate the risk of mercury toxicity as well. The discussion below focuses on selenium and on the species that are most sensitive and most likely to be exposed to selenium as a result of the delivery of federal water to the San Luis Unit.

San Joaquin kit fox (*Vulpes macrotis mutica*)

Status: The San Joaquin kit fox has been federally listed as endangered throughout its range since 1967 (32 FR 4001). It is endemic to the western San Joaquin Valley in the vicinity of the San Luis Unit (Figure 1).

Life history summary: Studies of kit fox and their small mammal prey in the vicinity of Kesterson Reservoir indicate that kit foxes are likely to forage in drainwater reuse areas and around evaporation ponds where selenium concentrations in their prey are likely to be well above levels known to cause adverse effects in members of the canid family of carnivores to which kit fox belong.

Risk of selenium exposure: No toxicity tests have been performed on kit fox. The most closely related surrogate species for which toxicity data are available is the domestic dog (*Canis familiaris*), which is in the same family (Canidae) as the San Joaquin kit fox. Dogs exposed to 7.2 µg/g (dry weight) dietary (organic) selenium suffered adverse effects, including reduced appetite, subnormal growth, and poorly developed ovaries and testes (Rhian and Moxon 1943). The 7.2 µg/g concentration is a Lowest Observed Adverse Effect Concentration (LOAEC); the actual toxicity threshold for domestic dogs must be an unknown amount below this value. Further, any extrapolation of dog toxicity data to kit foxes must include an uncertainty factor to account for the risk that kit foxes may be more sensitive than dogs. Therefore, given available data, an appropriate selenium dietary toxicity threshold for San Joaquin kit fox diet must be well below 7.2 µg/g.

Areas of the San Luis Unit supplied directly with relatively good quality federal water are probably best represented by the small mammals collected by Clark (1989) on the Volta Wildlife Management Area in 1984. Clark did not report whole-body selenium analyses of these mammals, but his reported analyses of liver selenium indicate that selenium concentrations in the small mammal prey of San Joaquin kit foxes at Volta were as much as two orders of magnitude less than concentrations at the drainwater evaporation ponds of Kesterson Reservoir. For example, the California voles captured at Volta Pond 5 in May 1984 (n=5) had a mean liver selenium concentration of 0.228 µg/g; the same species collected at Kesterson pond 2 at the same time (n=5) had a mean (geometric) liver selenium concentration of 119 µg/g (Clark 1989). Since background selenium concentrations in mammal livers are about 1-10 µg/g

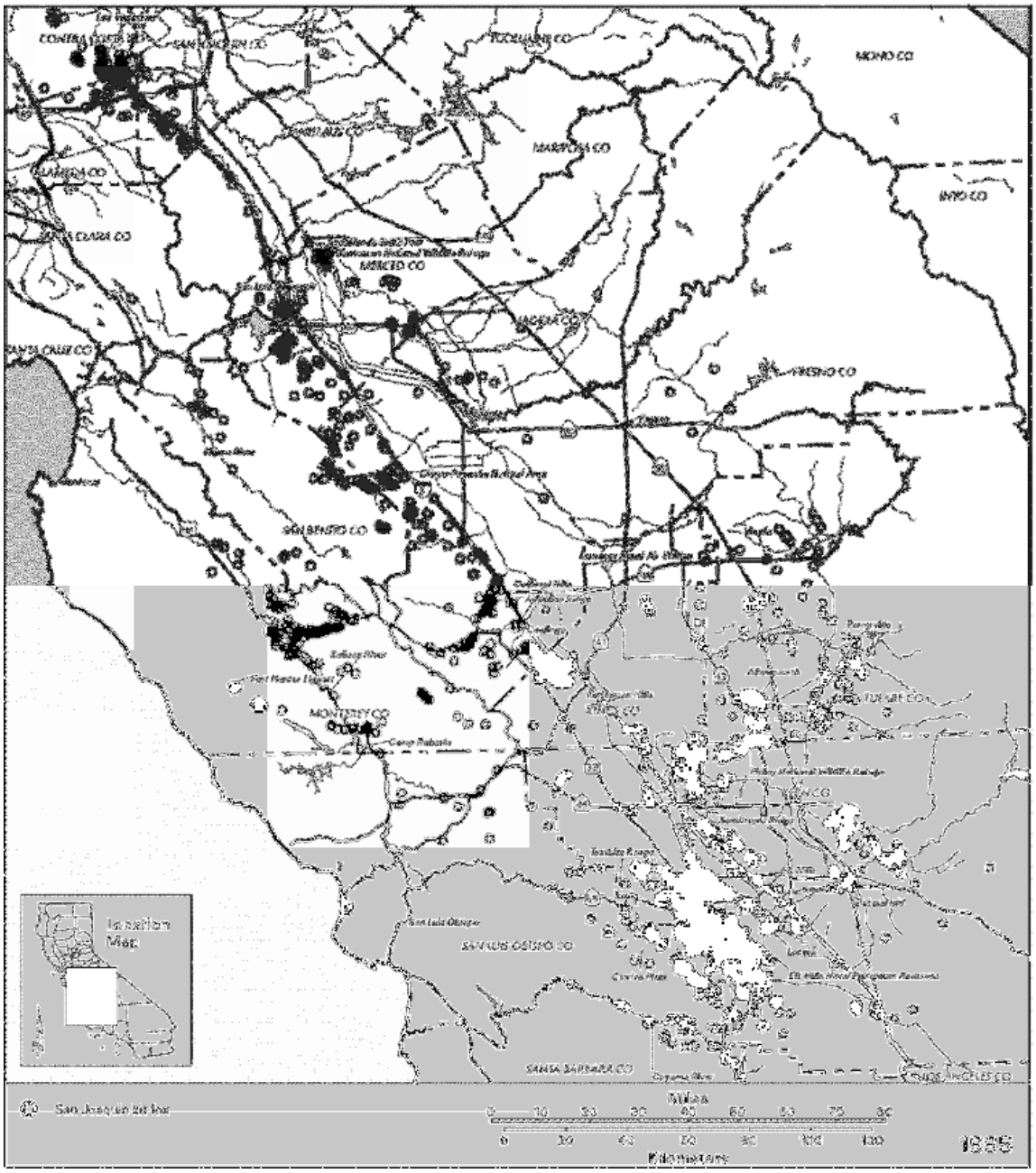


Figure 1. San Joaquin kit fox distributional records (Williams *et al.* 1998).

(NIWQP 1998), it seems likely that in portions of the Project area that are supplied with good quality water, selenium concentrations in prey pose no threat to the San Joaquin kit fox.

The San Luis Unit includes some localities that have (or are expected to have, as a consequence of application of federal water) elevated concentrations of selenium in soil and surface water or near-surface groundwater. Such localities include open ditches that convey subsurface drainwater, retired or fallowed seleniferous farm land, and drainwater reuse projects. Open drainwater conveyances are probably best represented by evaporation ponds of Kesterson Reservoir in the early 1980s.

The history of Kesterson Reservoir in the 1980s provides the best available information on potential exposure of the San Joaquin kit fox to contaminants due to the proposed action. Paveglio and Clifton (1988) sighted San Joaquin kit fox 39 times in 108 night surveys in the Kesterson Reservoir area between September 1986 and August 1988. They trapped and radio-tagged two kit fox within one mile of Kesterson Reservoir. They found that kit fox frequently used the San Luis Drain road, which formed the eastern boundary of Kesterson Reservoir. The California vole was the most important component of the diet of kit foxes in the Kesterson area (Paveglio and Clifton 1988). Clark (1987, 1989) collected small mammals, including California voles at Kesterson Reservoir in 1984. He found selenium concentrations of 13 and 33 µg/g (mean 23.0 µg/g) in California voles collected at Pond 2 of Kesterson Reservoir. The average selenium concentration in all California voles collected at all ponds of the reservoir (n=5) was 10.4 µg/g. The average selenium concentrations in prey items of kit fox collected at Kesterson Reservoir while the ponds were operational was as follows:

Species	**Number Collected**	**Mean Selenium Concentration** (µg/g whole body dry wt.)
House mouse	5	18.5
Western harvest mouse	5	12.5
Ornate shrew	4	47.9
California vole	5	10.4

Seleniferous uplands that usually lack ponded water are best represented by data from Kesterson after it was closed and low-lying areas were filled (CH2MHILL 1999). This data is as follows:

Species	**Number Collected**	**Mean Selenium Concentration** (µg/g whole body dry wt.)
House mouse	31	7.9
Western harvest mouse	17	7.7
Ornate shrew	1	7.5
Deer mouse	30	6.7
California vole	7	4.4

Because the mean concentrations of all San Joaquin kit fox prey items analyzed are about the level of the domestic dog LOAEC (7.2 µg/g, from above), it is likely that in any locations where San Joaquin kit fox range over upland portions of the Project area that may be contaminated with selenium (e.g. reuse areas), these foxes are potentially at risk from dietary intake of selenium. The average selenium concentration of each of the kit fox prey items sampled at Kesterson

Reservoir evaporation ponds was well above the dog LOAEC. Therefore, it is possible that selenium contamination in the small-mammal diet of kit foxes in the vicinity of Project evaporation ponds or solar evaporators may put San Joaquin kit foxes at risk.

If reuse areas and evaporation basins are fenced to exclude kit fox, or if other measures are taken to exclude kit fox from the project areas, recovery of remnant populations of kit fox may be impacted by loss of existing or potential habitat.

Kangaroo rats (*Dipodomys sp.*)
including:
Giant kangaroo rat (*Dipodomys ingens*)
Fresno kangaroo rat (*Dipodomys nitratoides exilis*)
Tipton kangaroo rat (*Dipodomys nitratoides nitratoides*)

Status: Three kangaroo rats in the vicinity of the San Luis Unit have been federally listed as endangered throughout their respective ranges: the Fresno kangaroo rat since 1985 (50 FR 4222-4226), the giant kangaroo rat since 1987 (52 FR 283-288), and the Tipton kangaroo rat since 1988 (53 FR 25608-25611). All three species are endemic to the San Joaquin Valley and found only in the vicinity of the San Luis Unit. The ranges of the giant and Tipton kangaroo rats extend farther south to the west side of the Tulare Basin (**Figure 2**).

Life history summary: All three species of kangaroo rat are primarily seed eaters, but also eat insects as well as green plants. All three species are found in annual grassland and saltbush scrub in alkaline soils (Williams *et al*. 1998).

Risk of selenium exposure: We are not aware of any selenium toxicity studies with kangaroo rats. Sublethal liver changes have been found in laboratory rats (*Rattus norvegicus*) following lifetime exposure to natural selenium in the diet at a concentration of 1.4 µg/g (dry weight) and reduced longevity was found at 3 µg/g in the lifetime diet (Eisler 1985). Olson (1986) also reported reproductive selenosis in rats that consumed wheat with a concentration of 3 µg/g. Halverson *et al*. (1966) found a dietary selenium threshold of about 4.8 µg/g for growth retardation in rats.

All three species of kangaroo rat were probably displaced from historic scrub and grassland habitat that was converted into irrigated crop land in the San Luis Unit with the application of federal water. All three species are not likely to be impacted by selenium in high quality irrigation water delivered to primary fields because (1) such crop land habitat is not favored by kangaroo rats, and (2) this applied water generally has relatively low concentrations of selenium. However, in retired seleniferous land, along drainwater conveyances, near evaporation ponds, and especially in drainwater re-use areas, habitat that is attractive but toxic to kangaroo rats may occur, and individuals may attempt to recolonize the habitat.

Observers performing wildlife surveys at the Atwell Island Land Retirement Program pilot site found a population of the endangered Tipton's Kangaroo Rat (USBR, 2007). The mean selenium concentration in 20 species of plants collected from Atwell Island varied from less than

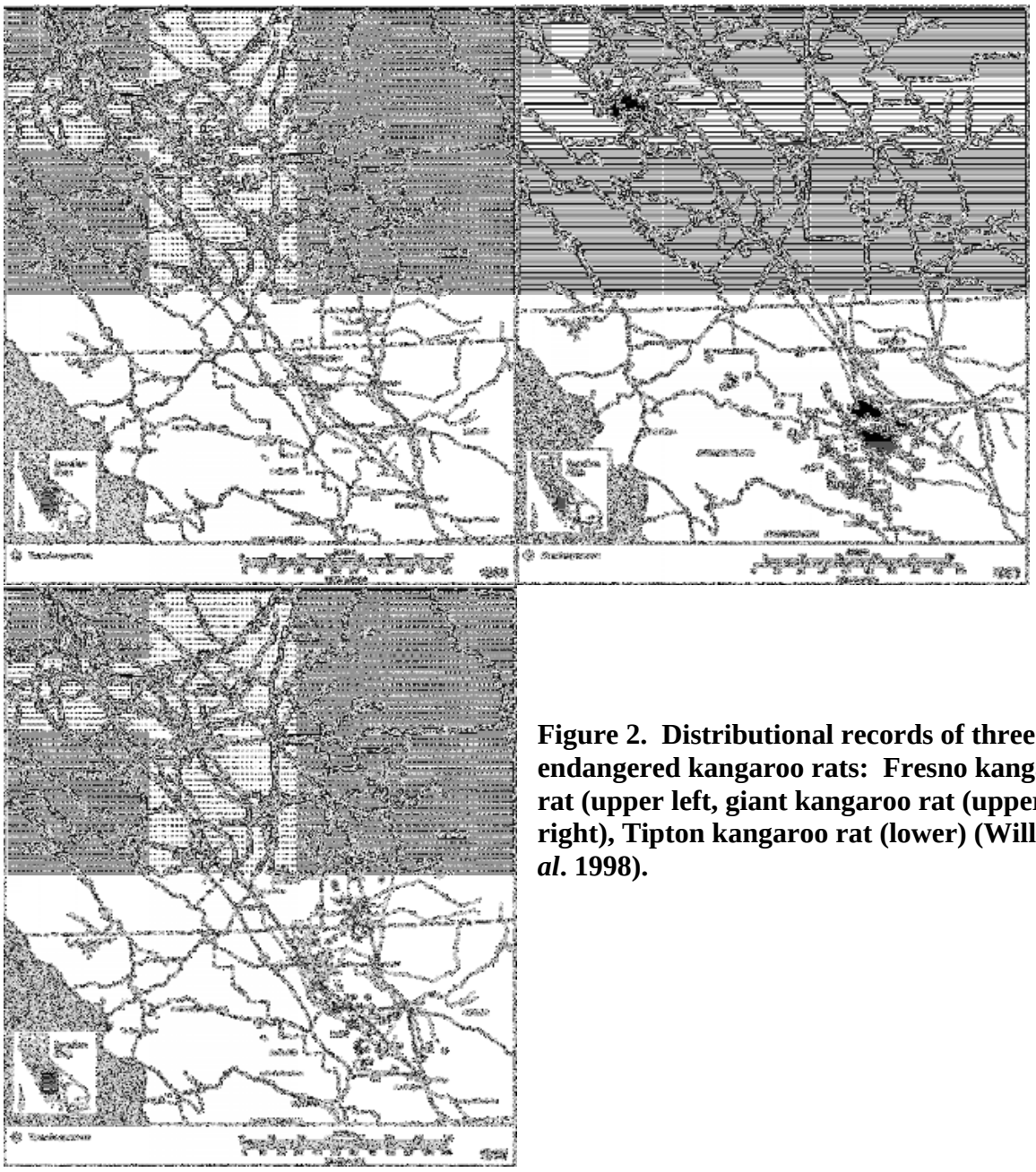


Figure 2. Distributional records of three endangered kangaroo rats: Fresno kangaroo rat (upper left, giant kangaroo rat (upper right), Tipton kangaroo rat (lower) (Williams *et al*. 1998).

0.17 to 0.5 mg/kg and none of the samples were above the 2 mg/kg threshold recommended for the project by the Service (USBR, 2005). There were no discernable differences in the selenium concentration between plant parts (whole, vegetation, fruits) at the Atwell Island site.

Agroforestry projects operated in the western San Joaquin Valley since the 1980's serve as pilot projects for the more extensive drainwater reuse areas that are likely to be established in the San Luis Unit to enable sustained irrigated agriculture there. Monitoring of agroforestry projects by the California Department of Fish and Game indicates that in reuse areas, selenium concentrations in dietary items of kangaroo rats are likely to exceed thresholds for adverse effects (Figure 3 and Figure 4).

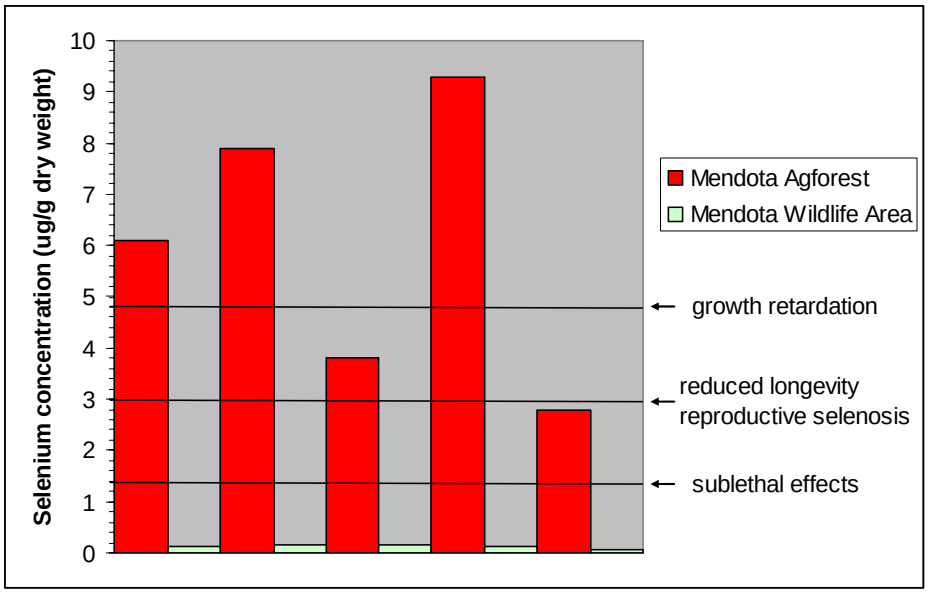


Figure 3. Selenium in rabbitfoot grass (*Polypogon monspeliensis*) collected in the Mendota agroforestry area and the Mendota Wildlife Area in May 1997 (Dunne pers. com.). Effect thresholds for rats (*Rattus norvegicus*) are from Eisler 1985, Olsen 1986, and Halverson *et al.* 1966 (See text).

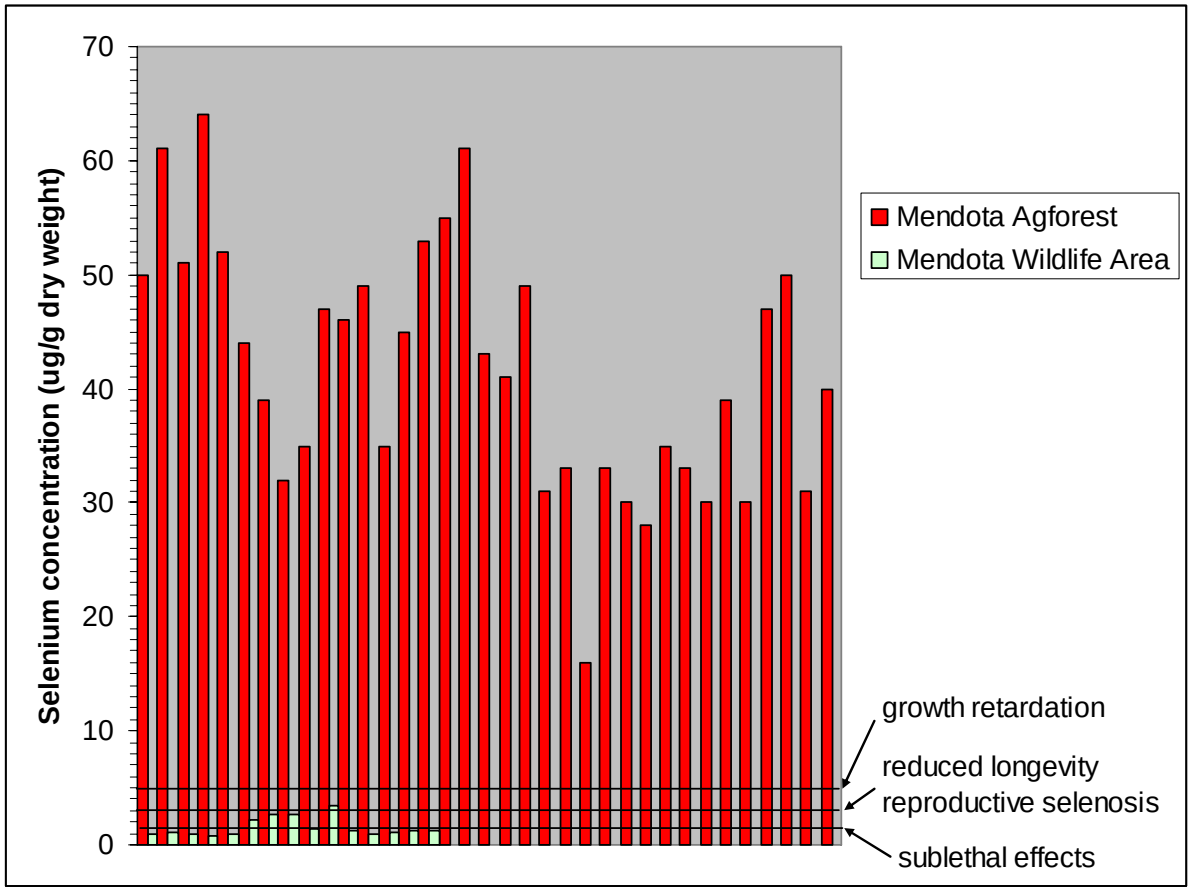


Figure 4. Selenium in sowbugs collected in the Mendota agroforestry area and the Mendota Wildlife Area in 1997 and 1998 (Dunne pers. com.) Effect thresholds for rats (*Rattus norvegicus*) are from Eisler 1985, Olsen 1986, and Halverson *et al.* 1966 (See text).

Giant garter snake (*Thamnophis gigas*)

Status: The giant garter snake was listed as threatened in 1993 (58 FR 54053-54066). It is endemic to the wetlands of the Central Valley from Butte County in the north to Kern County in the south (USFWS 1999). A 5 year review completed in September 2006 recommended no change in the listing status for the snake (USFWS 2006a). Most populations of giant garter snakes are found in the Sacramento Valley while small isolated populations are found in northern San Joaquin Valley (primarily Merced County and western Fresno County).

Life history summary: Fish and amphibians (tadpoles and adults) are the primary food items of giant garter snakes (58 FR 54053-54066). Giant garter snakes prefer marshes, sloughs, ponds, small lakes, and low gradient streams. Currently agricultural wetlands such as irrigation and drainage canals and rice fields provide key habitat for the snake (USFWS 1999). These wetland habitats must include sufficient water through the summer; emergent vegetation for escape cover; grassy banks and openings for basking; and higher elevation uplands for cover and refuge from flood waters (USFWS 1999, 58 FR 54053-54066).

Risk of selenium exposure: Very little research has been done on the toxicity of selenium to reptiles (Hopkins 2000); no such studies have been done on giant garter snakes or on any other species of garter snake (Campbell and Campbell, 2001). Hopkins *et al*. (2002) found that in another species of aquatic snake, the banded water snake (*Nerodia fasciata*), bioaccumulation of dietary selenium was most notable (greatly exceeding toxicity thresholds that have been established for other vertebrates) compared to other elevated trace elements at a site contaminated with coal ash. At the same selenium-contaminated site, Roe et al. (2004) found clutch viability to be reduced in alligators (*Alligator mississippiensis*; viability 30-54%, egg selenium 2.1-7.8 µg/g dry weight) compared to a reference site (viability 67-74%, egg selenium 1.4-2.3 µg/g). Average selenium concentrations in common prey items of alligators (fish and frogs) in the contaminated site ranged from 10 to 27 µg/g (dry weight), with an average concentration of 14.3 µg/g in mosquitofish (*Gambusia affinis*). Average concentrations in the same prey items from the reference site ranged from 1.12 to 3.43 µg/g, with an average concentration of 1.82 µg/g in mosquitofish (Hopkins *et al*. 1999). Other contaminant in prey species varied between the sites, so the role of selenium in reduced clutch viability is not unequivocal.

These data suggest that dietary selenium concentrations of 10 to 27 µg/g may have a negative impact on reptiles that are dependent on an aquatic food chain. It should be noted that interpretation of these field data is confounded by the co-occurrence of other contaminants that could also affect egg viability. However, in such coal ash-contaminated sites, as in subsurface drainwater-contaminated sites, selenium has been implicated as the chief cause of toxicity to wildlife. If, as is most likely, selenium is the principal cause of reduced clutch viability, then the corresponding selenium concentration in prey items must be treated as a dietary LOAEC for a single effect on a single species of aquatic reptile. The actual toxicity threshold for alligators is an unknown amount below this LOAEC value (10 µg/g). Further, any extrapolation of alligator toxicity data to giant garter snakes must include an uncertainty factor to account for the risk that

giant garter snakes may be more sensitive than alligators. This accords with findings by a study of dietary selenium effects on the brown house snake (*Lamprophis fulginosus*), a common terrestrial snake found in southern Africa. Female snakes exposed to a diet containing 10 µg/g seleno-D,L-methionine produced about half as many eggs as control females exposed to 1 µg/g (Hopkins *et al*. 2004). Also, the dietary selenium toxicity threshold for the avian descendants of reptiles is about 3 to 7 µg/g (dry weight; Wilber 1980, Martin 1988, Heinz 1996). Therefore, given the above data, an appropriate dietary selenium toxicity threshold for the giant garter snake is probably well below 10 µg/g.

Historical exposure: Open ditches in the Northerly Area of the San Luis Unit have in the past carried subsurface drainwater with elevated concentrations of selenium. Green sunfish (*Lepomis cyanellus*) in this drainwater have been found to have concentrations of selenium ranging from 12 to 23 µg/g (geometric mean: 17.3 µg/g) (Saiki 1998), within the range of concentrations associated with adverse effects on predatory aquatic reptiles (see above). Since 1996, subsurface drainwater has been discharged, via the Grassland Bypass Project, into lower Mud Slough North, where selenium concentrations in small fish, such as mosquitofish, inland silversides (*Menidia beryllina*), red shiners (*Cyprinella lutrensis*), and fathead minnows (*Pimephales promelas*), frequently reach 10-15 µg/g (Beckon *et al*. 2003). Most of the remaining water supply channels such as Salt Slough now have fish selenium levels that are below concern thresholds (Beckon *et al*. 2003).

Potential Project-related exposure: Dietary uptake is the principle route of toxic exposure to selenium in wildlife, including giant garter snakes. Giant garter snakes feed primarily on aquatic prey such as fish and amphibians (Miller and Hornaday 1999). The extent to which they may take aquatic invertebrates is unknown.

Open drainwater ditches may constitute risks of exposure of giant garter snakes to selenium in the aquatic food chain. In addition, these conveyances could provide routes of dispersal of giant garter snakes from existing habitat to evaporation ponds. The drainwater conveyances and ponds of Kesterson Reservoir in the early 1980s serve as the best available prototype for estimation of the effects on giant garter snakes of selenium contamination associated with water deliveries to the San Luis Unit. Mosquitofish were the only fish species that survived in the ponds of Kesterson Reservoir after September 1983 (Saiki 1986). Concentrations of selenium ranged up to 366 µg/g in samples of mosquitofish collected from the San Luis Drain and up to 293 µg/g in the ponds of Kesterson Reservoir in May and August, 1983; aquatic insects collected in these localities had selenium concentrations of up to 326 and 295 µg/g respectively (Saiki 1986). These concentrations are far above dietary selenium concentrations associated with adverse effects in aquatic reptiles (see above).

Gopher snakes (*Pituophis melanoleucus*) collected at Kesterson Reservoir in April-June 1984 and April-July 1985 had liver selenium concentrations ranging from 8.2 to 19 µg/g (dry weight; geometric mean 10.9; Ohlendorf *et al*. 1988). Such a range of liver concentrations corresponds to a selenium concentration range of about 7 to 20 µg/g in eggs in the brown house snake (*Lamprophis fuliginosus*) (Hopkins *et al*. 2005), the closest relative of the giant garter snake for which data are available linking liver and egg concentrations. Therefore the eggs of gopher snakes at Kesterson Reservoir were probably within or above the range (2.1-7.8 µg/g) associated

with adverse effects in reptiles (see above). Gopher snakes have a more terrestrial diet than giant garter snakes, but the gopher snake data provide an additional indication that reptiles in an agricultural drainwater evaporation pond environment may be at risk.

Isolation of evaporation ponds from existing giant garter snake habitat may reduce the likelihood that the ponds could serve as attractive population sinks. Such isolation may be accomplished by positioning of drainwater treatment facilities in locations remote from existing habitat and by conveyance of Project drainwater exclusively through closed pipes rather than open ditches. However, it is not known how far giant garter snakes may disperse overland to new aquatic habitats.

Blunt-nosed leopard lizard (*Gambelia sila*)

Status: The Blunt-nosed leopard lizard was federally listed as endangered in 1967 (32 FR 4001). It is endemic to the San Joaquin Valley, and several remaining populations are found in the vicinity of the San Luis Unit (Figure 5).

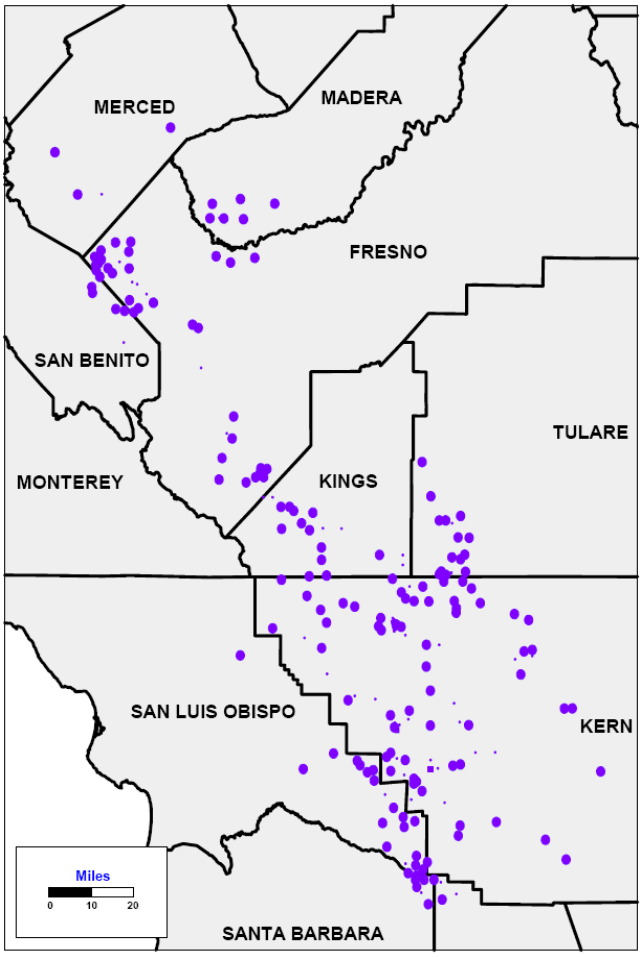


Figure 5. Currently occupied habitat of the blunt-nosed leopard lizard (http://www.cdpr.ca.gov/docs/es/espdfs/bnllall.pdf)

General life history: Blunt-nosed leopard lizards are most commonly found in open vegetated habitats dominated by non-native grasses or by low, alkali-tolerant shrubs of the family Chenopodiaceae, such as iodine bush, and seepweeds, which grow on saline and alkaline soils (Williams *et al*. 1998).

Risk of selenium exposure: Very little is known of the toxicity of selenium to reptiles (see giant garter snake discussion above); even less is known of the effects of selenium on lizards in particular. The effects of selenium on birds are better known, and birds are closely related to reptiles (Hedges 1994; Hedges and Poling 1999). Like birds, most other reptiles are oviparous (egg-laying); therefore, it is likely that in reptiles the maternal transfer of selenium to eggs is critical to the expression of selenium toxicity because the most selenium-sensitive life stage is the development of the embryo in the egg. Some of the mechanisms of maternal transfer of selenium to eggs in lizards are somewhat different from the mechanisms in birds (Unrine *et al*. 2006), but these mechanisms could be at least as efficient in moving selenium from the mother to her eggs. Roe *et al.* (2004) documented maternal transfer of selenium in alligators. Eggs from the contaminated sites had selenium concentrations ranging from 2.1 to 7.8 µg/g and lower viability (30-54 %) compared to reference sites (eggs, 1.4 to 2.3 µg/g: viability, 67 to 74 %). Alligator prey items at the contaminated sites ranged from 10 to 37 µg/g (Roe *et al.* 2004). Female western fence lizards bioaccumulated selenium in their gonads to a level (14.1 µg/g dry weight) that is toxic to bird reproduction after being fed crickets (15 µg/g Se dry weight) that had been fed on commercial feed spiked with seleno-D,L-methione (30 µg/g dry weight) (Hopkins *et al*. 2005). Therefore, lizards foraging in seleniferous habitats must be regarded as potentially at risk to selenium toxicity.

Blunt-nosed leopard lizards are likely to be exposed to selenium by feeding on insects in the vicinity of agricultural drainwater conveyances, evaporation ponds, retired seleniferous land, and re-use areas. At land retirement pilot project lands mean selenium concentrations in crickets ranged from 0.13 to 0.81 mg/kg; in beetles from 0.14 to 1.35 mg/kg; in spiders from 0.25 to 2.24 mg/kg; and in isopods 0.13 to 3.47 mg/kg (USBR 2005). These concentrations are generally within the range for terrestrial invertebrates found in non-seleniferous soils in the western United States (2.5 mg/kg, USDI 1998) although isopods at the Tranquillity site exceeded this range in most years. The selenium levels in all invertebrate groups collected from the land retirement sites are approximately an order of magnitude less than corresponding invertebrate groups collected between 1988 and 1992 in upland habitat at the closed Kesterson Reservoir (USBR 2005). The selenium exposure in invertebrates seen at the closed Kesterson Reservoir may be the best comparison data for drainwater reuse areas. Reuse areas used to grow salt-tolerant grasses and other salt-tolerant forage crops may provide habitat that is attractive to blunt-nosed leopard lizards but so enriched in selenium that it presents a risk of adverse effects.

Bald eagle (*Haliaeetus leucocephalus*)

Status: The bald eagle was federally listed as endangered on February 14, 1978 (43 FR 6233) in all of the conterminous United States except Minnesota, Wisconsin, Michigan, Oregon, and Washington, where it was classified as threatened. On August 15, 1995 (60 FR 36010), the bald eagle was down-listed to threatened throughout its range. On July 9, 2007 the Service, removed

the bald eagle in the lower 48 States of the United States from the Federal List of Endangered and Threatened Wildlife (72 FR 37346). The bald eagle remains protected under the Bald and Golden Eagle Protection Act (BGEPA) and the Migratory Bird Treaty Act (MBTA) and a new permitting process will authorize limited take under BGEPA.

General life history: Breeds in coastal and aquatic habitat with forested shorelines or cliffs in North America, including the Pacific Northwest as far south as the northern Sierra Nevada Mountains in California. Wintering areas include coastal estuaries and river systems of northern California (Buehler 2000).

Risk of selenium exposure: Wintering bald eagles have been observed on occasion in the Project area and vicinity (USBR 1991). In addition, bald eagles forage for fish along waterways and the estuary downstream of the Project.

Lillebo *et al*. (1988) derived levels of selenium to protect various species of waterbirds. Based on an analysis of bioaccumulation dynamics and an estimated critical dietary threshold for toxicity of 3 µg/g, they concluded that piscivorous birds would be at substantially greater risk of toxic exposure than mallards (*Anas platyrhynchos*). The calculated water criterion to protect piscivorous birds was 1.4 µg/L as opposed to 6.5 µg/L for mallards. It should also be noted that the 6.5 µg/L calculated criterion for mallards exceeds the actual threshold point for ducks in the wild which is somewhere below 4 µg/L (Skorupa 1998). Thus, the 1.4 µg/L calculated criterion for piscivorous birds may be biased high compared to the wild as well.

Applying an energetics modeling approach, modified from the Wisconsin Department of Natural Resources, Peterson and Nebeker (1992) calculated a chronic criterion specifically for bald eagles. Peterson and Nebeker's estimate of a protective criterion is 1.9 µg/L. Peterson and Nebeker calculated a mallard criterion (2.1 µg/L) that was much closer to their bald eagle criterion than Lillebo *et al.*'s (1988) results would suggest. Peterson and Nebeker's mallard criterion is consistent with real-world data (cf. Skorupa 1998) and therefore their bald eagle criterion may also be reliable.

Even after considerable dilution, waters receiving agricultural drainwater from the west side of the San Joaquin Valley frequently exceed 1.4 µg/L selenium; however, bald eagle dietary exposure to fish from these waters is expected to be low.

California clapper rail (*Rallus longirostris obsoletus*)

Status: The California clapper rail was federally listed as endangered on October 13, 1970 (35 FR 16047-16048).

General life history: The California clapper rail inhabits salt marshes surrounding the San Francisco Bay, California. Principal habitats are low portions of coastal wetlands dominated by cordgrass and pickleweed (USFWS 1984). Nesting habitat in San Francisco Bay is characterized by tidal sloughs, abundant invertebrate populations, pickleweed, gum plant, and wrack in upper zone. Individuals do not migrate far from the breeding grounds (Eddleman and Conway 1998).

Risk of selenium exposure: California clapper rails feed largely on benthic invertebrates, including filter-feeding mussels and clams (Moffitt 1941), a well-documented pathway for bioaccumulation of selenium (Pease *et al*. 1992, Stewart *et al*. 2004). Lonzarich *et al*. (1992) reported that eggs of California clapper rails collected from the north bay in 1987 contained up to 7.4 µg/g selenium. Water data from this time and location are not available. The *in ovo* threshold for selenium exposure that causes toxic effects on embryos of California clapper rails is unknown. For another benthic-foraging marsh bird, the black-necked stilt, the *in ovo* threshold for embryotoxicity is 6 µg/g selenium (Skorupa 1998). The most widely-used biphasic model (Brain and Cousens 1989) applied to Heinz *et al*. (1989) data from laboratory experiments with mallard reproduction indicates that in mallards, a selenium concentration of 7.4 µg/g (dry weight) in the eggs would be associated with a 32 percent reduction in hatchability of the eggs (Figure 6).

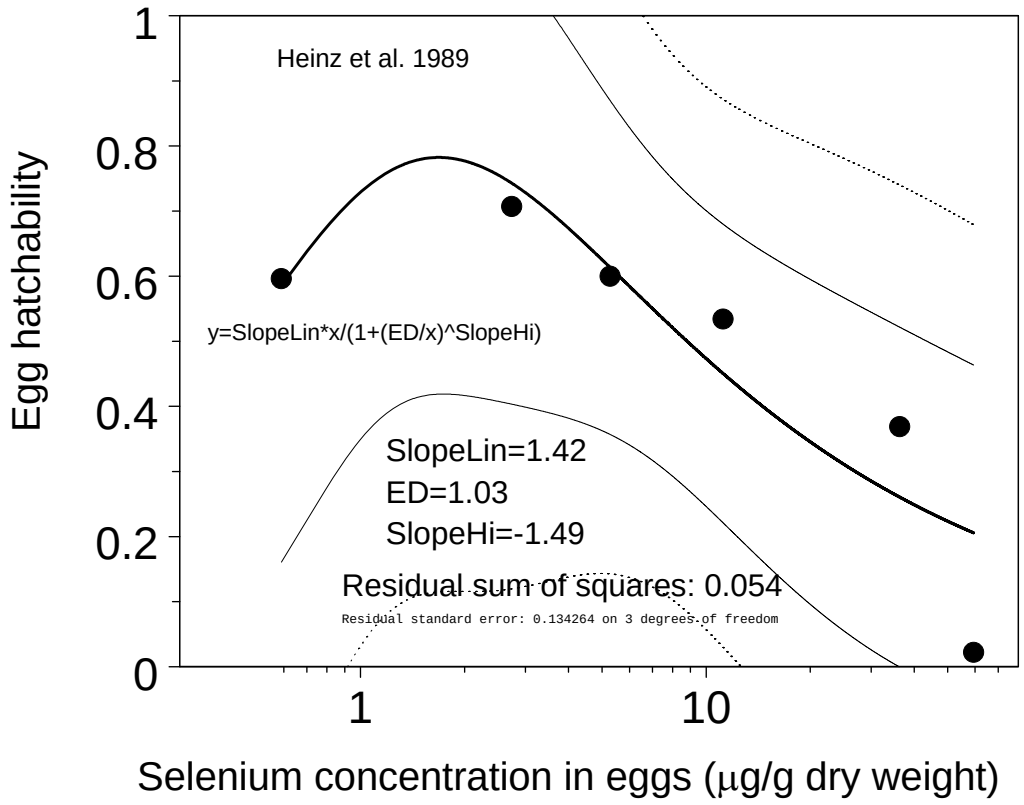


Figure 6. The hatching success of mallard eggs as a function of selenium concentration in the eggs, with the Brain-Cousens biphasic model fitted by least squares regression. Confidence intervals of 95% and 99% are shown.

It has been demonstrated for mallard ducks that interactive effects of selenium and mercury can be super-toxic with regard to embryotoxic effects (Heinz and Hoffman 1998). Lonzarich *et al*. (1992) also reported potentially embryotoxic concentrations of mercury in eggs of California clapper rails. Abnormally high numbers of nonviable eggs, 13.7-22.9 percent (Schwarzbach 1994) and 31 percent (Schwarzbach *et al.* 2006), have also been reported for the California clapper rail.

Based, in part, on the data for California clapper rails, staff technical reports prepared for the San Francisco Bay Regional Water Quality Control Board recommend decreasing current selenium loading to the estuary by 50 percent or more (Taylor *et al*. 1992, Taylor *et al*. 1993). The California clapper rail is particularly vulnerable to any locally elevated effluent concentrations of selenium as the rail generally occupies small home ranges of only a few acres. As selenium loads to the San Joaquin River and hence to the estuary are reduced over time due to implementation of selenium total maximum daily load limits and the Grassland Bypass Project, potential impacts to clapper rails due to delivery of water to the San Luis Unit will diminish.

California least tern (*Sterna antillarum browni*)

Status: The California least tern has been federally listed as endangered throughout its range since 1970 (35 FR 8491-8498, 35 FR 16047-16048). Distributed along the Pacific coast from the San Francisco Bay to Baja California, it is widely separated from the four other subspecies of least tern (Thompson *et al*. 1997). A 5-year review was completed in 2006 which recommended down listing the species to threatened (USFWS 2006b).

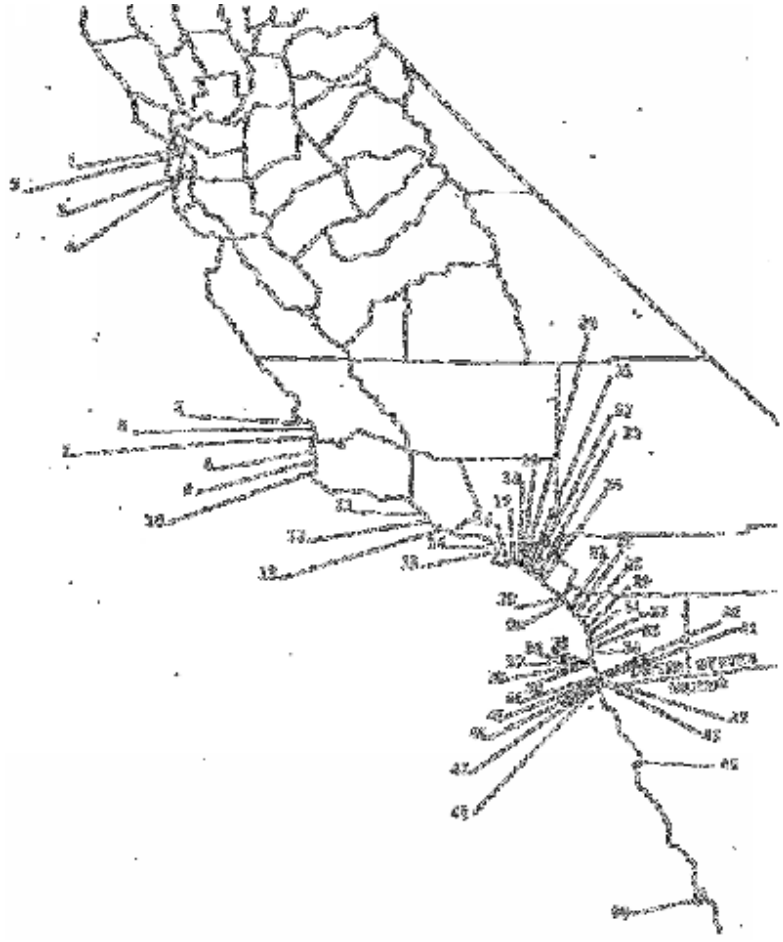


Figure 7. Nesting sites of the California least tern recorded since 1970 (USFWS 1985).

Life history summary: California least terns are migratory, wintering along the southern coast of Mexico (Thompson *et al*. 1997). The primary nesting site in San Francisco Bay is located at the former Alameda Naval Air Station. Least terns primarily eat small fish species that are less than 8 cm in length and small young-of-year fish of larger species. Fish species include northern anchovy (*Engraulis mordax*), top smelt (*Atherinops affinis*), and yellowfin goby (*Acanthogobius flavimanus*). Up to 50 species of fish have been documented in their diet (USFWS 1985).

Risk of selenium exposure: Currently, breeding colonies of California least tern are confined to scattered, isolated locations on beaches along the coast of California and in the San Francisco estuary, where they feed on surface fish in adjacent waters. In these locations any agricultural drainwater from the San Luis Unit is well diluted. Therefore, the current risk of selenium to this bird is probably *de minimis*. However, it is possible that the creation of evaporation ponds for disposal of agricultural drainwater from the San Luis Unit could provide habitat attractive to California least terns. Least terns in North Carolina and the Caribbean are known to eat invertebrates, including shrimp (review in Thompson *et al*. 1997). Although unlikely, California least terns could learn to feed opportunistically on abundant brine shrimp and other invertebrates in evaporation ponds. Concentrations of selenium in evaporation pond invertebrates are likely to be sufficiently elevated to cause reproductive impacts in least terns. Forster's tern eggs from San Joaquin Valley nests at evaporation ponds had an average of 7.1 μg/g dw of selenium (n=10, range 2.6 to 12 μg/g) while Caspian tern eggs averaged 2.4 μg/g (n=7, range 1.9 to 3.3 μg/g) (USFWS unpublished data). Methods of configuring evaporation ponds to discourage shorebird usage (deepening and steepening sides) will be ineffective in deterring foraging by least terns.

Chinook Salmon (*Oncorhynchus tshawytscha*)

Status: The National Marine Fisheries Service (NMFS) has identified 17 Evolutionarily Significant Units (ESUs) of Chinook salmon from Washington, Oregon, Idaho, and California (Myers *et al*. 1998; 63 FR 11482). Three of these use the San Francisco Estuary: the Sacramento River winter-run ESU, the Central Valley spring-run ESU, and the Central Valley fall/late fall-run ESU. The Sacramento River winter-run ESU was listed as endangered on January 4, 1994 (59 FR 440). On September 16, 1999, NMFS listed the Central Valley spring-run ESU as threatened (64 FR 50394). In the same rulemaking, NMFS also determined that the Central Valley fall/late fall ESU is not warranted for listing at that time; however, with recent record declines of salmon fall runs in California listing of this ESU may occur in the future.

Life history summary: Chinook salmon are anadromous and semelparous. That is, as adults they migrate from a marine environment into the fresh water streams and rivers of their birth (anadromous) where they spawn only once and die (semelparous). Juvenile Chinook may spend from 3 months to 2 years in freshwater after emergence before migrating to estuarine areas as smolts, and then into the ocean to feed and mature. The timing and duration of the migratory movements of Chinook salmon are important in assessing their exposure to selenium and estimating consequent risks. Natal streams and estuary rearing habitat vary seasonally in selenium concentration and the salmon evidently vary in sensitivity to selenium across stages in their life histories. A more detailed life history discussion is provided for salmon in order to

more clearly define the selenium exposure risks to the various ESUs and to identify the ones at greatest risk to selenium exposure resulting from irrigation deliveries to the San Luis Unit.

Freshwater migration: Once their downstream migration begins, Chinook salmon fry may stop migrating and take up residence in the stream for a period of two weeks to a year or more (Healey 1991).

Use of estuarine habitat: On their migration downstream, many Chinook salmon fry take up residence in the river estuary where they rear to smolt size (about 70 mm fork length) before resuming their migration to the ocean. The proportion of fry that rear in the estuary is not known. On Vancouver Island, BC, about 30 percent of the estimated downstream migrants could be accounted for in the estuary; the fate of the remaining 70 percent is unknown, but they probably suffered mortality due to unknown agents (Healey 1991). The maximum residence time of Chinook salmon fry in the Sacramento-San Joaquin River delta was estimated to be 64 days in 1980 and 52 days in 1981 (Kjelson *et al*. 1981)

Life history types: Chinook salmon exhibit two generalized freshwater life history types (Healey 1983, Healey 1991). "Stream-type" Chinook salmon, enter freshwater months before spawning and reside in freshwater for a year or more following emergence, whereas "ocean-type" Chinook salmon spawn soon after entering freshwater and migrate to the ocean as fry or parr within their first year. Spring-run Chinook salmon exhibit a stream-type life history. Adults enter freshwater in the spring, hold over summer, spawn in fall, and the juveniles typically spend a year or more in freshwater before emigrating. Winter-run Chinook salmon are somewhat anomalous in that they have characteristics of both stream- and ocean-type races (Healey 1991). Adults enter freshwater in winter or early spring, and delay spawning until spring or early summer (stream-type). However, juvenile winter-run Chinook salmon migrate to sea after only four to seven months of river life (ocean-type). Adequate instream flows and cool water temperatures are more critical for the survival of Chinook salmon exhibiting a stream-type life history due to over summering by adults and/or juveniles. The stream-type life history also increases selenium exposure risks during the critical egg development stage of the adult and the growth stage of juveniles.

Runs: Salmon runs (separate ESUs) are designated on the basis of adult migration timing; however, distinct runs also differ in the degree of maturation at the time of river entry, thermal regime and flow characteristics of their spawning site, and the actual time of spawning (Myers *et al.* 1998). Both spring-run and winter-run Chinook salmon tend to enter freshwater as immature fish, migrate far upriver, and delay spawning for weeks or months. For comparison, fall-run Chinook salmon enter freshwater at an advanced stage of maturity, move rapidly to their spawning areas on the mainstem or lower tributaries of the rivers, and spawn within a few days or weeks of freshwater entry (Healey 1991).

Run-specific downstream migration: Winter-run Chinook salmon fry begin to emerge from the gravel in late June to early July and continue through October (Fisher 1994). Spring-run Chinook salmon fry emerge from the gravel from November to March and spend about 3 to 15 months in freshwater habitats prior to emigrating to the ocean (Kjelson *et al.* 1981). Post-emergent fry disperse to the margins of their natal stream, seeking out shallow waters with

slower currents, finer sediments, and bank cover such as overhanging and submerged vegetation, root wads, and fallen woody debris, and begin feeding on small insects and crustaceans.

When juvenile Chinook salmon reach a length of 50 to 57 mm, they move into deeper water with higher current velocities, but still seek shelter and velocity refugia to minimize energy expenditures. In the mainstems of larger rivers, juveniles tend to migrate along the margins and avoid the elevated water velocities found in the thalweg of the channel. When the channel of the river is greater than 9 to 10 feet in depth, juvenile salmon tend to inhabit the surface waters (Healey 1982). Emigration of juvenile winter-run Chinook salmon past Red Bluff Diversion Dam (RBDD) on the Sacramento River may begin as early as mid-July, typically peaks in September, and can continue through March in dry years (Vogel and Marine 1991; NMFS 1997). From 1995 to 1999, all winter-run Chinook salmon outmigrating as fry passed RBDD by October, and all outmigrating pre-smolts and smolts passed RBDD by March (Martin *et al.* 2001). The emigration timing of Central Valley spring-run Chinook salmon is highly variable (CDFG 1998). Some fish may begin emigrating soon after emergence from the gravel, whereas others over summer and emigrate as yearlings with the onset of intense fall storms (CDFG 1998). The emigration period for spring-run Chinook salmon extends from November to early May, with up to 69 percent of the young-of-the-year fish outmigrating through the lower Sacramento River and Delta during this period (CDFG 1998).

As Chinook salmon fry and fingerlings mature, they prefer to rear further downstream where ambient salinity is up to 1.5 to 2.5 parts per thousand (Healey 1980, 1982; Levings *et al.* 1986). Juvenile winter-run Chinook salmon occur in the Delta from October through early May based on data collected from trawls, beach seines, and salvage records at the Central Valley Project (CVP) and State Water Project (SWP) pumping facilities (CDFG 1998). The peak of listed juvenile salmon arrivals in the Delta generally occurs from January to April, but may extend into June. Upon arrival in the Delta, winter-run Chinook salmon spend the first two months rearing in the more upstream, freshwater portions of the Delta (Kjelson *et al.* 1981, Kjelson *et al.* 1982). Data from the CVP and SWP salvage records indicate that most spring-run Chinook salmon smolts are present in the Delta from mid-March through mid-May depending on flow conditions (CDFG 2000).

Winter-run Chinook salmon fry remain in the estuary (Delta/Bay) until they reach a fork length of about 118 mm (*i.e.*, 5 to 10 months of age) and then begin emigrating to the ocean perhaps as early as November and continuing through May (Fisher 1994; Myers *et al.* 1998). Little is known about estuarine residence time of spring-run Chinook salmon. Juvenile Chinook salmon were found to spend about 40 days migrating through the Delta to the mouth of San Francisco Bay and grew little in length or weight until they reached the Gulf of the Farallones (MacFarlane and Norton 2002). Based on the mainly ocean-type life history observed (*i.e.*, fall-run Chinook salmon) MacFarlane and Norton (2002) concluded that unlike other salmonid populations in the Pacific Northwest, Central Valley Chinook salmon show little estuarine dependence and may benefit from expedited ocean entry. Spring-run yearlings are larger in size than fall-run yearlings and are ready to smolt upon entering the Delta; therefore, they are believed to spend little time rearing in the Delta.

Risk of selenium exposure: Due to water diversions and consequent loss of breeding and migrating habitat, California Central Valley Chinook salmon have been effectively extirpated

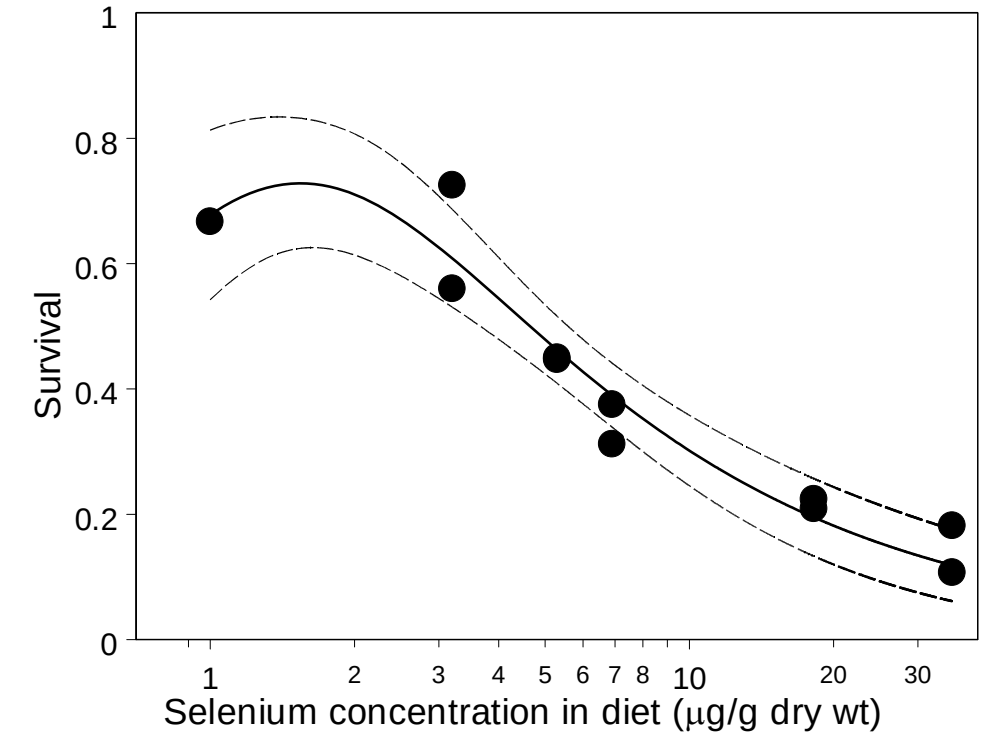
from the San Joaquin River above the confluence of the Merced River. Planning is underway to restore salmon to this river by increasing flows and restoring habitat. However, seepage and flood flows carrying agricultural drainwater from the San Luis Unit into the San Joaquin River may impact salmon and could impair efforts to restore them to this river.

California Central Valley Chinook salmon evidently are among the most sensitive of fish and wildlife to selenium. They are especially vulnerable during juvenile life stages when they migrate and rear in selenium-contaminated Central Valley rivers and the San Francisco Bay/Delta estuary.

In a laboratory experiment, measurements were made of the selenium bioaccumulation, weight and survival of juvenile (initially swim-up larvae) San Joaquin River fall run Chinook salmon that were exposed for 90 days in fresh water to two parallel graded series of dietary selenium treatments (Hamilton *et al*. 1990). In one series, the food was spiked with seleno-DL-methionine (SeMet); in the other series, the source of selenium was mosquitofish collected from the San Luis Drain (SLD), which carried seleniferous agricultural drainwater from a subsurface tile drainage system in the Westlands Water District in the San Joaquin Valley of California. Although the SLD mosquitofish diets may have included other contaminants, such as pesticides, the results of this experiment indicate that, once selenium is incorporated into fish tissue, there is no difference in the tissue concentration-response relationship due to the different sources of selenium (SLD or SeMet). Therefore, all data from both diet series were combined in the analysis presented here.

The effects of selenium on animals (including fish) are well known to be biphasic (beneficial at low doses; toxic at high doses; see, for example, Beckon *et al*. 2008), and in the Hamilton *et al*. (1990) experiment, the 90-day survival data appear to confirm a biphasic dose-response relationship with respect to the survival endpoint (Figure 8). Therefore, we fitted a biphasic model (Brain and Cousens 1989) to the data by least squares regression. This regression provides a weight-of-evidence estimate of the maximum survival rate (0.7, or 70 percent) of young salmon under these experimental conditions at the estimated optimal selenium concentration in the fish (about 1 µg/g whole body dry weight). It also provides an estimate of the survival rate at any given selenium concentration above the optimum. Any such survival rate estimate can be compared to the maximum survival rate to yield an estimate of the mortality (inverse of survival) specifically attributable to selenium. For example, at a fish tissue concentration of 7.9 µg/g (whole body dry weight) the regression curve predicts a survival of 0.29 (29 percent). As a proportion of the maximum survival this is 0.29/0.7 = 0.41, or 41 percent. Therefore our best weight-of-evidence estimate of the mortality due to selenium toxicity at a tissue concentration of 7.9 µg/g is the inverse of 0.41, which is 0.59, or 59 percent. Similarly, the model predicts that fish with a selenium concentration of 2.45 µg/g (whole body dry weight) after 90 days of exposure would experience 20 percent mortality due to selenium (Figure 8 lower graph).

In the Hamilton *et al*. (1990) experiment, the concentrations of selenium in the food that was provided to the salmon were about the same as the concentrations reached by the salmon themselves. This experiment indicates that, in sloughs that carry agricultural drainwater, concentrations of selenium in invertebrates, small (prey) fish, and larger predatory fish



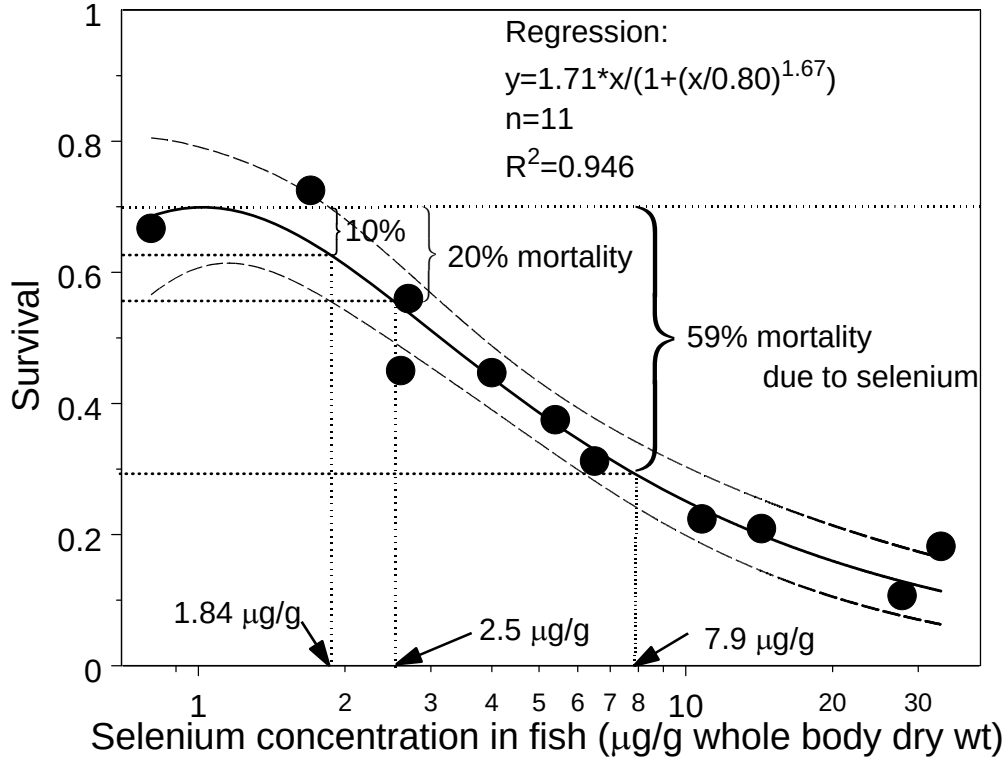


Figure 8. Survival as a function of selenium concentration in diet (above) and tissue (below) of juvenile Chinook salmon after 90 days of exposure to dietary selenium. A biphasic model (Brain and Cousens 1989) was fitted by least squares regression. Dashed lines indicate 95% confidence bands around the regressions.

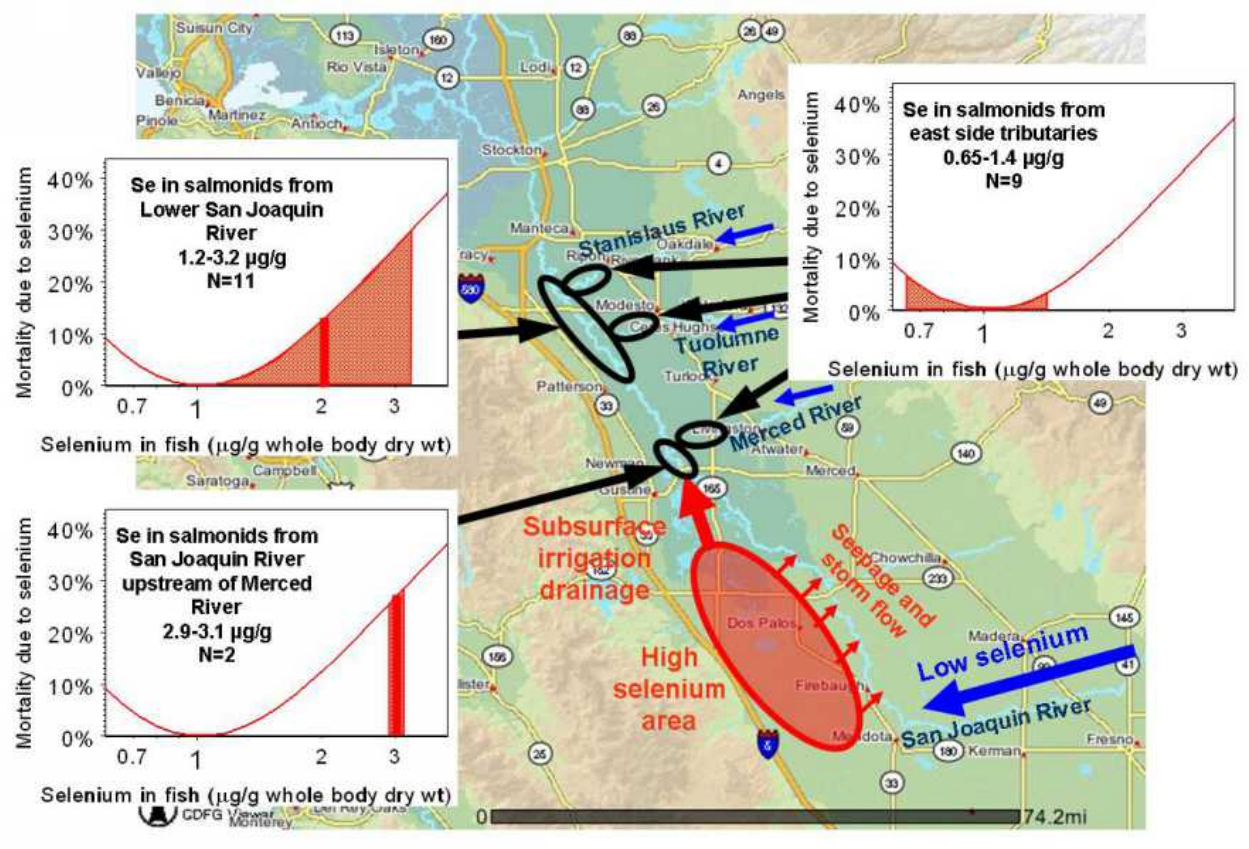


Figure 9. Risk of mortality to juvenile Chinook salmon based on selenium measured in the salmon (Saiki, *et al.* 1991) and the toxicity data shown in Figure 8 (presented here as mortality). Solid red bars represent the geometric mean selenium concentration in sampled fish at each location or cluster of locations. The stippled red areas span the ranges of concentrations in fish at the respective locations.

commonly reach levels (Beckon *et al*. 2003) that could kill a substantial portion of young salmon (Figure 8 upper graph) if the salmon, on their downstream migration, are exposed to those selenium-laden food items for long enough for the salmon themselves to bioaccumulate selenium to toxic levels.

Available data (Saiki *et al*. 1991) confirm that young salmon migrating down the San Joaquin River in 1987 bioaccumulated selenium to levels (about 3 µg/g whole body dry wt.) that were likely to kill more than 25% (**Figure 9**).

Concentrations of selenium in the San Joaquin River have been reduced since juvenile Chinook salmon were sampled in 1987 (Saiki *et al*. 1991). However, the relationship between selenium in water and in young salmon in 1987 (Figure 10) indicates that there remains a substantial ongoing risk to migrating juvenile Chinook salmon in the San Joaquin River (Figure 11).

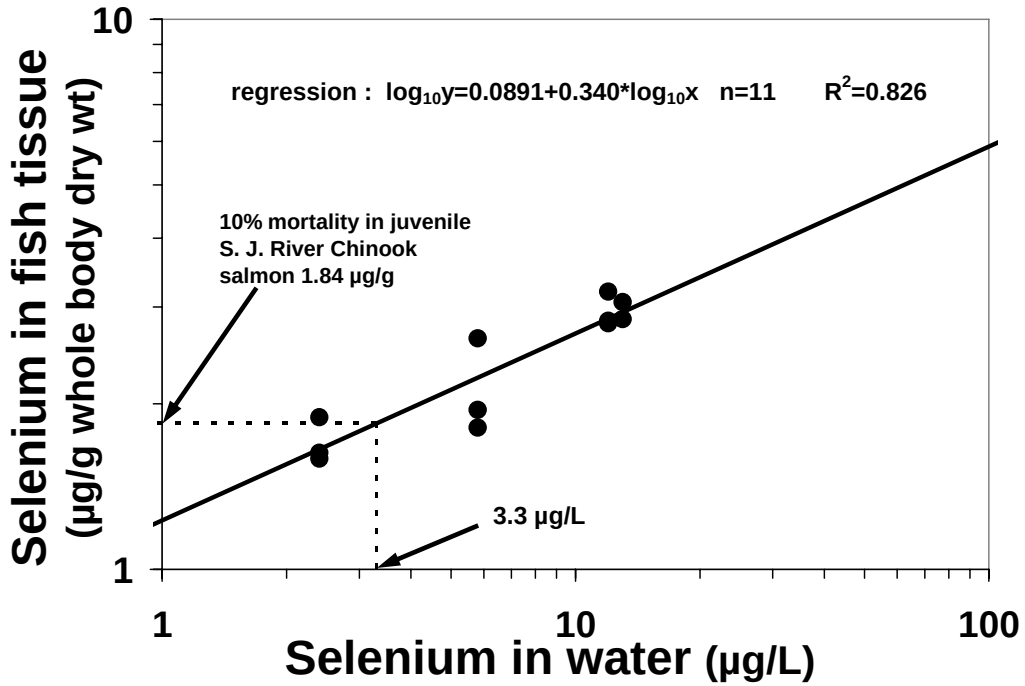


Figure 10. Relationship between selenium in juvenile Chinook salmon (Saiki *et al*. 1991, Saiki pers. com.) and water (Central Valley Regional Water Quality Control Board "Flat File") in the San Joaquin River and its tributaries.

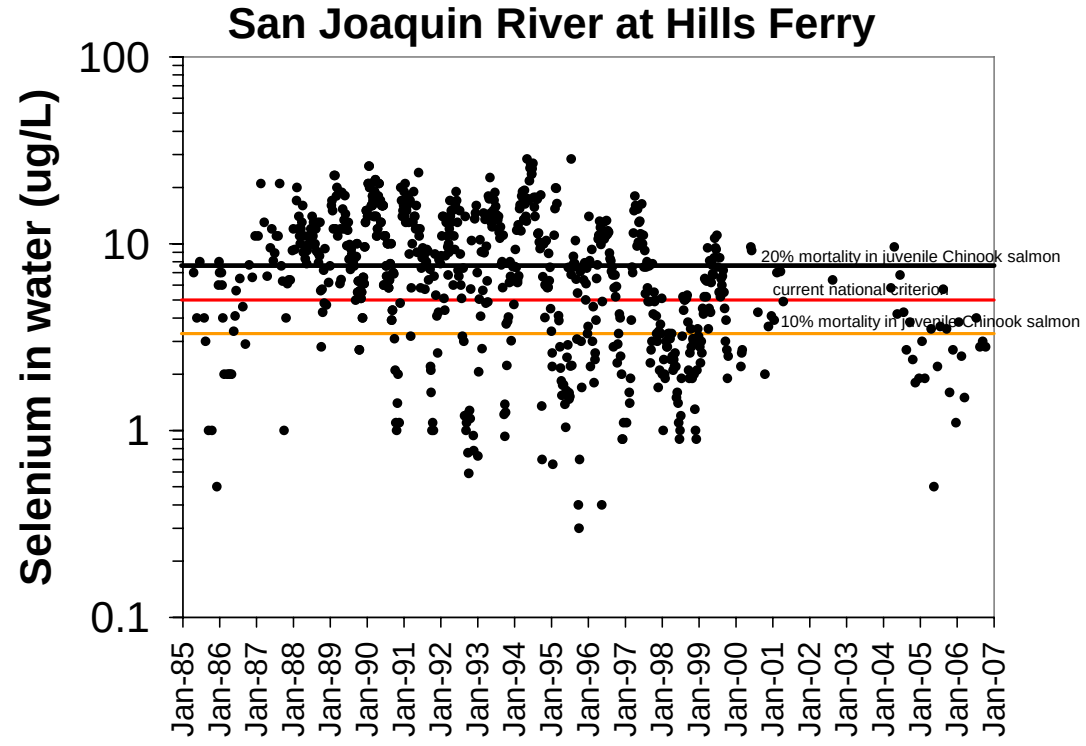


Figure 11. Selenium concentrations measured in the San Joaquin River at Hills Ferry, just upstream of the confluence of the Merced River. The data are from the Central Valley Regional Water Quality Control Board.

Steelhead Trout (*Oncorhynchus mykiss*)

Status: Steelhead trout are the anadromous form of the rainbow trout species. Central Valley steelhead were listed as threatened under the ESA on March 19, 1998 (63 FR 13347). This ESU consists of steelhead populations in the Sacramento and San Joaquin River (inclusive of and downstream of the Merced River) basins in California's Central Valley.

The breeding of wild steelhead in the Central Valley is mostly confined to the Sacramento River and its tributaries, including Antelope, Deer, and Mill Creeks and the Yuba River. Populations may exist in Big Chico and Butte Creeks and a few wild steelhead are produced in the American and Feather Rivers (McEwan and Jackson 1996).

Steelhead were thought to be extirpated from the San Joaquin River system. Monitoring has detected small self sustaining populations of steelhead in the Stanislaus, Mokelumne, Calaveras, and other streams previously thought to be devoid of steelhead (McEwan 2001).

General Life History: Steelhead can be divided into two life history types, stream-maturing and ocean-maturing, based on their state of sexual maturity at the time of river entry and the duration of their spawning migration. Stream-maturing steelhead enter freshwater in a sexually immature condition and require several months to mature and spawn, whereas ocean-maturing steelhead enter freshwater with well-developed gonads and spawn shortly after river entry. These two life history types are more commonly referred to by their season of freshwater entry (*i.e.* summer [stream-maturing] and winter [ocean-maturing] steelhead). Only winter steelhead currently are found in the rivers and streams of Central Valley and San Francisco Bay area (McEwan and Jackson 1996).

Winter steelhead generally leave the ocean from August through April, and spawn between December and May (Busby *et al.* 1996). Timing of upstream migration is correlated with higher flow events and associated lower water temperatures. In general, the preferred water temperature for adult steelhead migration is 46 ^{o}F to 52 ^{o}F (McEwan and Jackson 1996; Myrick 1998; and Myrick and Cech 2000).

Unlike Pacific salmon, steelhead are iteroparous, or capable of spawning more than once before death (Busby *et al.* 1996). However, it is rare for steelhead to spawn more than twice before dying; most that do so are females (Nickleson *et al.* 1992; Busby *et al.* 1996). Iteroparity is more common among southern steelhead populations than northern populations (Busby *et al.* 1996). Although one-time spawners are the great majority, Shapovalov and Taft (1954) reported that repeat spawners are relatively numerous (17.2 percent) in California streams. Most steelhead spawning takes place from late December through April, with peaks from January though March (Hallock *et al.* 1961). Steelhead spawn in cool, clear streams featuring suitable gravel size, depth, and current velocity, and may spawn in intermittent streams as well (Everest 1973; Barnhart 1986).

The length of the incubation period for steelhead eggs is dependent on water temperature, dissolved oxygen concentration, and substrate composition. In late spring and following yolk

sac absorption, fry emerge from the gravel and actively begin feeding in shallow water along stream banks (Nickelson *et al.* 1992).

Steelhead rearing during the summer takes place primarily in higher velocity areas in pools, although young-of-the-year also are abundant in glides and riffles. Winter rearing occurs more uniformly at lower densities across a wide range of fast and slow habitat types. Productive steelhead habitat is characterized by complexity, primarily in the form of large and small woody debris. Cover is an important habitat component for juvenile steelhead both as velocity refugia and as a means of avoiding predation (Shirvell 1990; Meehan and Bjornn 1991). Some older juveniles move downstream to rear in large tributaries and mainstem rivers (Nickelson *et al.* 1992). Juveniles feed on a wide variety of aquatic and terrestrial insects (Chapman and Bjornn 1969), and older juveniles sometimes prey upon emerging fry.

Steelhead generally spend two years in freshwater before emigrating downstream (Hallock *et al.* 1961; Hallock 1989). Rearing steelhead juveniles prefer water temperatures of 45˚ F to 58˚ F and have an upper lethal limit of 75˚ F. They can survive up to 81˚ F with saturated dissolved oxygen conditions and a plentiful food supply.

Juvenile steelhead emigrate episodically from natal streams during fall, winter, and spring high flows. Emigrating Central Valley steelhead use the lower reaches of the Sacramento River and the Delta for rearing and as a migration corridor to the ocean. Some may utilize tidal marsh areas, non-tidal freshwater marshes, and other shallow water areas in the Delta as rearing areas for short periods prior to their final emigration to the sea. Barnhart (1986) reported that steelhead smolts in California range in size from 140 to 210 mm (fork length). Hallock *et al.* (1961) found that juvenile steelhead in the Sacramento River Basin migrate downstream during most months of the year, but the peak period of emigration occurred in the spring, with a much smaller peak in the fall.

Risk of selenium exposure: Planning is underway to restore salmon to the San Joaquin River by increasing flows and restoring habitat. Such restoration efforts would likely improve the small steelhead population in the San Joaquin Valley. However, as with salmon, seepage and flood flows carrying agricultural drainwater from the San Luis Unit into the San Joaquin River may impact steelhead and may confound efforts to restore them to this river.

Because steelhead are regarded as a life-history variant or "form" of the rainbow trout species, studies of the non-anadromous form of rainbow trout may provide a good indication of the risks of the exposure of steelhead to selenium. Such studies indicate that rainbow trout are among the more sensitive of fish to selenium. One of these studies examined the effects of selenium on fry of rainbow and brook trout exposed in streams in Alberta, Canada (Holm 2002, Holm *et al*. 2003). In summary, this study indicates that maternal selenium would result in 20 percent mortality of fry if female rainbow trout have a tissue selenium concentration of 2.93 µg/g wholebody dry weight (Figure 12). The USEPA (2004) has proposed that a fish tissue chronic criterion of 7.9 µg/g selenium (wholebody) would be protective. However, female rainbow trout in the wild with a concentration of about 8 µg/g selenium in their (wholebody) tissue would produce eggs that suffer 44.2 percent mortality by swimup stage (Figure 12). Among the

swimup survivors, 96 percent would suffer edema (Figure 13) and 42 percent would have craniofacial deformities (Figure 14) (for details, see USFWS 2005).

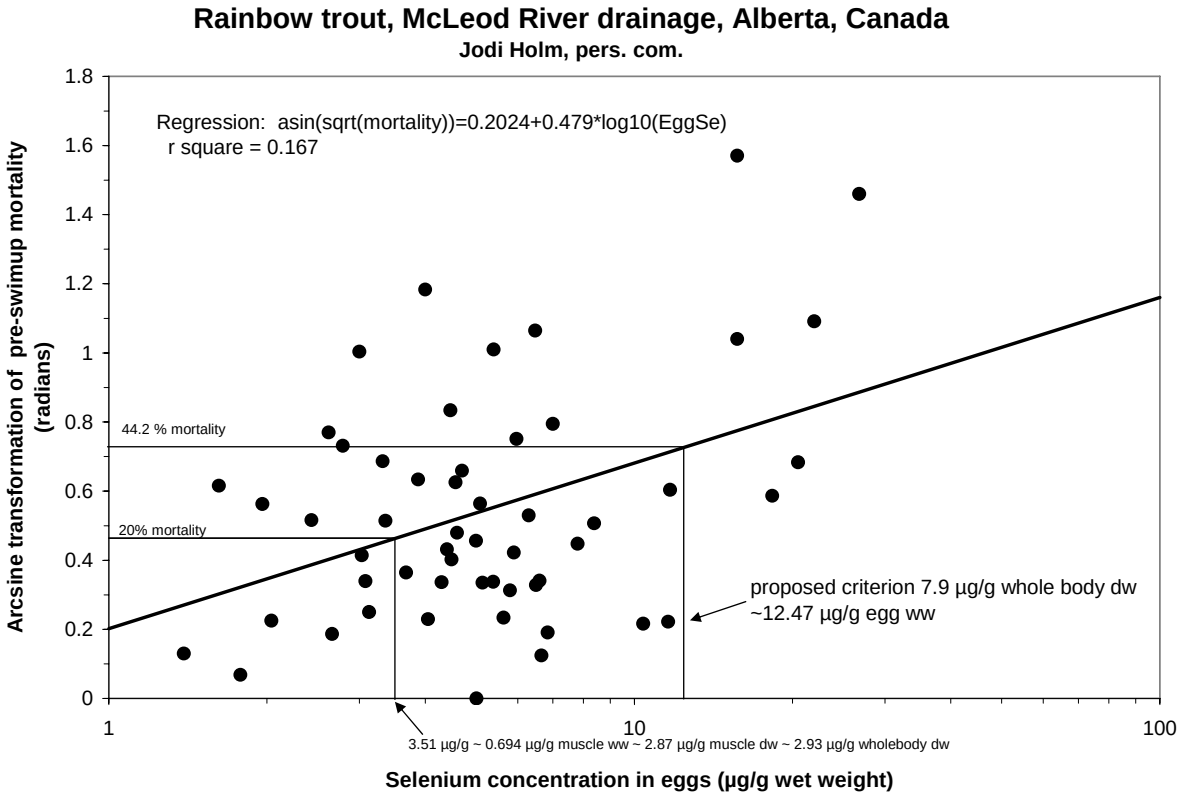


Figure 12. Relationship between selenium in rainbow trout eggs and mortality of eggs and fry by swimup stage. The arcsine transformation is applied to mortality data, as appropriate for linear regressions with percents or proportions (Sokol and Rohlf 1981). Data are from the years 2000-2002.

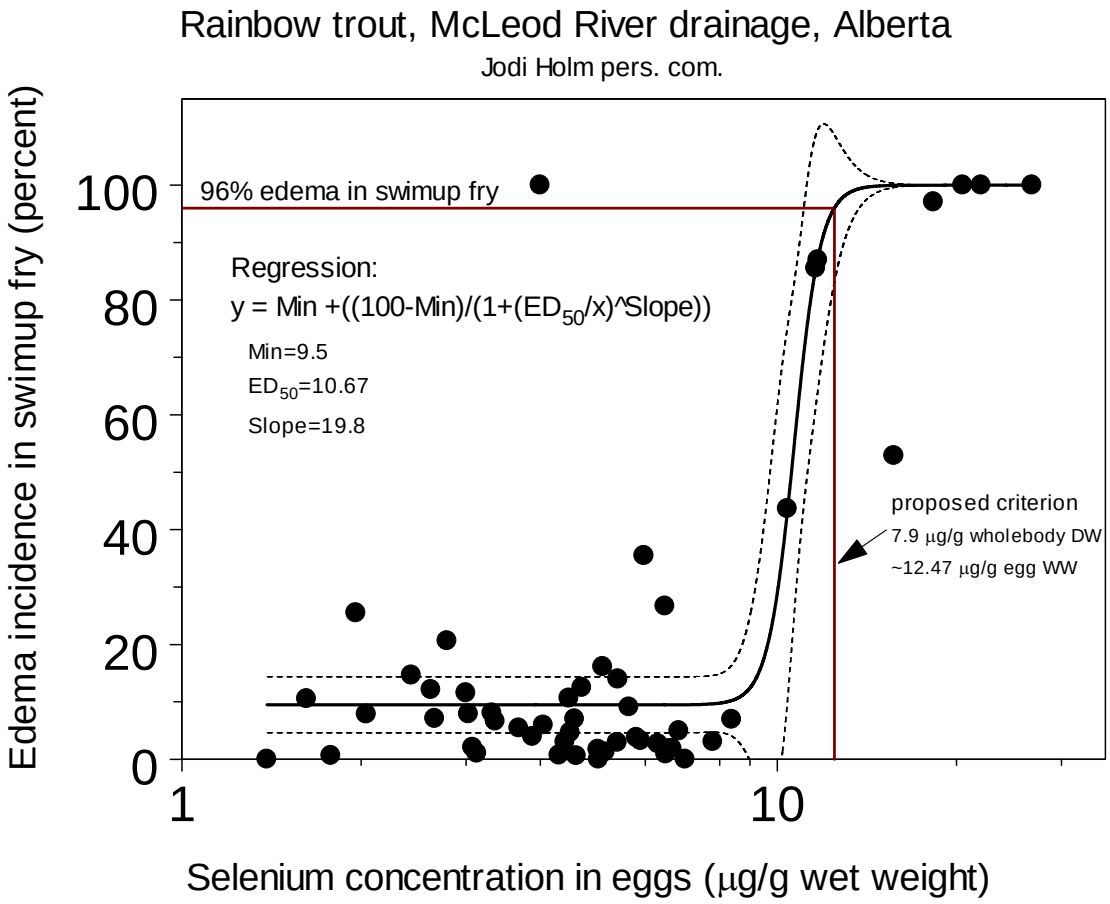


Figure 13. Relationship between selenium in rainbow trout eggs and edema in surviving swimup fry. Data from the years 2000-2002.

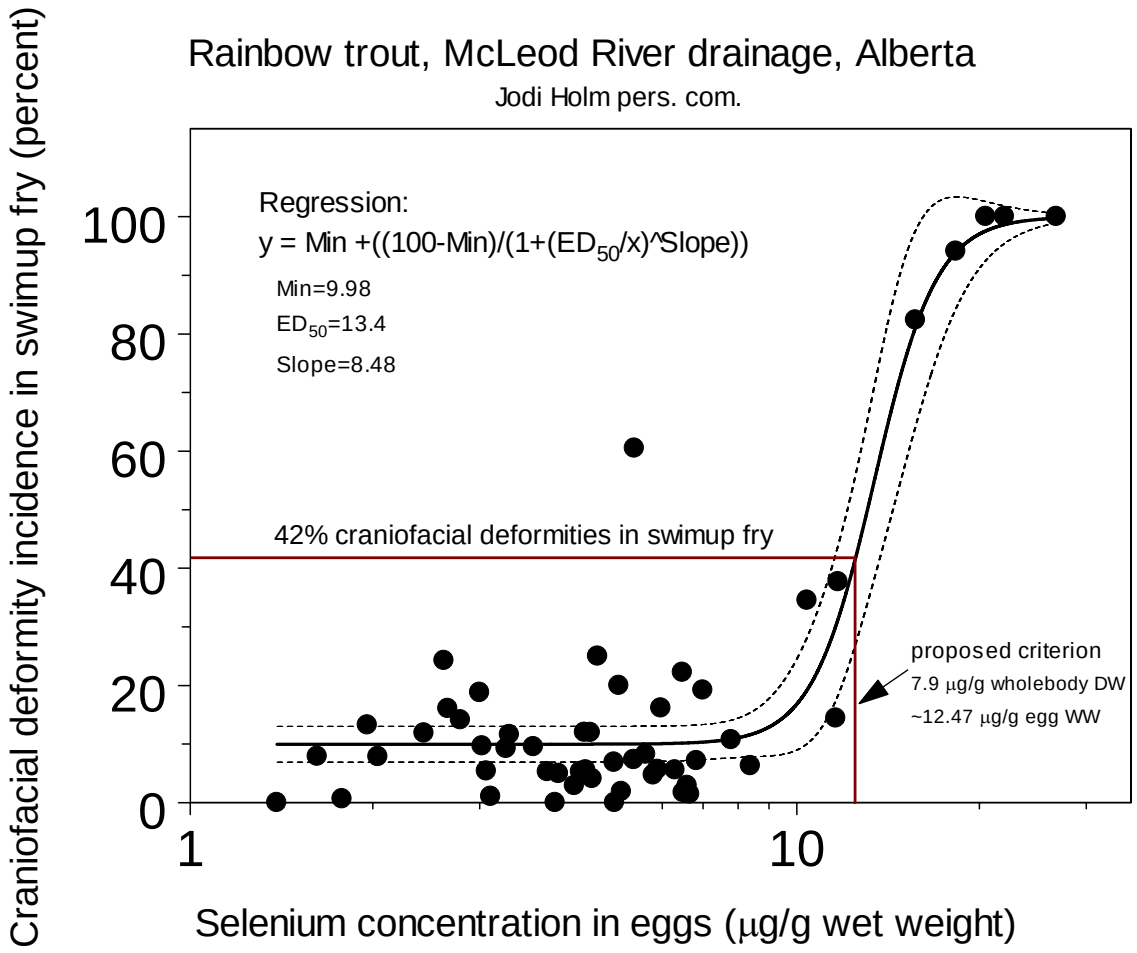


Figure 14. Relationship between selenium in rainbow trout eggs and craniofacial deformities in surviving swimup fry. Data from the years 2000-2002.

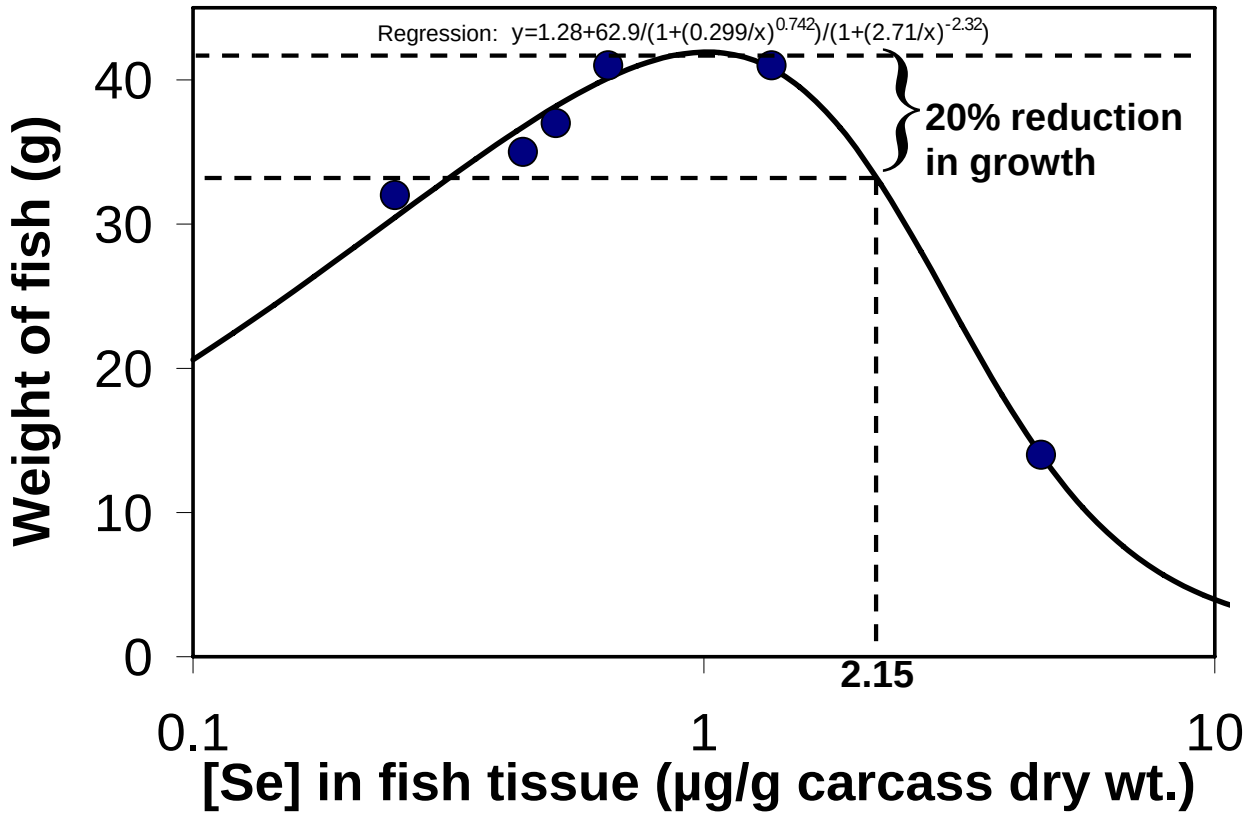


Figure 15. Average weights of juvenile rainbow trout after 20 weeks of exposure to diets spiked with sodium selenite (Hilton *et al*. 1980). The data were fitted with a biphasic model (Beckon *et al*. 2008). In the model it was assumed that at extremely high and extremely low selenium concentrations, the fish would have failed to grow at all, i.e. they would have remained at the initial average weight of 1.28 g. Carcass concentrations are from Fig. 2 of Hilton *et al*. 1980.

A laboratory experiment monitored the growth of juvenile rainbow trout exposed to a diet spiked with selenium in the form of sodium selenite (Hilton *et al*. 1980). This experiment indicates that juvenile rainbow trout that reach a selenium concentration of about 8 µg/g (carcass dry weight) by exposure for 20 weeks to dietary selenium in the form of sodium selenite will experience at least an 86 percent reduction in weight relative to the weight they would gain if their exposure to dietary sodium selenate were optimal (Figure 15). A weight reduction of 20 percent would be associated with a tissue selenium concentration of 2.15 µg/g (carcass dry weight).

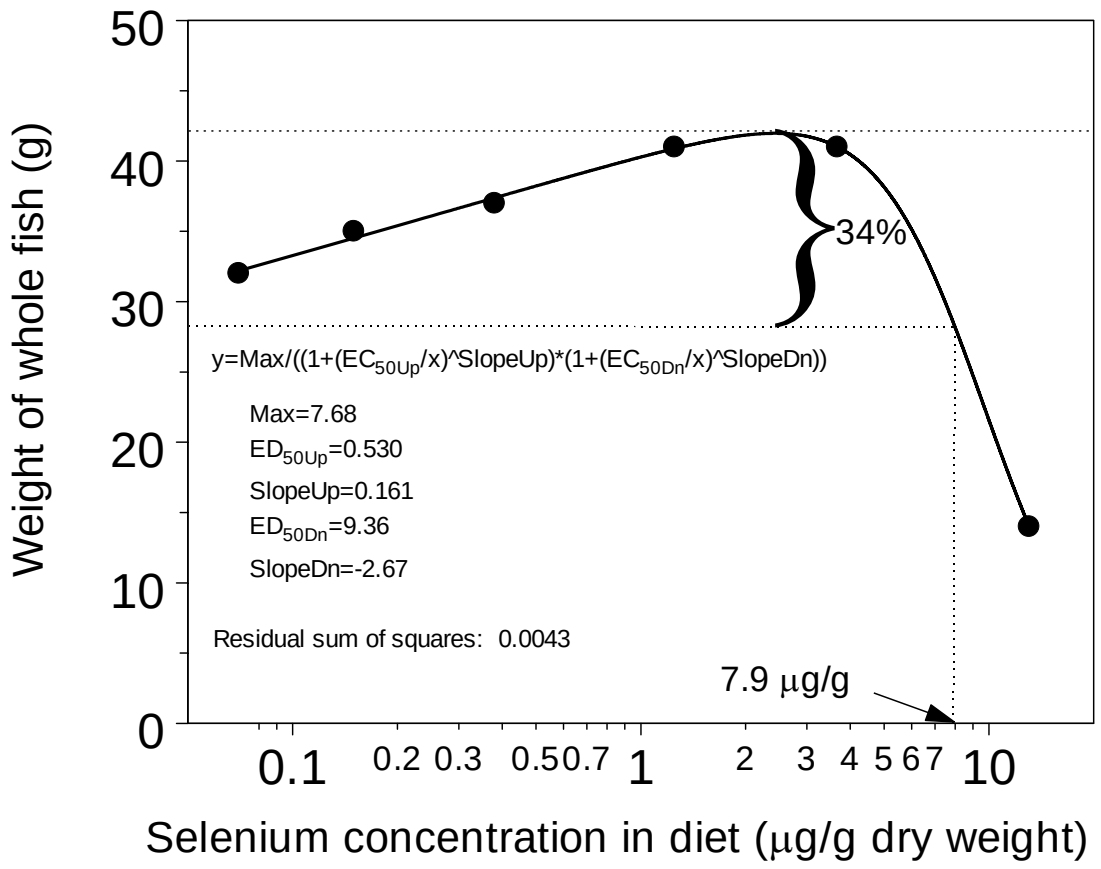


Figure 16. Average weights of juvenile rainbow trout after 20 weeks dietary exposure to sodium selenite (Hilton *et al*. 1980). A biphasic model (Beckon *et al*. 2008) is fitted to the data by least squares non-linear regression.

This experiment also indicates that if young rainbow trout feed on tissue that has a selenium concentration of about 8 µg/g (in the form of sodium selenite) they will suffer a reduction in growth of about 34 percent (Figure 16). Because the form of selenium administered to the fish in this experiment was sodium selenite, this analysis may yield an underestimate of the adverse effects of the more bioavailable organic forms of selenium that fish consume in the wild.

The experiments summarized above indicate that the larval survival and the health and growth of young steelhead trout would be impaired by a concentration of selenium (about 8 µg/g) commonly exceeded in invertebrates, small (prey) fish, and larger predatory fish in waterways that carry agricultural drainwater in the vicinity of the San Luis Unit (Beckon *et al*. 2003).

Green sturgeon (*Acipenser medirostris*)

Status: The southern distinct population segment, or DPS, of north American green sturgeon was federally listed as threatened under the Endangered Species Act on Apr. 7, 2006 (71 FR 17757). The range of the southern DPS extends southward from the Eel River, in northern California, and includes the green sturgeon inhabiting the San Francisco Bay/Delta estuary.

General life history: The ecology and life history of the anadromous green sturgeon have received comparatively little study, evidently because of their generally low abundance and their low commercial and sport-fishing value in the past. The adults are more marine than white sturgeon, spending limited time in estuaries or fresh water.

Green sturgeon migrate up the Klamath River between late February and late July. The spawning period is March-July, with a peak from mid-April to mid-June (Emmett *et al*. 1991). Spawning times in the Sacramento River are probably similar, based on times when adult sturgeon have been caught there. Spawning takes place in deep, fast water. Female green sturgeon produce 60,000-140,000 eggs (Moyle 1976). Based on their presumed similarity to white sturgeon, green sturgeon eggs probably hatch around 196 hours (at 12.7 degrees Celsius [54.9 degrees Fahrenheit]) after spawning, and larvae should be 8-19 millimeters (0.3-0.7 inch) long. Juveniles likely range in size from 2.0-150 centimeters (1-59 inches) (Emmett *et al*. 1991). Juveniles migrate out to sea before 2 years of age, primarily during summer-fall (Emmett *et al*. 1991). Length-frequency analyses of sturgeon caught in the Klamath Estuary by beach seine indicate that most green sturgeon leave the system at lengths of 30-70 centimeters (12-28 inches), when they are up to 4 years old, although a majority leave as yearlings (USFWS 1996). They remain near estuaries at first, but can migrate considerable distances as they grow larger (Emmett *et al*. 1991). Individuals tagged by DFG in San Pablo Bay (part of the San Francisco Bay system) have been recaptured off Santa Cruz, California, in Winchester Bay on the southern Oregon coast, at the mouth of the Columbia River and in Gray's Harbor, Washington (Chadwick 1959; Miller 1972). Most tags for green sturgeon in the San Francisco Bay system have been returned from outside that estuary (D. Kohlhorst, DEG, personal communication, cited in USFWS 1996).

Risk of selenium exposure: Little is known of the risk of selenium to green sturgeon, but white sturgeon (*Acipenser transmontanus*), a representative surrogate species for the green sturgeon, have been the subject of detailed studies within the San Francisco Bay estuary. See the discussion for white sturgeon below.

White Sturgeon (*Acipenser transmontanus*)

Status: According to the World Conservation Union (Duke *et al*. 2004), in general the white sturgeon species is not threatened, but some subpopulations are endangered (Kootenai River and Upper Fraiser River) or critically endangered (Nechako River, Upper Columbia River). The Kootenai River population of the white sturgeon in Montana and Idaho was federally listed as endangered under the Endangered Species Act on September 6, 1994 (59 FR 45989). The California Department of Fish and Game (CDFG) established a daily bag and possession limit of one fish, which must be between 46 and 72 inches total length (CDFG 2007). Temporary (120

days) emergency regulations issued by the CDFG in March 2006 restricted fishing in California to individuals between 46 and 56 inches total length.

General life history: Like green sturgeon, white sturgeon are anadromous, but the adults are less marine than green sturgeon, spending more time in estuaries or fresh water. At sea, white sturgeon have been found from Ensenada, Baja California (Mexico) to the Gulf of Alaska (Fry 1973). The majority of white sturgeon rear in the Columbia-Snake River and Sacramento-San Joaquin basins (Duke *et al*. 2004). White sturgeon have been the subject of detailed studies within the San Francisco Bay estuary (e.g., Kohlhorst *et al*. 1991, Linares *et al*. 2004, Linville 2006). White sturgeon are long-lived, large-bodied, and demersal (bottom-dwelling) fish. For most species of sturgeon, females require several years for eggs to mature between spawnings (Conte *et al*. 1988). White sturgeon in the San Francisco Bay estuary congregate in Suisun and San Pablo Bays where they remain year-round except for a small fraction of the population that moves up the Sacramento River, and to a lesser extent the San Joaquin River, to spawn in late winter and early spring (Kohlhorst *et al*. 1991).

Risk of selenium exposure: Many individuals of this species remain year-round in San Pablo Bay, the part of the San Francisco Bay estuary with the highest selenium concentrations (up to 2.7 µg/L). Clams predominated in the esophageal and stomach contents of white sturgeon caught by anglers in San Pablo Bay (213 fish) and Suisun Bay/Carquinez Strait (142 fish) in 1965-1967 (McKechnie and Fenner 1971). More recently with the change in the benthic food structure of the estuary (Feyrer *et al*. 2003) white sturgeon may depend more on the introduced Asian clam, *Potamocorbula amurensis*, which is an extraordinarily efficient bioaccumulator of selenium (Stewart *et al*. 2004). The median concentration of selenium in Asian clams from San Pablo Bay was found to be above 10 µg Se/g (Stewart *et al*. 2004). Based on histopathological alterations in the kidney, Tashjian *et al.* (2006) estimated that for juvenile white sturgeon a threshold dietary selenium toxicity concentration lies between 10 and 20 µg Se/g. It is uncertain at what point in their life white sturgeon begin feeding on Asian clams.

Linares *et al*. (2004) found concentrations of selenium as high as 46.7 µg/g in gonads of 39 white sturgeon captured in the San Francisco Bay. Kroll and Doroshov (1991) reported that developing ovaries of white sturgeon from San Francisco Bay contained as much as 71.8 µg/g selenium or 7-times the threshold for reproductive toxicity in fish (Lemly 1996a, 1996b) of 10 µg/g. An effect threshold in white sturgeon eggs has been estimated to be between 9 µg/g and about 16 µg/g in experiments in which seleno-L-methionine was injected into yolk sac larvae of white sturgeon (Linares *et al*. 2004). Linville (2006) showed that significant developmental defects and mortality occurred in white sturgeon eggs at a threshold of around 11–15 µg/g selenium. A hazard threshold of around 3–8 µg/g in developing white sturgeon was suggested by Linville (2006).

Sampling of pallid sturgeon (*Scaphirhynchus al*bus) in the Missouri River system suggests that normal selenium levels in sturgeon eggs are 2-3 µg/g (Ruelle and Keenlyne 1993) as has been found for many other fish species (see review in Skorupa *et al*. 1996 and in USDI-BOR/FWS/GS/BIA 1998). Thus, white sturgeon in the San Francisco Bay estuary are producing eggs with as much as 35-times normal selenium content. Based on studies regarding toxicity response functions for avian and fish eggs (e.g., Lemly 1996a, 1996b; Skorupa *et al.* 1996;

USDI-BOR/FWS/GS/BIA 1998) and assuming that sturgeon are as sensitive to selenium as birds and other fish, it is highly probable that these fish are reproductively impaired due to selenium exposure. For example, bluegill embryos resulting from ovaries containing 38.6 µg/g selenium exhibited 65 percent mortality (Gillespie and Bauman 1986).

Considering the high bioaccumulation efficiency of Asian clams and their importance in the diet of white sturgeon any selenium reaching the estuary from upstream sources likely contributes to the exposure risk of white sturgeon. As selenium loads to the San Joaquin River and hence to the estuary are reduced over time due to implementation of selenium total maximum daily load limits and the Grassland Bypass Project, potential impacts to sturgeon due to delivery of water to the San Luis Unit should diminish.

Delta smelt (*Hypomesus transpacificus*)

Status: Delta smelt were federally listed as a threatened species on March 5, 1993, (58 FR 12854). The Service completed a 5-year review in March 2003 (USFWS 2003) and recommended no change in its listing status; however, there has been a recent dramatic decline in Delta smelt numbers since 2005.

Life History: Delta smelt of all sizes are found in the main channels of the Delta and Suisun Marsh and the open waters of Suisun Bay where the waters are well oxygenated and temperatures relatively cool, usually less than 20˚-22˚ C in summer. When not spawning, they tend to be concentrated near the zone where incoming salt water mixes with out flowing freshwater (mixing zone). This area has the highest primary productivity and is where zooplankton populations (on which delta smelt feed) are usually most dense (Knutson and Orsi 1983; Orsi and Mecum 1986). At all life stages delta smelt are found in greatest abundance in the top two meters of the water column and usually not in close association with the shoreline.

Delta smelt inhabit open, surface waters of the Delta and Suisun Bay. In most years, spawning occurs in shallow water habitats in the Delta. Shortly before spawning, adult smelt migrate upstream from the brackish-water habitat associated with the mixing zone to disperse widely into river channels and tidally-influenced backwater sloughs (Radtke 1966; Moyle 1976, 2002; Wang 1991). Some spawning probably occurs in shallow water habitats in Suisun Bay and Suisun Marsh during wetter years (Sweetnam 1999 and Wang 1991). Spawning has also been recorded in Montezuma Slough near Suisun Bay (Wang 1986) and also may occur in Suisun Slough in Suisun Marsh (P. Moyle, UCD, unpublished data).

The spawning season varies from year to year, and may occur from late winter (December) to early summer (July). Pre-spawning adults are found in Suisun Bay and the western delta as early as September (DWR and USDI 1994). Moyle (1976, 2002) collected gravid adults from December to April, although ripe delta smelt were common in February and March. In 1989 and 1990, Wang (1991) estimated that spawning had taken place from mid-February to late June or early July, with peak spawning occurring in late April and early May.

Delta smelt spawn in shallow, fresh, or slightly brackish water upstream of the mixing zone (Wang 1991). Most spawning occurs in tidally-influenced backwater sloughs and channel edgewaters (Moyle 1976, 2002; Wang 1986, 1991; Moyle *et al*. 1992). Laboratory observations have indicated that delta smelt are broadcast spawners (DWR and USDI 1994) and eggs are demersal (sink to the bottom) and adhesive, sticking to hard substrates such as: rock, gravel, tree roots or submerged branches, and submerged vegetation (Moyle 1976, 2002; Wang 1986). Growth of newly-hatched delta smelt is rapid and juvenile fish are 40-50 mm long by early August (Erkkila *et al.* 1950; Ganssle 1966; Radtke 1966). By this time, young-of-year fish dominate trawl catches of delta smelt, and adults become rare. Delta smelt reach 55-70 mm standard length in 7-9 months (Moyle 1976, 2002). Growth during the next 3 months slows down considerably (only 3-9 mm total), presumably because most of the energy ingested is being directed towards gonadal development (Erkkila *et al*. 1950; Radtke 1966). There is no correlation between size and fecundity, and females between 59-70 mm standard lengths lay 1,200 to 2,600 eggs (Moyle *et al.* 1992). The abrupt change from a single-age, adult cohort during spawning in spring to a population dominated by juveniles in summer suggests strongly that most adults die after they spawn (Radtke 1966 and Moyle 1976, 2002). However, in El Nino years when temperatures rise above 18˚ C before all adults have spawned, some fraction of the unspawned population may also hold over as two-year-old fish and spawn in the subsequent year. These two-year-old adults may enhance reproductive success in years following El Nino events.

In a near-annual fish like delta smelt, a strong relationship would be expected between number of spawners present in one year and number of recruits to the population the following year. Instead, the stock-recruit relationship for delta smelt is weak, accounting for about a quarter of the variability in recruitment (Sweetnam and Stevens 1993). This relationship does indicate, however, that factors affecting numbers of spawning adults (*e.g.*, entrainment, toxics, and predation) can have an effect on delta smelt numbers the following year.

Risk of selenium exposure: The Recovery Plan for the Sacramento/San Joaquin Delta Native Fishes (USFWS 1996) states that Delta Smelt are ecologically similar to larval and juvenile Striped Bass (*Morone saxitilis*). Saiki and Palawski (1990) sampled juvenile striped bass in the San Joaquin River system including three sites in the San Francisco Bay estuary. Striped Bass from the estuary contained up to 3.3 µg/g whole-body selenium, a value just below Lemly's 4 µg/g toxicity threshold, even though waterborne selenium typically averages <1 µg/L (ppb) and has been measured no higher than 2.7 µg/L (ppb) within the estuary (Pease *et al*. 1992). Striped Bass collected from Mud Slough in 1986, when the annual median selenium concentration in water was 8 µg/L (ppb) (Steensen *et al*. 1997), contained up to 7.9 µg/g whole-body selenium and averaged 6.9 µg/g whole-body selenium.

Delta smelt, salvaged from the Chipps Island area during the springs of 1993 and 1994, had whole-body selenium concentrations of 1.5 µg/g dw (n=41, range 0.7 - 2.3 µg/g) (Bennett *et al.* 2001). Delta Smelt spawning sites are almost entirely restricted to the north-Delta channels associated with the selenium-normal Sacramento River and are nearly absent from the south-Delta channels associated with the selenium-contaminated San Joaquin River (USFWS 1996). Therefore, Delta smelt would appear to be at low risk to selenium exposure.

Sacramento splittail (*Pogonichthys macrolepidotus*)

Status: The Sacramento splittail was listed as threatened on February 8, 1999 (FR 64:5963). The listing was challenged in Federal District Court, and rescinded on September 22, 2003 (FR 68:55139). However, they remain a species of concern and are included in the report.

Sacramento splittail are endemic to certain waterways in California's Central Valley, where they were once widely distributed (Moyle 1976, Moyle 2002). Sacramento splittail currently occur in Suisun Bay, Suisun Marsh, the San Francisco Bay-Sacramento-San Joaquin River Estuary (Estuary), the Estuary's tributaries (primarily the Sacramento and San Joaquin rivers), the Cosumnes River, the Napa River and Marsh, and the Petaluma River and Marsh.

General life history: Splittail are relatively long-lived (about 5-7 years) and are highly fecund (up to 100,000 eggs per female). Their populations fluctuate on an annual basis depending on spawning success and strength of the year class (Daniels and Moyle 1983). Both male and female splittail mature by the end of their second year (Daniels and Moyle 1983), although occasionally males may mature by the end of their first year and females by the end of their third year (Caywood 1974). Fish are about 180-200 millimeters (7-8 inches) standard length when they attain sexual maturity (Daniels and Moyle 1983), and the sex ratio among mature individuals is 1:1 (Caywood 1974).

There is some variability in the reproductive period, with older fish reproducing first, followed by younger fish that tend to reproduce later in the season (Caywood 1974). Generally, gonadal development is initiated by fall, with a concomitant decrease in somatic growth (Daniels and Moyle 1983). By April, ovaries reach peak maturity and account for approximately 18 percent of the body weight. The onset of spawning seems to be associated with increasing water temperature and day length and occurs between early March and May in the upper Delta (Caywood 1974). However, Wang (1986) found that in the tidal freshwater and euryhaline habitats of the Sacramento-San Joaquin estuary, spawning occurs by late January and early February and continues through July. Spawning times are also indicated by the salvage records from the SWP pumps. Adults are captured most frequently in January through April, when they are presumably engaged in spawning movements, while young-of-year are captured most abundantly in May through July (Meng 1993). These records indicate most spawning takes place from February through April.

Splittail spawn on submerged vegetation in flooded areas. Spawning occurs in the lower reaches of rivers (Caywood 1974), dead-end sloughs (Moyle 1976) and in the larger sloughs such as Montezuma Slough (Wang 1986). Larvae remain in the shallow, weedy areas inshore in close proximity to the spawning sites and move into the deeper offshore habitat as they mature (Wang 1986).

Strong year classes have been produced even when adult numbers are low, if outflow is high in early spring (e.g., 1982, 1986). Since 1988, recruitment has been consistently lower than expected, suggesting this relationship may be breaking down (Meng 1993). For example, both 1978 and 1993 were wet years following drought years, yet the young-of-year abundance in 1993 was only 2 percent of the abundance in 1978.

Risk of selenium exposure: Like white sturgeon, splittail are likely to be relatively vulnerable to selenium contamination because of their estuarine habitat, bottom-feeding habits, and high bioaccumulation rates of Asian clams. The Asian clam and other mollusks constituted 34 percent of the splittail diet (Feyrer and Matern 2000, Feyrer *et al*. 2003).

The median selenium liver level in splittail from the Suisun Bay area of the estuary was about 13 μg/g dw (Stewart *et al*. 2004) while background liver concentrations in fish are generally less than 5 μg/g (USDI-BOR/FWS/GS/BIA 1998). Deformities typical of Se exposure have been seen in splittail collected from Suisun Bay (Stewart *et al*. 2004). Teh *et al*. (2004) found that juvenile splittail are impacted (liver lesions) by chronic exposure (nine months) to a diet of 6.6 μg/g selenium.

In 1998, an above normal rainfall year type, splittail were collected from Mud and Salt Sloughs within the San Luis National Wildlife Refuge during quarterly fish sampling for the Grassland Bypass Project (GBP)(Beckon *et al*. 1999). This was the only time in the 14 year life of the project (1993-2007) that splittail were documented in these two sloughs. Selenium levels in splittail composite whole-body samples at the three Mud Slough sites were all above the GBP concern threshold of 4 μg/g dw with the site immediately downstream of the San Luis Drain having 7.1 μg/g dw (Beckon *et al*. 1999). At Salt Slough where drainwater no longer is discharged into the slough the splittail whole-body composite concentration was 3.1 μg/g dw (Beckon *et al*. 1999).

Considering the high bioaccumulation efficiency of Asian clams and their importance in the diet of splittail any selenium reaching the estuary from upstream sources likely contributes to the exposure risk of splittail. As selenium loads to the San Joaquin River and hence to the estuary are reduced over time due to implementation of selenium total maximum daily load limits and the Grassland Bypass Project, potential impacts to splittail due to delivery of water to the San Luis Unit should diminish.

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PART 1180–RAILROAD ACQUISITION, CONTROL, MERGER, CONSOLIDATION PROJECT, TRACKAGE RIGHTS, AND LEASE PROCEDURES

■ 8. The authority citation for part 1180 continues to read as follows:

Authority: 5 U.S.C. 553 and 559; 11 U.S.C. 1172; 49 U.S.C. 721, 10502, 11323–11325.

■ 9. Revise § 1180.4(g)(2)(i) and (g)(2)(ii) to read as follows:

§ 1180.4 Procedures.

* * * * *

(g) * * *

* * * * *

(2)(i) To qualify for an exemption under § 1180.2(d)(7) (acquisition or renewal of trackage rights agreements), in addition to the notice, the railroad must file a caption summary suitable for publication in the **Federal Register.** The caption summary must be in the following form:

Surface Transportation Board

Notice of Exemption

Finance Docket No.

(1)—Trackage Rights—(2)

(2) (3) to grant (4) trackage rights to (1) between (5). The trackage rights will be effective on (6).

This notice is filed under § 1180.2(d)(7). Petitions to revoke the exemption under 49 U.S.C. 10502(d) may be filed at any time. The filing of a petition to revoke will not stay the transaction.

Dated:

By the Board.

[Insert name],

Director, Office of Proceedings.

The following key identifies the information symbolized in the summary.

(1) Name of the tenant railroad.

(2) Name of the landlord railroad.

(3) If an agreement has been entered use "has agreed", but if an agreement has been reached but not entered use "will agree."

(4) Indicate whether "overhead" or "local" trackage rights are involved.

(5) Describe the trackage rights.

(6) State the date the trackage rights agreement is proposed to be consummated.

(ii) To qualify for an exemption under § 1180.2(d)(8) (acquisition of temporary trackage rights), in addition to the notice, the railroad must file a caption summary suitable for publication in the **Federal Register.** The caption summary must be in the following form:

Surface Transportation Board

Notice of Exemption

STB Finance Docket No.

(1)—Temporary Trackage Rights—(2)

(2) (3) to grant overhead temporary trackage rights to (1) between (4). The temporary trackage rights will be effective on (5). The authorization will expire on (6).

This notice is filed under § 1180.2(d)(8). Petitions to revoke the exemption under 49 U.S.C. 10502(d) may be filed at any time. The filing of a petition to revoke will not stay the transaction.

Dated:

By the Board.

[Insert name]

Director, Office of Proceedings.

The following key identifies the information symbolized in the summary.

(1) Name of the tenant railroad.

(2) Name of the landlord railroad.

(3) If an agreement has been entered use "has agreed," but if an agreement has been reached but not entered use "will agree."

(4) Describe the temporary trackage rights.

(5) State the date the temporary trackage rights agreement is proposed to be consummated.

(6) State the date the authorization will expire (not to exceed 1 year from the date the trackage rights will become effective).

[FR Doc. 2010–13130 Filed 6–1–10; 8:45 am]

BILLING CODE 4915–01–P

DEPARTMENT OF COMMERCE

National Oceanic and Atmospheric Administration

50 CFR Part 223

[Docket No. 070910507–0037–02]

RIN 0648–AV94

Endangered and Threatened Wildlife and Plants: Final Rulemaking To Establish Take Prohibitions for the Threatened Southern Distinct Population Segment of North American Green Sturgeon

AGENCY: National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration (NOAA), Commerce.

ACTION: Final rule and notice of availability of a final environmental assessment.

SUMMARY: This final ESA section 4(d) rule represents the regulations that we, the National Marine Fisheries Service (NMFS), believe necessary and advisable to conserve the threatened Southern Distinct Population Segment of North American green sturgeon (*Acipenser medirostris;* hereafter Southern DPS). We apply the prohibitions listed under ESA section 9 for the Southern DPS, and we highlight specific categories of activities that are likely to result in take of Southern DPS fish. We do not find it necessary and advisable to apply the take prohibitions to certain categories of activities that contribute to conserving the Southern DPS. We also provide a variety of methods by which take of the Southern DPS may be authorized. This document also announces the availability of a final draft environmental assessment (EA) that analyzes the environmental impacts of promulgating the 4(d) regulations for the Southern DPS.

DATES: The effective date of this final rule is July 2, 2010.

ADDRESSES: Reference materials regarding this final rule can be obtained via the Internet at *http://www.swr.nmfs.noaa.gov* or by submitting a request to the Assistant Regional Administrator, Protected Resources Division, Southwest Region, NMFS, 501 West Ocean Blvd., Suite 4200, Long Beach, CA 90802–4213.

FOR FURTHER INFORMATION CONTACT: Melissa Neuman, NMFS, Southwest Region (562) 980–4115, or Lisa Manning, NMFS, Office of Protected Resources (301) 713–1401.

SUPPLEMENTARY INFORMATION:

Background

We determined that the Southern DPS is at risk of extinction in the foreseeable future throughout all or a significant portion of its range and listed the species as threatened under the ESA on April 7, 2006 (71 FR 17757). At that time we summarized the process for considering the application of ESA section 9 prohibitions to the threatened Southern DPS. In the case of threatened species, ESA section 4(d) states that the Secretary shall decide whether, and to what extent, to extend the ESA section 9(a) prohibitions, including those regarding take of the species, and authorizes us to issue regulations we consider necessary and advisable for the conservation of the species. Such regulations may include any or all of the prohibitions that automatically apply to endangered species. Those prohibitions, in part, make it illegal for any person subject to the jurisdiction of the United States to take the listed species. The term "take" means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to engage in any such conduct. (16 U.S.C. 1532(19)). The term "harm" is defined as any act which actually kills or injures fish or wildlife. Such an act may include significant habitat modification or degradation which actually kills or injures fish or wildlife by significantly impairing essential behavioral patterns, including breeding, spawning, rearing, migrating, feeding, or sheltering. (50 CFR 222.102).

Whether take prohibitions or other protective regulations are necessary or advisable is in large part dependent on

the biological status of the species and potential impacts of various activities on the species. Green sturgeon have persisted for millions of years through cycles of naturally occurring perturbations that have likely presented short- and long-term challenges to the species' survival. We conclude that the threatened Southern DPS of North American green sturgeon is currently at risk of extinction primarily because of human-induced "takes" involving elimination of freshwater spawning habitat, degradation of freshwater and estuarine habitat quality, water diversions, fishing, and other causes. Therefore, we conclude that extending the take prohibitions to the Southern DPS is necessary and advisable.

When the final rule to list the Southern DPS was published on April 7, 2006, we solicited the public for information that would inform the ESA section 4(d) rulemaking. Specific information requested can be found in the final rule (71 FR 17757; April 7, 2006). No substantive additional comments, beyond those that had been received during prior solicitations for information, were received.

Public scoping workshops held on May 31 and June 1, 2006, helped advance our understanding of the threats that are likely to result in the take of Southern DPS fish. In cases where evidence of direct take due to a particular activity was lacking, activities that have caused take of species that use similar habitats (*i.e.,* migratory, spawning, and rearing), consume similar prey types, have similar morphologies and/or physiologies, and/or share other life history requirements (*e.g.,* white sturgeon (*Acipenser transmontanus*) and Chinook salmon (*Oncorhynchus tshawytscha*)) were identified and considered for their effects on Southern DPS fish. More detailed justification regarding the use of take information for surrogate species (*i.e.,* one that shares a similar life history or habitat requirements) to infer the take potential of an activity on the Southern DPS fish is provided in previous **Federal Register** notices (70 FR 17386, April 6, 2005; 71 FR 17757, April 7, 2006).

On May 21, 2009, we proposed protective regulations under section 4(d) of the ESA to extend the prohibitions listed under ESA sections 9(a)(1)(A) through 9(a)(1)(G) for the threatened Southern DPS, but included certain exceptions and exemptions from the take prohibitions for activities that we have determined to be adequately protective of the Southern DPS (74 FR 23822).

Summary of Comments and Information Received in Response to the Proposed Rule and Draft Environmental Assessment

The public comment period for the proposed rule and draft Environmental Assessment (EA) was open from May 21, 2009, through July 6, 2009. During the comment period, NMFS received 7 written comments on the proposed rule and draft EA from various agencies, non-governmental organizations, and individuals. A summary of the comments and NMFS' responses to those comments are presented here.

Comments and Responses

Comment 1: One commenter requested clarification in the draft EA regarding the exception for emergency fish rescue activities under Alternative B. Specifically, the commenter was unclear what 4(d) programs were referred to in the sentence stating that "[p]roject-related activities * * * would not be considered an emergency fish rescue activity and would be subject to review under ESA section 7 or 10, or under another 4(d) program."

Response: We corrected the sentence in the final EA to read "Project-related activities * * * would not be considered an emergency fish rescue activity and would be subject to review under ESA section 7 or 10." We removed the phrase "or under another 4(d) program" because the ESA 4(d) Rule does not include a 4(d) program to cover such project-related activities.

Comment 2: One commenter stated that the draft EA needs to describe the specific categories of activities to which the take prohibitions would be applied under Alternative C.

Response: The final EA was revised to clarify that under Alternative C, the take prohibitions would apply to the same specific categories of activities and in the same areas as described under Alternative A. Those categories of activities are: Commercial, recreational, and tribal fisheries; collecting or handling Southern DPS fish for any purpose; habitat-altering activities affecting passage or spawning and rearing habitat in the Central Valley, California; operation of water diversion, dredging, and power plant activities resulting in entrainment or impingement of Southern DPS fish; application or discharge of pollutants adjacent to or within waterways occupied by Southern DPS fish; and introduction or release of non-native species adjacent to or within waterways occupied by Southern DPS fish.

Comment 3: One commenter felt that the proposed rule listed dredging as a threat to only juvenile green sturgeon and wanted NMFS to acknowledge that adult Southern DPS fish have the potential to be found in dredging areas outside the Central Valley, San Francisco Bay, Suisun Bay and San Pablo Bay.

Response: The final rule was revised to acknowledge that dredging is a potential threat to adult green sturgeon. Dredging occurs in the following areas where adults also occur: The Lower Sacramento River, Sacramento-San Joaquin Delta, Elkhorn Slough, Suisun Bay, San Pablo Bay, San Francisco Bay, Noyo Harbor, and Humboldt Bay in California; Coos Bay, Yaquina Bay, Tillamook Bay, and Nehalem Bay in Oregon; the Lower Columbia River Estuary, the Lower Columbia River, Willapa Bay, Grays Harbor, and Puget Sound in Washington; and coastal U.S. marine waters (74 FR 52300, October 9, 2009). Although adults occur in areas where dredging takes place, we don't have any direct evidence of the effect that dredging has on adult green sturgeon.

Comment 4: One commenter asked why the draft EA specifically excludes the Channel Islands from the list of areas known to be occupied by Southern DPS green sturgeon, noting that this exclusion was not mentioned in the proposed critical habitat designation for the species (73 FR 52084, September 8, 2008).

Response: At this time we do not have any data showing that Southern DPS green sturgeon occur in waters around the California Channel Islands and we specifically noted this in the description of occupied areas in the draft EA. However, the protections under the ESA 4(d) rule would apply to Southern DPS green sturgeon wherever they are found. Thus, if a Southern DPS green sturgeon occurred in the waters around the Channel Islands, the take prohibitions under the ESA 4(d) rule would apply to that fish. Because of similarity of appearance, any green sturgeon occurring in the marine environment (including estuaries in Washington, Oregon, and Humboldt Bay) would be considered the listed species as they cannot be identified as belonging to a particular DPS unless genetic samples are taken and analyzed. The final EA was revised to include a statement clarifying this.

Comment 5: Two commenters felt that the five alternative approaches need to be described in greater detail and that the geographic limitations and distinctions of the proposed rule and alternatives are not clearly laid out. Further clarification was requested.

Response: The final EA was revised to more clearly describe the geographic limitations and distinctions between the various alternatives considered.

Comment 6: One commenter recommended that NMFS consult with the Pacific Fishery Management Council (PFMC) as early in the process as possible concerning the effects of the ESA 4(d) Rule on fisheries managed under the PFMC.

Response: NMFS is currently working with the PFMC regarding the potential effects of the West Coast groundfish bottom trawl fishery on the listed Southern DPS of green sturgeon and its designated critical habitat.

Comment 7: One commenter stated that the San Francisco Bay is not used as habitat for green sturgeon and that regulating take and requiring consultation on activities that are not limiting the recovery of the Southern DPS diverts staff resources from other permitting actions that would have positive effects.

Response: The best available data for the San Francisco Bay indicate that green sturgeon are present in both Central and South San Francisco Bay, albeit in low numbers compared to other parts of the San Francisco Bay/Delta Region. The survey methods and sampling gear used in studies within San Francisco Bay were not designed to target green sturgeon, and thus the data may not be truly representative of the relative levels of green sturgeon use among the bays and the Delta. For example, given that all green sturgeon must pass through Central San Francisco Bay in their migrations to and from the ocean, it is expected that larger numbers of green sturgeon are using this area at certain times of the year. In addition, the catch data do not provide information about the distribution of juvenile green sturgeon throughout the bays and the Delta. Based on the best available information, juvenile green sturgeon are believed to distribute widely throughout the bays and Delta for feeding and rearing and be present in all months of the year. Detailed fishery-dependent data for the San Francisco Bay is provided in the final critical habitat designation (74 FR 52300, October 9, 2009).

Comment 8: One commenter strongly supports the 4(d) rule and provided the information that green sturgeon are vulnerable to selenium toxicity from feeding on the overbite clam. The commenter stated that selenium toxicity can cause reproductive failure and the threat of reduced recruitment through selenium toxicity puts additional stress on the Southern DPS population.

Response: NMFS appreciates the information provided regarding green sturgeon vulnerability to selenium toxicity. Recent studies have shown that green sturgeon are more sensitive to selenium than white sturgeon and continued monitoring of selenium levels in sediments and research on the sensitivity of green sturgeon to this and other contaminants would be supported (Kaufman *et al.,* 2008).

Comment 9: One commenter felt that including marine coastal waters as green sturgeon critical habitat is unjustified as there is no reliable data on the take of the Southern DPS in coastal waters.

Response: Comments pertaining to critical habitat were addressed in the final critical habitat designation for green sturgeon (74 FR 52300, October 9, 2009). Activities that occur in coastal marine waters that may cause take of green sturgeon include bottom trawling, disposal of dredged material, hydrokinetic projects and pollution from commercial shipping.

Comment 10: One commenter stated that sand mining operations in San Francisco and Suisun Bays are highly regulated and there is very little evidence that sand mining in the San Francisco Bay-Delta Estuary negatively impacts green sturgeon or their habitat. The commenter requested that additional exceptions be included for activities such as sand mining that pose a low risk of take.

Response: In 2006, NMFS completed formal consultation with the U.S. Corps of Engineers under section 7 of the ESA for sand mining activities in the San Francisco and Suisun Bay region. The resulting biological opinion concluded that sand mining activities were not likely to jeopardize threatened green sturgeon (NMFS, 2006). An Incidental Take Statement (that remains discretionary until a 4(d) rule has been promulgated) was included with the biological opinion that provides protection to the sand miners for the entrainment of one green sturgeon per year for each of the three sand mining companies operating in the region at the time the biological opinion was written.

Comment 11: One commenter stated that we do not have data to differentiate between Northern DPS and Southern DPS green sturgeon in fisheries bycatch, but we require a Fisheries Management and Evaluation Plan (FMEP) to include measures specifically to protect Southern DPS green sturgeon.

Response: Acknowledging the fact that we cannot tell the difference between NDPS and SDPS fish due to similarity of appearance, the FMEPs must address green sturgeon and do not require that the DPS be determined.

Comment 12: One commenter stated that the green sturgeon fishery was mismanaged and that more care should have been taken to prevent the fishery from becoming overfished.

Response: NMFS acknowledges that a lack of monitoring and directed management of the green sturgeon has likely contributed to its current threatened status. However, since the listing, academic institutions, the states, NMFS and the tribes have been conducting more comprehensive studies that focus on green sturgeon in an effort to better understand its biology, status and recovery needs. It is our hope that finalizing this 4(d) rule and enforcing the take prohibitions will further the conservation of the species and aid in its recovery.

Comment 13: One commenter provided the information that there is a new surge in the green sturgeon population in Yaquina Bay, and feels that listing green sturgeon as threatened in this area is inaccurate and unfounded.

Response: NMFS appreciates the information provided regarding observations of green sturgeon in Yaquina Bay and agrees that additional studies are needed to better understand the use of coastal estuaries (including Yaquina Bay) and coastal marine waters by both DPSs of green sturgeon. Southern DPS presence in Yaquina Bay was confirmed in 2006 by the detection of one tagged Southern DPS green sturgeon (pers. comm. with Dan Erickson, ODFW, September 3, 2008). The Southern DPS was listed based on several threats, including the concentration of spawning to one river. Each Southern DPS green sturgeon carries the listing with it wherever it goes as the listing is not limited by geographic area. We acknowledge the commenter's observations suggesting that the number of green sturgeon using Yaquina Bay has increased. *While this news is promising:* (1) We recognize that green sturgeon may experience sporadic recruitment success depending on many factors that are not well understood; and (2) this uncertainty coupled with a lack of population abundance estimates and a limited understanding of population structure has led us to adopt regulations necessary and advisable for the conservation of the Southern DPS. We will conduct periodic status reviews of both DPSs and as more information becomes available we will revise our regulations if necessary.

Comment 14: One commenter felt that the requirement that research or monitoring that involves action, permitting or funding by a Federal agency must still comply with the

requirements of ESA section 7(a)(2) negates the exception from the take prohibitions for all researchers and stated that Federal employees who can fulfill all other requirements cannot use this exception. If non-Federal studies do not need to be analyzed in order to ensure that they would not jeopardize the species, then it seems counterintuitive that Federal studies with the same requirements would create jeopardy. The commenter also felt that the requirement that the activity must comply with required state reviews or permits negates the exception because as part of the application process, state permits require a copy of the authorization from NMFS when working with species listed under the ESA.

Response: Under the 4(d) Rule, we can exempt a non-Federal entity from the take prohibitions, but cannot exempt Federal agencies from the jeopardy standard under section 7 of the ESA. Compliance with section 7(a)(2) of the ESA would be required, but the consultation would be limited to an analysis of whether the activity may jeopardize the continued existence of the species or destroy or adversely modify critical habitat, and would not involve an assessment of take. Section 7 of the ESA does not apply to non-Federal entities. Although Federal employees are still subject to the section 7 jeopardy standard, under the exception they would not be required to obtain an ESA section 10(a)(1)(A) permit for their research/monitoring activities if conducted according to the exception criteria. The Federal biologists carrying out research activities would need to obtain state permits regardless of whether Federal take prohibitions are in place or not. The exception simplifies the NMFS review and approval process for research activities and relies on the state review and permits to minimize impacts related to the research activities. In the state application, applicants will need to identify that their activities meet the exception criteria and will need to indicate that they have submitted the information to NMFS or indicate that NMFS has confirmed that their activities meet the exception criteria.

Comment 15: One commenter felt that NMFS has not taken into account the extent of the existing regulatory programs and improvement to the health of the San Francisco Bay-Delta ecosystem that has taken place over the last 30 years and stated that certain activities are already regulated under other Federal, state and local programs that directly govern activities that NMFS stated could result in the take of green sturgeon. The commenter recommended that NMFS provide exceptions from the take prohibitions for navigation channel and harbor berths dredging, dredged material placement, mineral extraction and maintenance and installation of in-water and shoreline structures. The commenter also recommended that exceptions for the small business category of construction activities be considered.

Response: NMFS acknowledges that many of the activities that may cause take of green sturgeon are already regulated by existing Federal, state and local laws and regulations, and appreciates any efforts that have been made to protect and improve habitats where green sturgeon reside. However, these laws, regulations, and programs may not specifically address green sturgeon and may not be as protective of green sturgeon as the 4(d) Rule. For example, there is a 50-year dredging program in the San Francisco Bay region that currently has not implemented measures that would specifically protect green sturgeon. Construction activities conducted by small businesses may also not include measures that would be adequately protective of green sturgeon. However, any protections already afforded to green sturgeon through existing programs would be considered in NMFS' analyses under section 7 or section 10 of the ESA.

Comment 16: One commenter requested that a public hearing be held in coastal Oregon prior to publishing the final rule.

Response: A workshop to discuss the ESA 4(d) rule prohibitions and exceptions/exemptions with state fishery management agencies, NMFS, and representatives from the fishing industry was held in Newport, Oregon on March 15, 2010.

Comment 17: One commenter requested clarification on the Protection/Conservation Measures or Benefits under Table 1, as emergency rescue and habitat restoration indicates that there are no benefits provided to green sturgeon in these activities.

Response: The Note section under Table 1 was clarified to state that the "Protective/conservation measures or benefits" column refers to whether the activity, as it is currently conducted, includes protections or benefits to green sturgeon. Emergency rescue activities and habitat restoration activities that are not conducted according to the criteria under the exceptions do not provide benefits to green sturgeon and are therefore not covered under the exceptions. If these activities may cause take of green sturgeon, that take must be covered under section 7 or 10 of the ESA, or come under compliance with the exceptions criteria.

Comment 18: One commenter requested clarification in the draft EA regarding which states' recreational fishing regulations, prior to 2006, did not differentiate between white sturgeon and green sturgeon.

Response: The final EA was revised to clarify that, prior to 2006, state recreational fishing regulations in Washington, Oregon, and California did not differentiate between white sturgeon and green sturgeon.

Comment 19: One commenter suggested updating the 2005 reference for the Environmental Water Account because the program expired in 2007 and a revised program is currently in place with adjusted water amounts to augment instream flows.

Response: The final EA was updated to remove the outdated reference for the Environmental Water Account.

Spatial Context for ESA 4(d) Rule Application

As described in a **Federal Register** notice (68 FR 4433) published on January 23, 2003, we determined that based on genetic and behavioral information, North American green sturgeon is comprised of at least two DPSs that qualify as species under the ESA: (1) A northern DPS consisting of populations originating from coastal watersheds northward of and including the Eel River ("Northern DPS"); and (2) a southern DPS consisting of populations originating from coastal watersheds south of the Eel River ("Southern DPS") and the Central Valley of California. These geographic boundaries were largely defined by genetic evidence indicating that, among samples from rivers where green sturgeon are known to spawn (*i.e.*, the Rogue, Klamath, and Sacramento rivers), the Rogue and Klamath River fish were more similar to one another than to the Sacramento River fish (Israel *et al.,* 2004). Although the Southern DPS boundaries are defined by the species' genetic structure and its likely strong homing capabilities and spawning site fidelity, the spatial extent of the ESA listing and take prohibitions for the Southern DPS is not confined to areas south of the Eel River. Detailed information on occurrences of the Southern DPS green sturgeon is provided in the proposed 4(d) rule (74 FR 23822, May 21, 2009).

Sections 10(a)(1)(A) and 10(a)(1)(B) provide exceptions to the section 9 take prohibitions. NMFS can authorize research and enhancement through section 10(a)(1)(A) permits and

incidental take through section 10(a)(1)B) permits. While this rule applies the section 9 take prohibitions to any activity that takes the Southern DPS, we wanted to determine which activities would most likely impede efforts necessary to conserve and recover the Southern DPS. To do this, we considered the following questions: (1) For which activities do we have evidence of take of Southern DPS fish; (2) for those activities where evidence of Southern DPS take does not exist, is there evidence of take of surrogate species that share similar biological requirements with Southern DPS fish; (3) are protective/conservation measures underway to reduce or minimize take imposed by some activities; and (4) are there additional protective/conservation measures that, if taken, would reduce take to low enough levels such that particular activities could proceed without appreciably reducing the likelihood of survival and recovery of the Southern DPS?

Commercial and Recreational Fisheries Activities

Take of Southern DPS fish occurs during commercial and recreational fishing activities throughout the range of North American green sturgeon. However, quantifying fishery-related take reliably and assessing its effects is challenging because: (1) Northern and Southern DPS fish are morphologically indistinguishable from one another and when green sturgeon have been taken, they have rarely been identified to the DPS level; (2) until recently some fisheries did not report green sturgeon take; and (3) in cases where data on take of green sturgeon is available, methods for estimating the total annual take by a fishery are still being developed. The two DPSs co-inhabit some coastal areas and bays in Northern California, Oregon, and Washington, and the proportion of Southern DPS fish contributing to overall populations in these areas may be high (*e.g.,* 80 percent in the Columbia River; J. Israel, UC Davis, 2008, unpublished data). Thus, while we know that fisheries-related take is occurring, we are uncertain how this take is apportioned between the two DPSs, different locales, and different types of fisheries.

Green sturgeon are taken as bycatch in white sturgeon fisheries, salmon gillnet fisheries, coastal groundfish trawl fisheries, and coastal California halibut set net fisheries (Adams *et al.,* 2006; R. Rasmussen, NMFS, 2006, unpublished data; J. Ferdinand *et al.,* NMFS, 2006, unpublished data). These fisheries have taken large numbers of green sturgeon historically and have been cited as factors in the decline of the species (70 FR 17386, April 6, 2005; 71 FR 17757, April 7, 2006). For example, from 1985 to 1993, the harvest of green sturgeon in commercial fisheries in the Columbia River and in Washington ranged from 3,000 to over 7,500 fish per year. Sport fishing harvest during the same period ranged from less than 100 to over 500 fish, with the majority harvested from the Columbia River. Since 1993, commercial and sport harvest of green sturgeon has declined in the Columbia River and Washington fisheries to about 150 fish harvested in 2003 (Adams *et al.* 2006).

State recreational and commercial fishing regulations have been revised in response to evidence of recent sturgeon declines and to the listing of the Southern DPS. In California, the California Fish and Game Commission approved revised regulations, effective March 1, 2007, to prohibit retention of green sturgeon, alter the slot (size) limit (142 cm) and bag limit (one individual daily; 3 individuals annually) for white sturgeon, and require implementation of a sturgeon report card system. Recently, the California Fish and Game Commission approved revised regulations, effective March 1, 2010, that prohibit all sturgeon fishing in the upper Sacramento River where southern DPS green sturgeon spawn. The Washington Fish and Wildlife Commission adopted a permanent rule to prohibit retention of green sturgeon in recreational fisheries statewide effective May 1, 2007. In addition, the Washington Department of Fish and Wildlife and Oregon Department of Fish and Wildlife voted to prohibit the retention of green sturgeon in Columbia River recreational fisheries from Bonneville Dam to the mouth of the river, effective January 1, 2007. For commercial fisheries, the retention of green sturgeon has been prohibited in the Columbia River by emergency rule since July 2006 and statewide in Washington by permanent rule since January 26, 2007. The Oregon Fish and Wildlife Commission voted to prohibit the retention of green sturgeon in commercial nearshore fisheries, effective January 1, 2010, and is prohibiting the retention of green sturgeon in recreational fisheries statewide, effective April 1, 2010. The State of California has prohibited commercial fishing for sturgeon since 1917. While these emergency and permanent rules offer Southern DPS fish protection, it is unclear whether the state closures will remain in effect over the long-term and ultimately what overall effect the closures will have on the Southern DPS.

Commercial groundfish trawl fisheries occurring in coastal waters along the West Coast of North America take green sturgeon. Fish are primarily caught as bycatch off the coast of California. Over a 6-year period, from 2001–2007, 450 green sturgeon were reported as by-catch in trawls off the California coast. Almost all green sturgeon caught in this fishery are released alive (J. Majewski, NMFS, 2006, unpublished data), but the long-term fate of these individuals remains unknown. A program for monitoring green sturgeon take was established with the NMFS Observer Program in January 2007 to determine the amount of take, the DPS of the green sturgeon that are caught (through genetic analysis), and in the future to address the long-term fate of these individuals through tagging. Additional measures that may be implemented to protect green sturgeon and the Southern DPS include zero retention of green sturgeon in all fisheries, minimizing incidental catch, monitoring of incidental catch, increased enforcement, fisheries closures in areas important to the species, and outreach and education on proper catch and release methods and green sturgeon conservation issues.

Tribal Fisheries

Green sturgeon are taken as bycatch in tribal salmon and sturgeon fisheries conducted by the Quinault Tribe in coastal Washington waters. Tribal harvest of green sturgeon occurs in Grays Harbor and at the mouth of tributaries, primarily the Chehalis and Humptulips rivers. The number of green sturgeon taken annually from 1985 to 2003 ranged from less than 10 to almost 200 fish (Adams *et al.,* 2006). In 2006, the Quinault Tribe implemented zero retention of green sturgeon for the Grays Harbor fishery (J. Schumacker, Quinault Indian Tribe, 2006, personal communication). A large proportion of green sturgeon caught in Grays Harbor may be Southern DPS fish, based on hydroacoustic tracking information (Lindley and Moser, 2006) and a genetic study indicating that approximately 50 percent of green sturgeon sampled in Grays Harbor belong to the Southern DPS (J. Israel and B. May, UC Davis, 2006, unpublished data).

Green sturgeon are also taken, though rarely, in tribal commercial and subsistence salmon fisheries occurring in freshwater and coastal marine waters of Washington, including the Strait of Juan de Fuca, Georgia and Rosario straits, and Puget Sound (W. Beattie, NW Indian Fisheries Commission, 2008, personal communication). The Yurok

and Hoopa Tribes harvest green sturgeon in the Klamath River in California, but most of the fish are believed to be Northern DPS green sturgeon (J. Israel, UC Davis, 2006, unpublished data). Overall, the take of green sturgeon in tribal fisheries has been low compared to non-tribal fisheries. Measures that may be implemented to conserve the Southern DPS include a commitment by the Quinault Tribe, and perhaps other Tribes within the occupied range of the Southern DPS, to minimize take and monitor incidental catch of green sturgeon over the long-term.

Poaching

Poaching is a potential threat to the Southern DPS. In recent years, several arrests have been made for illegal harvest of white sturgeon for their meat and roe from the Sacramento River (CDFG, 2003 and 2006), the Sacramento-San Joaquin Delta (CDFG, 2004), and the lower Columbia River (Cohen, 1997). In the lower Columbia River, an estimated 2,000 sturgeon were killed over a 5-year period by poachers to produce caviar (Cohen, 1997). Poaching may be less significant than incidental take associated with white sturgeon sportfishing (Williamson, 2003). However, the tendency for green sturgeon to form aggregations for long periods of time may make them easy targets for poachers (Erickson *et al.,* 2002). Increased public outreach and awareness, increased enforcement, and heavier sentences and fines for poachers may help to protect green sturgeon from the threats of poaching.

Research and Monitoring Activities

Scientific research and monitoring of the Southern DPS contributes valuable information for the management, conservation, and future status reviews of the species. However, collection or handling associated with scientific research and monitoring constitutes take and may result in stress, injuries, or mortality of Southern DPS fish. In recent years, much research and monitoring effort has been placed on: (1) Tracking the movements and habitat use of Southern DPS fish by using a variety of non-lethal tagging techniques; and (2) identifying the DPS of origin using non-lethal genetic sampling techniques. These two research and monitoring activities provide information crucial to the development of an effective recovery strategy for the species. The best available information indicates that these procedures, when done according to accepted protocols, result in minimal short-term stress to the fish and do not result in lethal take. Important scientific information (*e.g.,* genetic, pathologic, taxonomic, meristic) is also gathered from already dead individuals, thereby providing valuable data without putting the species at further risk.

Emergency Rescue and Salvage Activities

Emergency fish rescue activities, including aiding sick, injured, or stranded fish, disposing of dead fish, or salvaging dead fish for use in scientific studies, are forms of take. Rescue activities would benefit the Southern DPS in the event of emergency situations that result from natural disasters, man-made habitat alterations, national defense activities, security emergencies, etc. Allowing take of the Southern DPS for emergency rescue and salvage activities is likely to enhance survival and recovery of the listed species. However, it is important that measures be taken to investigate emergency events during or after they have occurred in order to determine whether a non-ESA-compliant action(s) necessitated the rescue or salvage.

Habitat-Altering Activities

Dams and water diversion structures have caused the elimination, obstruction, or delay of passage for green sturgeon and other sturgeon species and may reduce body condition and reproductive success. For example, dams and water diversion structures have been observed to obstruct or disrupt the upstream spawning migrations of shortnose sturgeon in the lower Cape Fear River, NC (Moser and Ross, 1995). White sturgeon have also been found stranded behind the Fremont Weir in the Yolo Bypass, CA (Harrell and Sommer, 2006). Disruptions in migration may cause fish to stop their upstream migration or may delay access to spawning habitats (Moser and Ross, 1995). The inability to reach spawning habitats may cause fish to spawn in habitats of lower quality, resulting in decreased recruitment (Cooke and Leach, 2004). Several dams and water diversion structures exist along the spawning migration route of the Southern DPS and would be expected to have detrimental effects similar to those observed in surrogate species. Fish passage studies at the Red Bluff Diversion Dam (RBDD) in the Sacramento River show that the RBDD blocks the upstream migration of the Southern DPS when the gates are lowered between May 15 and September 15 (Heublein *et al.,* 2006; Brown, 2007). Mitigation measures have been implemented, including the raising of RBDD gates from September 15 to June 15 each year to allow fish passage and the protection and restoration of spawning and rearing habitat along the Sacramento River, bays, and the Sacramento-San Joaquin Delta. However, when the gates are raised, green sturgeon may become disoriented or suffer injuries due to the high velocity of water passing under the gates (M. Tucker, NMFS, 2007, personal communication). Between May 18 and June 10, 2007, carcasses of 10 adult Southern DPS fish (168–226 cm total length) were found at (n=2) or downstream (n=8) of RBDD (E. Campbell, USFWS, 2007, unpublished data). Locations of the retrieved carcasses and necropsy results suggest that the fish suffered mortality due to injuries inflicted by the gates at RBDD. Installation of adequate fish passage facilities, modification of existing passage facilities, or other provisions to specifically aid sturgeon passage at dams and diversions, and application of other mitigation measures, such as salvage operations, would contribute to the protection of the Southern DPS.

The elimination, obstruction, or delay of downstream passage is a concern for larval and juvenile stages of the Southern DPS, as are habitat-altering activities that destroy, modify, or curtail spawning or rearing habitats for egg, larval, or juvenile stages. Specific concerns include, but are not limited to: Increased sediment input or runoff into streams; filling in or isolation of stream channels, side channels, and intermittent waters; direct removal or alteration of physical structures; and obstruction of downstream migration.

Increased input or runoff of fine sediments into streams may result from a number of activities including, but not limited to, mining, logging, farming, grazing, and bridge and road construction. Increased erosion and sediment input or runoff into streams caused by land use and other human activities have been found to reduce the survival and successful development of eggs and embryos of salmon and other fish species (Scrivener and Brownlee, 1989; Owen *et al.,* 2005). The effects on green sturgeon eggs and embryos are likely to be similar. Green sturgeon eggs are large and dense and likely sink into rock crevices or attach to hard surfaces (Deng *et al.,* 2002; Kynard *et al.,* 2005). Once hatched, green sturgeon embryos remain near the bottom and use rocks as cover (Kynard *et al.,* 2005). Excess fine sediments can compromise successful development by burying already-deposited eggs, reducing interstitial dissolved oxygen available for eggs (Scrivener and Brownlee, 1989), or filling areas used by embryos for cover. Thus, Southern DPS eggs or embryos

may be taken due to habitat-altering activities that increase input of fine sediments or runoff into spawning or rearing habitat. The effect that increased input of fine sediments or runoff has at the individual, population and species levels will depend on the temporal and spatial extent of habitat change. The only way to determine this is to analyze particular activities on a case-by-case basis.

The filling in or isolation of stream channels, side channels, and intermittent waters may destroy or block access to rearing habitats, or impede or delay downstream migration by trapping larvae and juveniles that have entered these areas. Activities that fill in or isolate waters include, but are not limited to, the installation of tide gates, culverts, and debris- or sediment-trapping road crossing structures. These activities and their effects are a concern for listed salmon and steelhead and may also affect larval and juvenile Southern DPS fish. However, we currently lack the information needed to quantitatively assess these effects. Although relatively large numbers of juveniles have been collected in shallow areas of the Santa Clara shoal in the Sacramento-San Joaquin Delta (Radtke, 1966), the use of stream channels, side channels, and intermittent waters as rearing habitat by green sturgeon larvae and juveniles has not been documented. Information regarding the use of these habitats by early life stages of green sturgeon is needed.

Direct removal or alteration of physical structures essential to the integrity and function of the Southern DPS's spawning or rearing habitat, including rocks, soil, gravel, and vegetation, may adversely affect the growth and survival of larvae and juveniles. Green sturgeon likely use specific substrate types at different life stages, but observations of early life stages of green sturgeon in the field are lacking. Studies suggest that spawning most likely occurs over cobble substrates that provide crevices and cover for eggs (Kynard *et al.,* 2005; Nguyen and Crocker, 2006). However, in a laboratory study of substrate use by post-hatch larval green sturgeon, growth and survival was greatest in flat slate-rock substrates that provided cover and sufficient foraging opportunities (Nguyen and Crocker, 2006). Survival was low in cobble substrates, because larvae became trapped in crevices and died; whereas in sand substrates, the cause of lower survival and growth was attributed to the ingestion of sand particles similar in size to food particles (Nguyen and Crocker, 2006). Juveniles likely use deep pool habitats with rock structure during the winter (Kynard *et al.,* 2005). Removal or alteration of these physical structures (*i.e.* cobble for spawning and egg development; flat rock for larval rearing; deep pool habitats with rock structure for juvenile rearing) may reduce spawning or rearing success rates. Additional studies regarding the use of spawning habitats by Southern DPS early life stages and the effects of removing or altering physical components of Southern DPS spawning habitat on recruitment success are encouraged.

The construction and maintenance of dams and water diversion structures may impede or delay downstream migration and alter habitats important to larval and juvenile stages of the Southern DPS. Dams and water diversions may block downstream migration of larvae and juveniles, unless fish transport or bypass facilities exist. Passage across dams and water diversion structures may also disorient or injure larvae and juveniles and make them more vulnerable to predation, as has been observed for juvenile salmonids at RBDD (Bigelow and Johnson, 1996; Gaines and Martin, 2002). The actual construction of dams and water diversion structures may cause increased erosion and sedimentation and disrupt or alter physical structures in spawning or rearing habitats, with effects as described in the previous paragraphs.

While existing laws require mining, timber harvest, and other resource use plans to address erosion and other adverse impacts on stream habitats, these laws may not be adequate to protect the Southern DPS. Additional measures that would help reduce potential adverse impacts on Southern DPS fish are: (1) Protection of riparian habitat by limiting activities that cause erosion, sediment input or runoff into streams, or roadway and other linear development near or across streams; (2) construction of fish protection and passage facilities; and (3) limiting the temporal and/or spatial scopes of habitat alteration activities that occur in and near spawning and rearing locations.

Habitat Restoration

The primary purpose of habitat restoration is to restore natural aquatic or riparian habitat conditions or processes over the long-term. Specifically, we define habitat restoration as the process of reestablishing a self-sustaining habitat that closely resembles natural conditions in terms of structure and function for the Southern DPS. A variety of habitat-altering activities such as barrier removal or modification to restore natural water flows, river and estuarine bed restoration, natural bank protection, restoration of native vegetation, removal of non-native species, and removal of contaminated sediments has been used to reestablish natural river and estuarine functions over the long-term. Although take of green sturgeon could potentially occur during the course of completing restoration activities, we do not have evidence that these types of activities have taken the Southern DPS or a surrogate species. It is likely that these activities are important to the conservation and recovery of the Southern DPS.

Entrainment and Impingement Risks

The operation of water diversions, power generating projects, and dredging activities pose entrainment and impingement threats to all life stages of the Southern DPS. We define entrainment to mean the incidental trapping of any life stage of fish within waterways or structures that carry water being diverted for anthropogenic use. We define impingement to mean the entrapment of any life stage of fish on the outer part of any structure (*e.g.,* intake structures, screening devices) that separates water traveling a natural course of passage from water that is being diverted for anthropogenic use. Unscreened water diversions number in the hundreds to thousands in the Sacramento River and the Sacramento-San Joaquin Delta (Herren and Kawasaki, 2001). Factors that determine the entrainment risk of fish at diversions include the location and size of fish. A study of fish entrainment at an unscreened diversion in the Sacramento River documented entrainment of fish ranging in size from 9 to 59 mm fork length (FL) in July 2000 and 2001 (Nobriga *et al.,* 2004). Green sturgeon were not among the species documented in the study, but Southern DPS larvae and small juveniles within the size range of 9–59 mm FL occur in the Sacramento River at that time of year and are believed to also be at risk of entrainment at unscreened diversions. Entrainment of juvenile green sturgeon has been documented at the state and Federal fish facilities in the south Sacramento-San Joaquin Delta, where fish are salvaged before they enter the pumps (Adams *et al.,* 2006). Programs to install fish screens at water diversions are being implemented and many major diversions have already been screened. Installation of fish screens, construction of bypass and other fish protection facilities (Bigelow and Johnson, 1996; Gaines and Martin, 2002), adjustments

in the timing of operations, and continuation of fish salvage operations, where applicable, would help minimize and mitigate entrainment of Southern DPS fish at water diversions.

Evidence exists for the impingement of green sturgeon in the operation of coastal power plants using cooling water intake systems, and there is a possibility that green sturgeon are also entrained at power plants. Two juvenile green sturgeon were impinged and died on cooling water intake screens at the now retired Contra Costa Plant Units 1–5 in 1978–1979 and at the Moss Landing Power Plant in 2006 (C. Raifsnider and J. Steinbeck, Tenera Environmental, 2006, personal communication). Current conservation efforts include the installation of screens to reduce entrainment, studies of fish impingement and entrainment at power plants, and laws that require the minimization of fish impingement and entrainment. Other actions that can be taken to reduce impingement and entrainment include altering the time of day when water intake pumps are operated, altering the velocity of water intake, and the use of alternative cooling systems that do not require water intake.

Dredging operations in freshwater rivers, bays, and estuaries where Southern DPS fish occur may pose entrainment risk. Although entrainment of green sturgeon in dredging operations has not been documented, the effects could be significant. Approximately 2,000 juvenile white sturgeon were entrained during operation of a large suction dredge in the lower Columbia River (Buell, 1992). Juvenile green sturgeon would be expected to face similar entrainment risks from dredging operations because they are also bottom-oriented and occur in habitats similar to white sturgeon. Dredging may also be a potential threat to adult green sturgeon because they occur in areas where dredging operations take place. Dredging stirs up the sediments causing the release of contaminants that would have adverse impacts on growth, reproductive development, and reproductive success of green sturgeon. Long-term management strategies for San Francisco Bay dredging operations have established regional environmental work windows, or periods of time when certain fish species are not likely to be present in a location. Currently, it is believed that Southern DPS juveniles reside in San Francisco, Suisun, and San Pablo bays year-round so environmental work windows will likely not be effective in reducing the risks of dredging operations to the Southern DPS in these locations (Ganssle, 1966; Miller, 1972; CDFG, 2002; Jahn, 2006; BDAT, 2009). However, the use of specific types of dredging equipment with modified designs would reduce the entrainment risk to Southern DPS fish from dredging operations.

Pesticides and Discharge of Pollutants

The application of pesticides adjacent to or within waterways that contain any life stage of the Southern DPS may adversely affect their growth and reproductive success. Several pesticides have been detected in the Sacramento River Basin at levels that are likely to be harmful to aquatic life (Domagalski *et al.,* 2000). The accumulation of industrial chemicals and pesticides such as polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethanes (DDTs), and chlordanes in white sturgeon gonad, liver, and muscle tissues affects growth and reproductive development and results in lower reproductive success (Fairey *et al.,* 1997; Foster *et al.,* 2001a; Foster *et al.,* 2001b; Kruse and Scarnecchia, 2002; Feist *et al.,* 2005; Greenfield *et al.,* 2005). Green sturgeon are believed to experience similar risks from contaminants, although their exposure may be reduced because a greater proportion of their subadult and adult lives are spent in marine waters (70 FR 17386, April 6, 2005). Pesticides may also indirectly affect green sturgeon through effects on their prey species. For example, green sturgeon are believed to enter Willapa Bay to feed on burrowing ghost shrimp (*Neotrypaea californiensis*), which have declined in abundance due to the deliberate application of carbaryl (Moser and Lindley, 2006).

The discharge or dumping of toxic chemicals or other pollutants into waters and areas where Southern DPS fish occur would be expected to reduce their growth and reproductive success. Pollutants including mercury, selenium, and arsenic have been detected in white sturgeon gonad, liver, and muscle tissues and are believed to affect growth, reproductive development, and reproductive success (Fairey *et al.,* 1997; Davis *et al.,* 2002; Kruse and Scarnecchia, 2002; Greenfield *et al.,* 2005; Webb *et al.,* 2006). Again, the effects on green sturgeon are likely to be similar.

Under the Federal Clean Water Act, acceptable levels for contaminants in waterways have been established by the States and the U.S. Environmental Protection Agency (EPA). Entities must also obtain National Pollutant Discharge Elimination System (NPDES) permits to discharge contaminants. However, NPDES permits are not required for irrigated agriculture and agricultural stormwater runoff. Furthermore, the national standards for use of pesticides and toxic substances may not be conservative enough to adequately protect the Southern DPS as was found for listed salmonids in recent draft and final jeopardy biological opinions issued by NMFS to the EPA (NMFS 1998, NMFS 2000, NMFS 2008). Thus, programs to aid agricultural producers in meeting NMFS-imposed water quality standards may be required to minimize adverse impacts on the Southern DPS.

Non-Native Species Introductions

Non-native species are a continuing problem in freshwater rivers and coastal bays and estuaries and may affect the Southern DPS through trophic interactions. Introduced species, such as striped bass in the Sacramento River and the Sacramento-San Joaquin Delta, may prey on green sturgeon juveniles. Non-native species may also replace prey species of green sturgeon and result in greater bioaccumulation of contaminants. For example, *Potamocorbula amurensis,* a non-native bivalve, has become widespread in the San Francisco Bay and the Sacramento-San Joaquin Delta and has replaced other common prey items for white sturgeon. *P. amurensis* is an efficient bioaccumulator of selenium, a reproductive toxin that causes deformities in embryos and reduced hatchability of eggs, and has been linked with increased selenium levels in white sturgeon (Linville *et al.,* 2002). *P. amurensis* has also been identified in the gut contents of at least one green sturgeon (CDFG, 2002). Non-native species may also alter the Southern DPS' habitat or compete with the Southern DPS for space or food. Although existing laws prohibit the release of non-native species into the environment, accidental and intentional introduction of non-native species remains a problem. Eradication programs for non-native species, increased public education and outreach, and increased fines or penalties for the release of non-native species would help to alleviate this problem.

4(d) Protective Regulations for the Southern DPS

We apply the prohibitions listed under ESA sections 9(a)(1)(A) through 9(a)(1)(G) for the Southern DPS, including all the ESA section 9(a)(1)(B) and 9(a)(1)(C) prohibitions (the "take prohibitions") except for specific activities described below (see Exceptions, Criteria for Exceptions, and

Reporting Requirements). ESA section 9(a)(1)(A) states that it is unlawful to import or export endangered species into or from the United States; ESA section 9(a)(1)(B) states that it is illegal to take endangered species within the United States or the territorial sea of the United States; ESA section 9(a)(1)(C) states that it is illegal to take endangered species upon the high seas; ESA section 9(a)(1)(D) states that it is illegal to possess, sell, deliver, carry, transport, or ship, by any means whatsoever, endangered species taken in violation of 9(a)(1)(B) and 9(a)(1)(C); ESA section 9(a)(1)(E) states that it is illegal to deliver, receive, carry, transport, or ship in interstate or foreign commerce by any means whatsoever and in the course of a commercial activity, endangered species; ESA section 9(a)(1)(F) states that it is illegal to sell or offer for sale in interstate or foreign commerce, endangered species; and ESA section 9(a)(1)(G) states that it is illegal to violate any regulation pertaining to endangered species or to any threatened species of fish or wildlife listed pursuant to section 4 of the ESA and promulgated by the Secretary pursuant to authority provided by the ESA.

These prohibitions are necessary and advisable for the conservation of the Southern DPS because human "take" via activities including, but not limited to, detrimental habitat alteration, modification, and curtailment; fisheries catch and bycatch; application of pesticides, toxic chemicals, or other pollutants adjacent to or within waterways; entrainment or impingement of eggs or fish during water diversion operations, dredging, or power generation; unnecessary collection or handling; and introduction of non-native species that disrupt trophic pathways, has contributed to the decline of the Southern DPS and is likely to impede its conservation and recovery. Evaluation of activities that may occur throughout the area affected by the prohibitions for Southern DPS fish, eggs or larvae is shown in Table 1.

Exceptions, Criteria for Exceptions, and Reporting Requirements

We establish exceptions to the ESA section 9(a)(1)(B) and 9(a)(1)(C) prohibitions (the "take prohibitions") for specific activities. These exceptions encompass specific activities that may be excluded from the take prohibitions for the Southern DPS through the relatively informal coordination process described below. In determining that it is necessary and advisable to not impose take prohibitions on certain activities, we are mindful that new information may require a reevaluation of that conclusion at any time. For any of the exceptions to the take prohibitions described below, we would evaluate on a regular basis the effectiveness of the activities in conserving and protecting the Southern DPS. If the activities are not effective in conserving and protecting the Southern DPS, we would identify ways in which the activities need to be altered or strengthened. For habitat-related exceptions to the take prohibitions, changes may be required if the activities are not achieving desired habitat functionality or the habitat is not supporting population productivity levels needed to conserve the Southern DPS. If the agency or entity carrying out the activity does not make changes to respond adequately to the new information, we would publish notification in the **Federal Register** announcing the intention to impose take prohibitions on those activities. Such an announcement would provide for a comment period of not less than 30 days, after which we would make a final determination whether to extend the ESA section 9(a)(1)(B) and (C) take prohibitions to the activities. If the activities do not meet the exception criteria any take must be covered under an ESA section 7 incidental take statement (*i.e.* for activities with a Federal nexus) or ESA section 10(a)(1)(B) incidental take permit. The take of the Southern DPS will not be prohibited during the course of the following activities:

(1) Federal, state, or private-sponsored research or monitoring activities if they adhere to all of the following: (a) The activity must comply with required state reviews or permits; (b) the research or monitoring activity must be directed at the Southern DPS and not be incidental to research or monitoring of another species; (c) take of live mature adults in the lower Feather River from the confluence with the Sacramento River to the Oroville Dam (rkm 116), the lower Yuba River from the confluence with the Feather River to the Daguerre Dam (rkm 19), or Suisun, San Pablo, and San Francisco Bays or the Sacramento-San Joaquin Delta from the Golden Gate Bridge up into the Sacramento River to Keswick Dam (rkm 483) may only occur from July 1 through March 1 so as to substantially increase the likelihood that uninterrupted upstream spawning migrations of adults will occur; (d) take must be non-lethal; (e) take involving the removal of any life stage of the Southern DPS from the wild must not exceed 60 minutes; (f) take must not involve artificial spawning or enhancement activities; (g) a description of the study objectives and justification, a summary of the study design and methodology, estimates of the total non-lethal take of Southern DPS fish anticipated, estimates of incidental take of other ESA listed species anticipated and proof that those takes have been authorized by NMFS or the USFWS, identification of funding sources, and a point of contact must be reported to the NMFS Southwest Regional Office (see **ADDRESSES:** above) at least 60 days prior to the start of the study, or, for ongoing studies, by August 31, 2010; (h) reports that include the total number of Southern DPS and any other ESA listed species taken, information that supports that take was non-lethal, and a summary of the project results must be submitted to NMFS on a schedule to be determined by NMFS staff; (i) research or monitoring that involves action, permitting, or funding by a Federal agency must still comply with the requirements of ESA section 7(a)(2) in order to ensure that the action will not jeopardize the continued existence of the threatened Southern DPS. NMFS will respond in a letter either confirming the activities meet the exception criteria or stating that the activities do not meet the exception criteria and are subject to the take prohibitions. The letter would acknowledge receipt of the project information and provide the schedule for submission of research/progress reports and technical assistance to clarify when the ESA section 9 prohibitions apply.

(2) Emergency fish rescue and salvage activities that include aiding sick, injured, or stranded fish, disposing of dead fish, or salvaging dead fish for use in scientific studies, if they adhere to all of the following: (a) The activity must comply with required state or other Federal reviews or permits; (b) activities may only be conducted by an employee or designee of NMFS or the U.S. Fish and Wildlife Service (USFWS), any Federal land management agency, or California Department of Fish and Game (CDFG), Oregon Department of Fish and Wildlife (ODFW), Washington Department of Fish and Wildlife (WDFW), or Alaska Department of Fish and Game (ADFG); (c) the emergency rescue must benefit the Southern DPS; (d) a report must be submitted to the NMFS Southwest Regional Office (*see* **ADDRESSES:** above) that includes, at a minimum, the number and status of fish handled, the location of rescue and/or salvage operations and the potential cause(s) of the emergency situation within 10 business days after carrying out the rescue.

(3) Habitat restoration activities, including barrier removal or

modification to restore water flows, riverine or estuarine bed restoration, natural bank stabilization, restoration of native vegetation, removal of non-native species, or removal of contaminated sediments, that reestablish self-sustaining habitats for the Southern DPS, if they adhere to all of the following: (a) Compliance with required state and Federal reviews and permits; (b) a detailed description of the restoration activity sent to the NMFS Southwest Regional Office (*see* **ADDRESSES:** above) at least 60 days prior to the start of the restoration project, or, for ongoing studies, by August 31, 2010, which includes: the geographic area affected; when activities will occur; how they will be conducted; and the severity of direct, indirect, and cumulative impacts of activities on the Southern DPS; identification of funding sources; demonstration that all state and Federal regulatory requirements have been met; a description of methods used to ensure that the likelihood of survival or recovery of the listed species is not reduced; a plan for minimizing and mitigating any adverse impacts to Southern DPS spawning or rearing habitat; an estimate of the amount of incidental take of the listed species that may occur and a description of how that estimate was made; a plan for effective monitoring and adaptive management; a pledge to use best available science and technology when conducting restoration activities; and a point of contact; (c) progress reports that include the total number of Southern DPS fish taken, information regarding whether the take was lethal or non-lethal, a summary of the status of the project, and any changes in the methods being employed, must be submitted to NMFS on a schedule to be determined by NMFS staff; (d) activities that involve action, permitting, or funding by a Federal agency must still comply with the requirements of ESA section 7(a)(2) in order to ensure that the action will not jeopardize the continued existence of the threatened Southern DPS. NMFS will respond in a letter either confirming the activities meet the exception criteria and are not subject to the take prohibitions, or stating that the activities do not meet the exception criteria and are subject to the take prohibitions and any take must be covered under an ESA section 7 incidental take statement or ESA section 10 permit. The letter would also provide the schedule for submission of progress reports and would provide technical assistance to clarify when the ESA section 9 prohibitions apply.

Exemptions Provided by NMFS-approved ESA 4(d) Programs

We provide exemptions from the take prohibitions for certain activities included within a NMFS-approved 4(d) program. Activities included in a 4(d) program would be excused from the take prohibitions for the Southern DPS through a formal NMFS 4(d) program approval process described below.

4(d) Program for Commercial and Recreational Fishery Management

Take of green sturgeon in commercial and recreational fisheries activities would be allowed if fisheries activities were conducted under approved Fisheries Management and Evaluation Plans (FMEPs). We expect that, in many cases, fisheries will have acceptably small impacts on the threatened Southern DPS as long as state fishery management programs are specifically tailored to meet certain criteria. NMFS-approved FMEPs must address limiting take of green sturgeon in order to protect the listed entity, the Southern DPS. We consider this necessary because discrimination between the non-listed Northern DPS and listed Southern DPS, via gear specificity, visual indicators, spatial distribution, etc., is not currently possible. In order for NMFS to exempt commercial or recreational fishing activities from the take prohibitions, an FMEP must: (1) Prohibit retention of green sturgeon (*i.e.,* zero bag limit); (2) set maximum incidental take levels; (3) include measures to minimize incidental take of green sturgeon (*e.g.,* temporal/spatial restrictions, size, gear); (4) provide a biologically based rationale demonstrating that the incidental take management strategy will not significantly reduce the likelihood of survival or recovery of the Southern DPS; (5) include effective monitoring and evaluation plans; (6) provide for evaluating monitoring data and making revisions to the FMEP; (7) provide for effective enforcement and education; (8) provide a timeframe for FMEP implementation; and (9) report the amount of incidental take and summarize the effectiveness of the FMEP to NMFS on a biannual basis. If we find that an FMEP meets these criteria, we will issue a letter of concurrence to the entity that sets forth the terms of the FMEP's implementation and the duties of the parties pursuant to the FMEP.

Section 9(a)(1)(B) and (a)(1)(C) take prohibitions would not apply to ongoing commercial and recreational fisheries activities until September 30, 2010 if a letter of intent to develop an FMEP addressing green sturgeon has been received by the NMFS Southwest Regional Office (*see* **ADDRESSES:** above) by July 2, 2010. The exemption will be suspended if the letter of intent is rejected without further review of an FMEP. If the letter of intent is received July 2, 2010, a draft FMEP must be received by NMFS within 6 months from the date of receipt of the letter of intent. A final FMEP must be received by NMFS within 3 months from the date of receipt of NMFS' comments on the draft FMEP. Ongoing commercial and recreational fisheries activities may continue until NMFS issues a letter of concurrence (or denial) for final FMEPs.

Once a final FMEP has been submitted to NMFS for review, NMFS will: (1) Provide a public comment period (≥30 days) before approval of new or amended FMEPs; (2) provide a letter of concurrence for approved FMEPs that specifies the implementation and reporting requirements; (3) evaluate FMEPs every 5 years and identify changes that would improve their effectiveness; and (4) provide a public comment period (≥30 days) before withdrawing approval of an FMEP.

4(d) Program for Tribal Fishery Management

Fishery harvest or other activities conducted by a tribe, tribal member, tribal permittee, tribal employee, or tribal agent in Willapa Bay, WA, Grays Harbor, WA, Coos Bay, OR, Winchester Bay, OR, Humboldt Bay, CA, and any other area where tribal treaty fishing occurs are eligible to obtain take authorization via the same method outlined in the NMFS final rule for authorizing take of threatened salmon and steelhead for actions under tribal resource management plans (July 10, 2000; 65 FR 42481). This method has been modified below for the Southern DPS. We consider current tribal fishing activities to have acceptably small impacts on the threatened Southern DPS, and if the tribes, either singly or jointly, develop tribal resource management plans for the Southern DPS, or incorporate the Southern DPS into existing tribal resource management plans, that current and future tribal activities are not likely to appreciably reduce the likelihood of survival and recovery of the species.

A tribe intending to exercise a tribal right to fish or undertake other resource management actions that may impact the threatened Southern DPS could create a tribal resource management plan (Tribal Plan) that would assure that those actions would not appreciably reduce the likelihood of survival and recovery of the species. Tribal Plans

should be sent to the NMFS Southwest Regional Office (*see* **ADDRESSES**). NMFS would stand ready to the maximum extent practicable to provide technical assistance to any tribe that so requests in examining impacts on the listed Southern DPS and in the development of Tribal Plans that meet tribal management responsibilities and needs. In making a determination whether a Tribal Plan will appreciably reduce the likelihood of survival and recovery of the threatened Southern DPS, the Secretary, in consultation with the tribe, would use the best available scientific and commercial data (including careful consideration of any tribal data and analysis) to determine the Tribal Plan's impact on the biological requirements of the species. The Secretary would also assess the effect of the Tribal Plan on survival and recovery in a manner consistent with tribal rights and trust responsibilities. Before making a final determination, the Secretary would seek comment from the public on his pending determination whether implementation of a Tribal Plan will appreciably reduce the likelihood of survival and recovery of the listed Southern DPS. The Secretary would publish notification in the **Federal Register** of any determination regarding a Tribal Plan and the basis for that determination.

4(d) Program for Scientific Research and Monitoring Activities

State-coordinated research activities for scientific research or enhancement purposes that do not fall into the exception category described above (*see* Exceptions, Criteria for Exceptions, and Reporting Requirements) may receive an exemption from the take prohibitions for the Southern DPS for activities included in a state-sponsored, ESA-compliant, scientific research program between state fishery agencies (*i.e.,* CDFG, ODFW, WDFW, or ADFG) and NMFS, hereafter referred to as a state 4(d) research program. Activities conducted as part of a state 4(d) research program must meet existing state and Federal laws and regulations and would include research and monitoring projects conducted by state employees or by recipients of state fishery agency-issued permits (including Federal and non-Federal entities) that directly or incidentally take Southern DPS green sturgeon. We find that in carrying out their responsibilities to manage state fisheries, state agencies conduct or sponsor research vital for improving our understanding of the status and risks facing the Southern DPS and other listed species that occur in overlapping habitat, and provide critical information for assessing the effectiveness of current and future management practices.

State 4(d) research programs have been developed and implemented in California, Oregon, and Washington for listed West coast salmon and steelhead and are consistent with ESA requirements for research-related take of these listed species. The Southern DPS would most likely be incorporated into the existing state 4(d) research programs established for listed salmon and steelhead, making use of the system already in place. Otherwise, the state would be required to prepare a program and submit it to the NMFS Southwest Regional Office (*see* **ADDRESSES:** above) for approval. NMFS may approve the program or return the program to the state agency for revision.

In general, we conclude that as long as state biologists and cooperating agencies carefully consider the benefits and risks of activities included in a state 4(d) research program, such programs would help streamline the take authorization process for researchers, state agencies, and NMFS by allowing state fishery agencies to maintain primary responsibility for coordination and oversight of research activities. Each year, researchers would be required to submit research applications to the state fishery agency preferably through the NMFS online application Web site Authorizations and Permits for Protected Species (APPS) at *https://apps.nmfs.noaa.gov. Research applications must include, at a minimum, the following information:* (1) An estimate of the total direct or incidental take of Southern DPS fish that is anticipated; (2) a description of the study design and methodology; (3) a justification for take of Southern DPS fish and the techniques to be used; and (4) a point of contact. The state agency would have access, via NMFS, to the submitted applications, evaluate and determine which projects are eligible for inclusion under the program, and approve or deny individual project applications. Once the state agency review is complete, the state agency would be required to provide for NMFS' review and approval a list of project applications approved for possible inclusion in a 4(d) research program for the coming year. After our review of the applications and follow-ups with the researchers to address concerns if necessary, we would analyze effects of the activities on the Southern DPS. Finally, we would complete the ESA section 7 consultation and NEPA documentation and issue an approval letter to the state fishery agency confirming that the research activities covered within the 4(d) research program are exempt from the ESA take prohibitions. A section 10(a)(1)(A) research or enhancement permit is not issued. Researchers have to comply with the conditions of the 4(d) research program and must submit an annual report, preferably through the NMFS online application Web site Authorizations and Permits for Protected Species (APPS) at *https://apps.nmfs.noaa.gov.* The annual report must include, for each project: (1) a summary of the number of green sturgeon taken directly or incidentally; and (2) a summary of the results of the project, in order for NMFS to evaluate the effects of the research project on the Southern DPS. We would continue to work with the state fishery agencies to ensure authorized research involving listed Southern DPS fish is both coordinated and conducted in a manner that does not jeopardize the conservation and recovery of the Southern DPS.

Section 9(a)(1)(B) and 9(a)(1)(C) take prohibitions would not apply to ongoing state-supported scientific research and enhancement activities seeking take authorization of the Southern DPS fish through a state 4(d) program, if the above information is provided to NMFS, preferably through the NMFS online application Web site Authorizations and Permits for Protected Species (APPS) at *https://apps.nmfs.noaa.gov,* during the mid-September through mid-October 2010 application period. The take prohibitions would take effect if the state 4(d) program package is rejected as insufficient or is denied. If the state 4(d) research program package is received during the mid-September to mid-October application period, ongoing state-supported scientific research activities may continue until NMFS issues a written decision of approval or denial. If approved, the state 4(d) program authorization will cover one calendar year and state supported researchers would have to renew authorizations annually during subsequent application periods.

Take Exemptions Provided By ESA Sections 7 or 10

Federally funded, authorized, or implemented activities that may require take coverage (*see* Proposed 4(d) Protective Regulations for the Southern DPS), and are not covered under Exceptions, Criteria for Exceptions, and Reporting Requirements or Exemptions Provided by NMFS-approved 4(d) Programs above, will be examined on a case-by-case basis through interagency consultation as prescribed by ESA section 7. All other activities (*i.e.,* those

not federally funded, authorized, or implemented) that may require take coverage, and are not covered under Exceptions, Criteria for Exceptions, and Reporting Requirements or Exemptions Provided by NMFS-approved 4(d) Programs above, will be examined on a case-by-case basis as prescribed by ESA section 10.

Federal, state, and private-sponsored research activities for scientific research or enhancement purposes that are not covered under Exceptions, Criteria for Exceptions, and Reporting Requirements or Exemptions Provided by NMFS-approved 4(d) Programs above, may take Southern DPS fish pursuant to the specifications of an ESA section 10 permit. Section 9(a)(1)(B) and (a)(1)(C) take prohibitions would not apply to ongoing research activities if an application for an ESA section 10 (a)(1)(A) permit is received by NMFS, preferably through the NMFS online application Web site *https://apps.nmfs.noaa.gov,* no later than November 29, 2010. The take prohibitions would take effect if the permit application is rejected as insufficient or a permit is denied. If the permit application is received by November 29, 2010, ongoing research activities may continue without take prohibitions until NMFS issues or denies a permit.

Evaluation of activities that may occur throughout the area affected by the prohibitions for Southern DPS fish, eggs, or larvae is shown in Table 1. Evidence of take of the Southern DPS during the course of an activity is indicated; if there is no such evidence, then evidence of take of a surrogate species is indicated. Existence of protective/conservation measures to minimize take of or benefit the Southern DPS fish during the course of the activity as it is currently conducted is indicated. Based on best available information, whether an activity requires take authorization or is illegal according to other laws and therefore cannot be authorized is indicated, and whether methods for allowing take resulting from a particular activity exist through ESA sections 7 or 10 or through an ESA section 4(d) Program is specified. This is not an exhaustive list of all activities that occur throughout the area affected by the take prohibitions. Please *see 4(d) Protective Regulations for the Southern DPS* for the full range of activities for which NMFS is prohibiting take.

Table 1. This table indicates whether evidence of take of the Southern DPS or take of a surrogate species exist (yes or no; Y or N) and whether protective/conservation measures to minimize take are currently in place (Y or N). The table also indicates whether under this rule an activity requires take authorization (Y or N), or cannot be authorized (N/A), and whether methods that allow take exist through ESA sections 7 or 10 (Y or N) or through an ESA section 4(d) program (Y or N)

Activity	Take	Take of surrogate species	Protective/ Conservation measures or benefits	Take authorization necessary	Methods of take authorization	
					ESA section 7 or 10	4(d) Program
Fishing						
Commercial	Y		Y	Y	Y	Y
Recreational	Y		Y	Y	Y	Y
Tribal	Y		Y	Y	Y	Y
Poaching	N	Y	N	N/A	N	N
Collection or Handling						
Research/monitoring						
Federal, State or Private-sponsored (compliant with Exceptions)	Y		Y	N		
State-sponsored (outside scope of Exceptions)	Y		Y	Y	Y	Y
Federal or Private-sponsored (outside scope of Exceptions)	Y		Y	Y	Y	N
Emergency Rescue (compliant with Exceptions)	N	Y	Y	N		
Emergency Rescue (outside scope of Exceptions)	N	Y	N	Y	Y	N
Detrimental Habitat-Altering Activities						
Activities that Eliminate, Obstruct, or Delay Passage						
Dam installation, repair, modification, operation	Y		Y	Y	Y	N
Diversion installation, repair, modification, operation	Y		Y	Y	Y	N
Activities that Destroy, Modify, or Curtail Spawning or Rearing Habitat						
Input of fine sediments/runoff	N	Y	Y	Y	Y	N
Dam installation, repair, modification, operation	Y		Y	Y	Y	N
Diversion installation, repair, modification, operation	Y		Y	Y	Y	N
Filling/isolation of channels/intermittent waters	N	N	Y	Y	Y	N
Removal/alteration of physical structure that provides spawning/rearing habitat	N	N	Y	Y	Y	N
Habitat Restoration (compliant with Exceptions)						
Barrier removal/modification to restore flows	N	N	Y	N		

Activity	Take	Take of surrogate species	Protective/ Conservation measures or benefits	Take authorization necessary	Methods of take authorization	
					ESA section 7 or 10	4(d) Program
Riverine or estuarine bed restoration	N	N	Y	N		
Natural bank protection	N	N	Y	N		
Restoration of native vegetation	N	N	Y	N		
Removal of non-native species	N	N	Y	N		
Removal of contaminated sediments	N	N	Y	N		
Habitat Restoration (outside scope of Exceptions)	N	N	N	Y	Y	N
Entrainment/Impingement						
Water diversions	Y		Y	Y	Y	N
Power generating projects	Y		Y	Y	Y	N
Dredging	N	Y	Y	Y	Y	N
Pesticide/Pollutant Discharge	N	Y	Y	Y	Y	N
Non-native Species Introductions	N	Y	Y	N/A	N	N

Under section 9(b)(1) of the ESA, people holding Southern DPS fish in captivity or in a controlled environment prior to the ESA listing are exempt from the prohibitions of section 9(a)(1)(A) and (a)(1)(G) of the ESA and would therefore also be exempt from the prohibitions of this regulation, provided that holding and any subsequent holding or use of the fish is not for commercial activity. The burden of proof that Southern DPS fish were taken prior to listing lies with the individual holding the animals. The prohibitions of this regulation would, however, apply to any progeny of Southern DPS fish taken prior to listing. Any activity involving Southern DPS fish taken pre-listing that is authorized, funded, or carried out by a Federal agency would also be subject to the consultation requirements of section 7 of the ESA.

We apply the section 9 take prohibitions to the Southern DPS, while providing exceptions for some activities (*i.e.,* some types of research/monitoring, enforcement, emergency rescue/salvage, and habitat restoration; see Exceptions, Criteria for Exceptions, and Reporting Requirements) that NMFS finds will not impede, and in most cases will promote, the conservation of the species. However, if the activity is federally funded, authorized, or implemented, it will still be subject to NMFS' review under the ESA jeopardy standard (*i.e.,* ESA section 7(a)(2)). Apart from the subset of activities defined in "Exceptions, Criteria for Exceptions, and Reporting Requirements" above, if the Southern DPS is anticipated to be taken during the course of an activity, several methods may be pursued to obtain take authorization depending on the specific circumstances of the activity. For federally funded, authorized, or implemented activities, the traditional method of seeking take coverage is through ESA section 7. For activities that are not federally funded, authorized, or implemented, take authorization may be obtained through ESA section 10, by establishing a NMFS-approved 4(d) program (*i.e.,* for commercial or recreational fishing activities or state-sponsored research outside the scope of those activities defined in Exceptions, Criteria for Exceptions, and Reporting Requirements) that adequately protects the Southern DPS, or by developing a tribal resource management plan that will not appreciably reduce the likelihood of survival and recovery of the Southern DPS (see Exemptions Provided by NMFS-approved ESA 4(d) Programs). Take of the Southern DPS due to poaching and non-native species introductions is illegal according to existing state and/or Federal laws, thus no method of take authorization is being provided for these activities.

Peer Review

In December 2004, the Office of Management and Budget (OMB) issued a Final Information Quality Bulletin for Peer Review (Peer Review Bulletin) establishing minimum peer review standards, a transparent process for public disclosure, and opportunities for public input. The Peer Review Bulletin, implemented under the Information Quality Act (Pub. L. 106 554), is intended to provide public oversight on the quality of agency information, analyses, and regulatory activities. The text of the Peer Review Bulletin was published in the **Federal Register** on January 14, 2005 (70 FR 2664). The Peer Review Bulletin requires Federal agencies to subject "influential" scientific information to peer review prior to public dissemination. Influential scientific information is defined as "information the agency reasonably can determine will have or does have a clear and substantial impact on important public policies or private sector decisions," and the Peer Review Bulletin provides agencies broad discretion in determining the appropriate process and level of peer review. The Peer Review Bulletin establishes stricter standards for the peer review of "highly influential" scientific assessments, defined as information whose "dissemination could have a potential impact of more than $500 million in any one year on either the public or private sector or that the dissemination is novel, controversial, or precedent-setting, or has significant interagency interest." We do not consider the scientific information underlying the protective regulations to constitute influential scientific information as defined in the Peer Review Bulletin. The information is not novel; similar information for listed salmonids whose range substantially overlaps with that of the Southern DPS has been used in support of protective regulations that have been in existence for a number of years. Therefore the agency expects the information to be non-controversial and have minimal impacts on important public policies or private sector decisions.

References

A complete list of the references used in this final rule is available upon request (*see* **ADDRESSES**) or via the Internet at *http://www.swr.noaa.gov.*

Classification

Regulatory Flexibility Act

This final ESA 4(d) rule has specific requirements for regulatory compliance and sets an enforceable performance standard (do not take listed fish) when conducting specific activities unless those activities are within a carefully circumscribed set of activities on which NMFS will not impose the take prohibitions. Hence, the universe of entities reasonably expected to be

directly or indirectly impacted by the prohibition is broad.

Based on the language of the 4(d) rule, as well as a review of existing section 7 consultations for the Southern DPS of green sturgeon and co-existing salmon and steelhead species, the FRFA identified the following activities that may be affected by this final rule: commercial, recreational and tribal fisheries; dams and water diversions; power production (electric services and gas distribution); crop agriculture and point source polluters (NPDES-permitted activities); habitat-altering activities; and in-water construction and dredging activities. A great deal of uncertainty exists with regard to how potentially regulated entities will attempt to avoid take of the Southern DPS. This is caused by two factors: relatively little data exist on green sturgeon abundance and behavior, and NMFS has a short history of managing the Southern DPS. In addition, the spatial distribution of the Southern DPS overlaps nearly entirely with habitat for salmon and steelhead species. Several key variables, such as whether current fish passage facilities and fish screens designed to protect salmon species will be considered adequate to provide passage for the Southern DPS over the long term, remain undetermined at this time. Thus, while baseline protections are expected to be afforded to the Southern DPS on behalf of salmon and steelhead species, the degree to which incremental measures would be required for the Southern DPS has not been determined. As such, the FRFA does not provide estimates of total costs of conservation measures likely to be undertaken for the Southern DPS. Instead, the analysis characterizes potential impacts on affected industries.

In formulating this rule, we considered five alternative approaches, described in more detail in the FRFA. These are: (1) A No Action Alternative where no ESA section 9(a)(1) prohibitions or any other protective regulations are applied to the Southern DPS; (2) a Full Action Alternative where all ESA section 9(a)(1) prohibitions are applied to the Southern DPS; (3) Alternative A where the prohibitions listed under ESA section 9(a)(1)(A) and 9(a)(1)(D) through 9(a)(1)(G) are applied to the Southern DPS and the take prohibitions (ESA section 9(a)(1)(B) and 9(a)(1)(C)) are applied to specific categories of activities that either cause take of Southern DPS fish; (4) Alternative B (Proposed Action) where ESA section 9(a)(1) prohibitions are applied to the Southern DPS as in the Full Action Alternative, but with exceptions and exemptions for activities that NMFS has determined to be adequately protective of the Southern DPS; and (5) Alternative C where the ESA section 9(a)(1) prohibitions are applied as described in Alternative A, but with exceptions from the take prohibitions (ESA section 9(a)(1)(B) and 9(a)(1)(C)) for activities that NMFS has determined to be adequately protective of the Southern DPS.

The comparative analysis of the alternatives is described in more detail in the FRFA. In summary, the Full Action Alternative and Alternative B (Proposed Action) are anticipated to affect the largest number of industries, but the impacts Alternative B will have on those industries is expected to be less severe because certain activities may be allowed to continue (*e.g.,* some habitat restoration, emergency rescue, and research/monitoring activities) under this alternative. Alternatives A and C are anticipated to affect a smaller number of industries than the Full Action Alternative and Alternative B. For reasons similar to those explained above, Alternative C is expected to have a less severe impact on the affected industries than Alternative A.—The No Action Alternative will have no effect on industries.

Executive Order (E.O.) 12866—Regulatory Planning and Review

This rule has been determined to be not significant for the purposes of E.O. 12866.

E.O. 12988—Civil Justice Reform

We have determined that this final rule does not unduly burden the judicial system and meets the requirements of sections 3(a) and 3(b)(2) of E.O. 12988. We are providing protective regulations pursuant to provisions in the ESA using an existing approach that improves the clarity of the regulations and minimizes the regulatory burden of managing ESA listings while retaining the necessary and advisable protections to provide for the conservation of threatened species.

E.O. 13175—Consultation and Coordination with Indian Tribal Governments

E.O. 13175 requires that, if NMFS issues a regulation that significantly or uniquely affects the communities of Indian tribal governments and imposes substantial direct compliance costs on those communities, NMFS must consult with those governments, or the Federal Government must provide the funds necessary to pay the direct compliance costs incurred by the tribal governments. This rule may impose substantial direct compliance costs on the communities of Indian tribal governments within the range of this DPS. Accordingly, the requirements of section 5(b) and (c) of E.O. 13175 may apply to this rule. During the development of the proposed and final rules, we provided drafts of relevant sections of the 4(d) Rule to potentially affected tribes and held conference calls with potentially affected tribes to discuss the 4(d) Rule and obtain the tribes' input.

E.O. 13132—Federalism

E.O. 13132 requires agencies to take into account any federalism impacts of regulations under development. It includes specific consultation directives for situations where a regulation will preempt state law, or impose substantial direct compliance costs on state and local governments (unless required by statute). Neither of those circumstances is applicable to this rule. In fact, this notice provides mechanisms by which NMFS, in the form of 4(d) exceptions to take prohibitions, may defer to state and local governments where they provide necessary protections for the Southern DPS. Even though this rule does not have federalism implications, we requested information from appropriate State resource agencies in California, Oregon, and Washington regarding the proposed action. As subsequent issues with ESA compliance and rulemaking arise (*e.g.,* issuance of permits, critical habitat designation, recovery planning), we will continue to communicate with the States, and other affected local or regional entities, giving careful consideration to all concerns and comments received.

Paperwork Reduction Act (PRA)

Notwithstanding any other provision of the law, no person is required to respond to, nor shall any person be subject to a penalty for failure to comply with, a collection of information subject to the requirements of the PRA, unless that collection of information displays a currently valid Office of Management and Budget (OMB) Control Number.

This final rule contains collection-of-information requirements subject to the PRA, which have been submitted to OMB for review and approval. Public reporting burden per response for this collection of information is estimated to average: (1) 40 hours for development of a Fisheries Management and Evaluation Plan; (2) 20 hours for development of a Tribal Fishery Management Plan; (3) 40 hours for development of a State-sponsored scientific research program; (4) 5 hours to prepare reports on emergency rescue, salvage, or disposal of Southern DPS fish; (5) 40 hours to prepare reports on restoration activities;

and (6) 40 hours to prepare reports on Federal and private-sponsored research and monitoring. These estimates include the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. We invite comments regarding these burden estimates, or any other aspect of this data collection, including suggestions for reducing the burden, to NMFS (*see* **ADDRESSES**) and to OMB at the Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, DC 20503 (Attention: NOAA Desk Officer).

National Environmental Policy Act (NEPA)

Whenever a species is listed as threatened, the ESA requires that we shall issue such regulations as we deem necessary and advisable to provide for its conservation. Accordingly, the promulgation of ESA section 4(d) protective regulations is subject to the requirements of NEPA, and we have prepared a final Environmental Assessment (EA) analyzing the 4(d) regulations and alternatives. The EA is available upon request (*see* **ADDRESSES**), via our Web site at *http://swr.nmfs.noaa.gov,* or via the Federal eRulemaking Web site at *http://www.regulations.gov.*

E.O. 13211—Energy Supply, Distribution, or Use

E.O. 13211 requires agencies to prepare Statements of Energy Effects when undertaking certain actions. According to E.O. 13211, "significant energy action" means any action by an agency that is expected to lead to the promulgation of a final rule or regulation that is a significant regulatory action under E.O. 12866 and is likely to have a significant adverse effect on the supply, distribution, or use of energy. NMFS has determined that this rule is not a significant energy action. First, this rule is not significant under E.O. 12866. Second, this rule would not be likely to result in significant adverse effects on the supply, distribution, or use of energy, because the spatial scope of this rule overlaps with areas where protections for ESA-listed salmonids are in effect and it is likely that the modifications required for ESA-listed salmonids are similar to those that would be required for the Southern DPS. Thus, no Statement of Energy Effects is required for this rule.

List of Subjects in 50 CFR Part 223

Endangered and threatened species, Exports, Imports, Transportation.

Dated: May 25, 2010.

Eric C. Schwaab,

Assistant Administrator for Fisheries, National Marine Fisheries Service.

■ For the reasons set out in the preamble, 50 CFR part 223 is amended as follows:

PART 223—THREATENED MARINE AND ANADROMOUS SPECIES

■ 1. The authority citation for part 223 continues to read as follows:

Authority: 16 U.S.C. 1531 1543; subpart B, § 223.201–202 also issued under 16 U.S.C. 1361 *et seq.;* 16 U.S.C. 5503(d) for § 223.206(d)(9).

■ 2. In subpart B of part 223, add § 223.210 to read as follows:

§ 223.210 North American green sturgeon.

(a) *Prohibitions.* The prohibitions of section 9(a)(1)(A) through 9(a)(1)(G) of the ESA (16 U.S.C. 1538) relating to endangered species apply to the threatened Southern Distinct Population Segment (DPS) of North American green sturgeon listed in § 223.102(c)(1).

(b) *Exceptions.* Exceptions to the take prohibitions described in section 9(a)(1)(B) and (C) of the ESA (16 U.S.C. 1538(a)(1)(B) and (C)) applied in paragraph (a) of this section to the threatened Southern DPS listed in section 223.102(c) are described in the following paragraphs (b)(1) through (b)(3).

(1) Scientific Research and Monitoring Exceptions. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to ongoing or future Federal, state, or private-sponsored scientific research or monitoring activities if:

(i) The scientific research or monitoring activity complies with required state reviews or permits;

(ii) The research or monitoring activity is directed at the Southern DPS and is not incidental to research or monitoring of another species;

(iii) Take of live mature adults in the lower Feather River from the confluence with the Sacramento River to the Oroville Dam (rkm 116), the lower Yuba River from the confluence with the Feather River to the Daguerre Dam (rkm 19), or Suisun, San Pablo, and San Francisco Bays or the Sacramento-San Joaquin Delta from the Golden Gate Bridge up into the Sacramento River to Keswick Dam (rkm 483) occurs from July 1 through March 1 so as to substantially increase the likelihood that uninterrupted upstream spawning migrations of adults will occur;

(iv) Take is non-lethal;

(v) Take involving the removal of any life stage of the Southern DPS from the wild does not exceed 60 minutes;

(vi) Take does not involve artificial spawning or enhancement activities;

(vii) A description of the study objectives and justification, a summary of the study design and methodology, estimates of the total non-lethal take of Southern DPS fish anticipated, estimates of incidental take of other ESA listed species anticipated and proof that those takes have been authorized by NMFS or the USFWS, identification of funding sources, and a point of contact is reported to the NMFS Southwest Regional Office in Long Beach at least 60 days prior to the start of the study, or by August 31, 2010 for ongoing studies;

(viii) Reports that include the total number of Southern DPS and any other ESA listed species taken, information that supports that take was non-lethal, and a summary of the project results is submitted to the NMFS Southwest Regional Office in Long Beach on a schedule to be determined by NMFS; and

(ix) Research or monitoring that involves action, permitting, or funding by a Federal agency still complies with the requirements of ESA section 7(a)(2) in order to ensure that the action will not jeopardize the continued existence of the threatened Southern DPS.

(2) Enforcement Exception. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to any employee of NMFS, when the employee, acting in the course of his or her official duties, takes the Southern DPS listed in § 223.102(c)(1) without a permit, if such action is necessary for purposes of enforcing the ESA or its implementing regulations.

(3) Emergency Fish Rescue and Salvage Exceptions. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to emergency fish rescue and salvage activities that include aiding sick, injured, or stranded fish, disposing of dead fish, or salvaging dead fish for use in scientific studies, if:

(i) The activity complies with required state or other Federal reviews or permits;

(ii) The activity is conducted by an employee or designee of NMFS or the U.S. Fish and Wildlife Service (USFWS), any Federal land management agency, or California Department of Fish and Game, Oregon Department of Fish and Wildlife, Washington Department of Fish and Wildlife, or Alaska Department of Fish and Game;

(iii) The activity benefits the Southern DPS; and

(iv) Those carrying out the activity submit a report to the NMFS Southwest Regional Office in Long Beach that includes, at a minimum, the number and status of fish handled, the location of rescue and/or salvage operations, and the potential causes(s) of the emergency situation within 10 days after conducting the emergency rescue.

(4) Habitat Restoration Exceptions. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to habitat restoration activities including barrier removal or modification to restore water flows, riverine or estuarine bed restoration, natural bank stabilization, restoration of native vegetation, removal of non-native species, or removal of contaminated sediments, that reestablish self-sustaining habitats for the Southern DPS, if:

(i) The activity complies with required state and Federal reviews and permits;

(ii) Those carrying out the activity submit a detailed description of the restoration activity to the NMFS Southwest Regional Office in Long Beach at least 60 days prior to the start of the restoration project, or, for ongoing studies, by August 31, 2010, which includes: the geographic area affected; when activities will occur; how they will be conducted; and the severity of direct, indirect, and cumulative impacts of activities on the Southern DPS; identification of funding sources; demonstration that all state and Federal regulatory requirements have been met; a description of methods used to ensure that the likelihood of survival or recovery of the listed species is not reduced; a plan for minimizing and mitigating any adverse impacts to Southern DPS spawning or rearing habitat; an estimate of the amount of incidental take of the listed species that may occur and a description of how that estimate was made; a plan for effective monitoring and adaptive management; a pledge to use best available science and technology when conducting restoration activities; and a point of contact;

(iii) Those carrying out the activity submit progress reports that include the total number of Southern DPS fish taken, information regarding whether the take was lethal or non-lethal, a summary of the status of the project, and any changes in the methods being used, to the NMFS Southwest Regional Office in Long Beach on a schedule to be determined by NMFS; and

(iv) An activity that involves action, permitting, or funding by a Federal agency complies with the requirements of ESA section 7(a)(2) in order to ensure that the action will not jeopardize the continued existence of the threatened Southern DPS.

(c) *Exemptions via ESA 4(d) Program Approval.* Exemptions from the take prohibitions described in section 9(a)(1)(B) and (C) of the ESA (16 U.S.C. 1538(a)(1)(B) and (C)) applied in paragraph (a) of this section to the threatened Southern DPS listed in § 223.102(c) are described in paragraphs (c)(1) through (c)(3) of this section.

(1) Scientific Research and Monitoring Exemptions. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to ongoing or future state-sponsored scientific research or monitoring activities that are part of a NMFS-approved, ESA-compliant state 4(d) research program conducted by, or in coordination with, state fishery management agencies (California Department of Fish and Game, Oregon Department of Fish and Wildlife, Washington Department of Fish and Wildlife, or Alaska Department of Fish and Game), or as part of a monitoring and research program overseen by, or coordinated by, one of these agencies. State 4(d) research programs must meet the following criteria:

(i) Descriptions of the ongoing and future 4(d) research or monitoring activity, as described in paragraph (c)(1)(ii) of this section, must be received by the NMFS Southwest Regional Office in Long Beach during the mid-September through mid-October 2010 application period. This exception to the section 9 take prohibitions expires if the proposal is rejected as insufficient or is denied. If the state 4(d) research program package is received during the mid-September to mid-October application period, ongoing state-supported scientific research activities may continue until NMFS issues a written decision of approval or denial. If approved, the state 4(d) program authorization will cover one calendar year and state-supported researchers would have to renew authorizations annually during subsequent application periods.

(ii) Descriptions of ongoing and future state-supported research activities must include the following information and should be submitted to NMFS by the State: an estimate of total direct or incidental take; a description of the study design and methodology; a justification for take and the techniques employed; and a point of contact.

(iii) NMFS will provide written approval of a state 4(d) research program.

(iv) The State agency will provide an annual report to NMFS that, at a minimum, summarizes the number of Southern DPS green sturgeon taken directly or incidentally, and summarizes the results of the project.

(2) Fisheries Exemptions. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to fisheries activities that are conducted in accordance with a NMFS-approved Fishery Management and Evaluation Plan (FMEP). If NMFS finds that an FMEP meets the criteria listed below, a letter of concurrence which sets forth the terms of the FMEP's implementation and the duties of the parties pursuant to the FMEP, will be issued to the applicant.

(i) An FMEP must prohibit retention of green sturgeon (*i.e.,* zero bag limit); set maximum incidental take levels, include restrictions to minimize incidental take of the green sturgeon (*e.g.,* temporal/spatial restrictions, size of fish, gear used); provide a biologically based rationale demonstrating that the incidental take management strategy will not significantly reduce the likelihood of survival or recovery of the Southern DPS; include effective monitoring and evaluation plans; provide for evaluating monitoring data and making revisions to the FMEP; provide for effective enforcement and education; provide a timeframe for FMEP implementation; and report the amount of incidental take and summarize the effectiveness of the FMEP to NMFS on a biannual basis.

(ii) The ESA section 9(a)(1)(B) and (a)(1)(C) take prohibitions will not apply to ongoing commercial and recreational fisheries activities until September 30, 2010 if a letter of intent to develop an FMEP that is protective of green sturgeon has been received by NMFS by July 2, 2010. The exemption will expire if the letter of intent is rejected without further review of a FMEP. If the letter of intent is received by August 31, 2010, a draft FMEP must be received by NMFS within 6 months from the date of receipt of the letter of intent. A final FMEP must be received by NMFS within 3 months from the date of receipt of NMFS' comments on the draft FMEP. Ongoing commercial and recreational fisheries activities may continue until NMFS issues a letter of concurrence or denial for final FMEPs.

(iii) NMFS will provide a public comment period (≥30 days) before approval of new or amended FMEPs; provide a letter of concurrence for

approved FMEPs that specifies the implementation and reporting requirements; evaluate FMEPs every 5 years and identify changes that would improve their effectiveness; and provide a public comment period (≥30 days) before withdrawing approval of an FMEP.

(3) Tribal Exemptions. The prohibitions of paragraph (a) of this section relating to the threatened Southern DPS listed in § 223.102(c)(1) do not apply to fishery harvest or other activities undertaken by a tribe, tribal member, tribal permittee, tribal employee, or tribal agent in Willapa Bay, WA, Grays Harbor, WA, Coos Bay, OR, Winchester Bay, OR, Humboldt Bay, CA, and any other area where tribal treaty fishing occurs, if those activities are compliant with a tribal resource management plan (Tribal Plan), provided that the Secretary determines that implementation of such Tribal Plan will not appreciably reduce the likelihood of survival and recovery of the Southern DPS. In making that determination the Secretary shall use the best available biological data (including any tribal data and analysis) to determine the Tribal Plan's impact on the biological requirements of the species, and will assess the effect of the Tribal Plan on survival and recovery, consistent with legally enforceable tribal rights and with the Secretary's trust responsibilities to tribes.

(i) A Tribal Plan may include, but is not limited to, plans that address fishery harvest, artificial production, research, or water or land management, and may be developed by one tribe or jointly with other tribes. The Secretary will consult on a government-to-government basis with any tribe that so requests and will provide, to the maximum extent practicable, technical assistance in examining impacts on the Southern DPS as tribes develop Tribal Plans. A Tribal Plan must specify the procedures by which the tribe will enforce its provisions.

(ii) Where there exists a Federal court proceeding with continuing jurisdiction over the subject matter of a Tribal Plan, the plan may be developed and implemented within the ongoing Federal Court proceeding. In such circumstances, compliance with the Tribal Plan's terms shall be determined within that Federal Court proceeding.

(iii) The Secretary shall seek comment from the public on the Secretary's pending determination whether implementation of a Tribal Plan will appreciably reduce the likelihood of survival and recovery of the listed Southern DPS.

(iv) The Secretary shall publish notification in the **Federal Register** of any determination regarding a Tribal Plan and the basis for that determination.

(d) The exceptions of section 10 of the ESA (16 U.S.C. 1539) and other exceptions under the ESA relating to endangered species, including regulations in part 222 of this chapter II implementing such exceptions, also apply to the threatened Southern DPS of North American green sturgeon listed in § 223.102(c)(1). Federal, state, and private-sponsored research activities for scientific research or enhancement purposes that are not covered under Scientific Research and Monitoring Exceptions as described in paragraph (b)(1) of this section or Scientific Research and Monitoring Exemptions as described in paragraph (c)(1) of this section, may take Southern DPS fish pursuant to the specifications of an ESA section 10 permit. Section 9(a)(1)(B) and (a)(1)(C) take prohibitions would not apply to ongoing research activities if an application for an ESA section 10(a)(1)(A) permit is received by NMFS, preferably through the NMFS online application Web site *https://apps.nmfs.noaa.gov,* no later than November 29, 2010. The take prohibitions would take effect if the permit application is rejected as insufficient or a permit is denied. If the permit application is received by November 29, 2010, ongoing research activities may continue without take prohibitions until NMFS issues or denies a permit.

(e) *Affirmative Defense.* In connection with any action alleging a violation of the prohibitions of paragraph (a) of this section with respect to the threatened Southern DPS of North American green sturgeon listed in § 223.102(c)(1), any person claiming that his or her take is authorized via methods listed in paragraph (b) of this section shall have a defense where the person can demonstrate that the take authorization is applicable and was in force, and that the person fully complied with the take authorization requirements at the time of the alleged violation. This defense is an affirmative defense that must be raised, pleaded, and proven by the proponent. If proven, this defense will be an absolute defense to liability under section 9(a)(1)(G) of the ESA with respect to the alleged violation.

[FR Doc. 2010–13233 Filed 6–1–10; 8:45 am]

BILLING CODE 3510–22–P

DEPARTMENT OF COMMERCE

National Oceanic and Atmospheric Administration

50 CFR Part 635

RIN 0648–XW54

Atlantic Highly Migratory Species; Atlantic Bluefin Tuna Fisheries

AGENCY: National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration (NOAA), Commerce.

ACTION: Temporary rule; inseason General category retention limit adjustment.

SUMMARY: NMFS has determined that the Atlantic tunas General category daily Atlantic bluefin tuna (BFT) retention limit should be adjusted for the June through August 2010 time period, based on consideration of the regulatory determination criteria regarding inseason adjustments. This action applies to Atlantic tunas General category permitted vessels and Highly Migratory Species Charter/Headboat category permitted vessels (when fishing commercially for BFT).

DATES: Effective June 1, 2010, through August 31, 2010.

FOR FURTHER INFORMATION CONTACT: Sarah McLaughlin or Brad McHale, 978–281–9260.

SUPPLEMENTARY INFORMATION: Regulations implemented under the authority of the Atlantic Tunas Convention Act (16 U.S.C. 971 *et seq.*) and the Magnuson-Stevens Fishery Conservation and Management Act (Magnuson-Stevens Act; 16 U.S.C. 1801 *et seq.*) governing the harvest of BFT by persons and vessels subject to U.S. jurisdiction are found at 50 CFR part 635. Section 635.27 subdivides the U.S. BFT quota recommended by the International Commission for the Conservation of Atlantic Tunas (ICCAT) among the various domestic fishing categories, per the allocations established in the 2006 Consolidated Highly Migratory Species Fishery Management Plan (2006 Consolidated HMS FMP) (71 FR 58058, October 2, 2006).

The 2010 BFT fishing year, which is managed on a calendar-year basis and subject to an annual calendar year quota, began January 1, 2010. The General category season, which was open for the month of January 2010, resumes on June 1, 2010, and continues through December 31, 2010. Starting on June 1, the General category daily retention limit (§ 635.23(a)(2)), is

Special Study Proposal: Selenium in Sturgeon Muscle Plugs

Summary: The Regional Water Board is currently developing a selenium TMDL for the North San Francisco Bay, which will establish a target concentration in white sturgeon muscle tissue as the basis for evaluating impairment. In 2014, the RMP successfully collaborated with CDFW to non-lethally collect white sturgeon muscle tissue for selenium analysis, and a follow-up study has been approved for 2015. This study proposes a continuation of this sampling in collaboration with CDFW in 2016, with the addition of blood plasma analyses for determination of fish sex and sexual maturity.

Estimated Cost: $42,000

Oversight Group: RMP Selenium Strategy Team

Proposed by: Jennifer Sun and Jay Davis

Background

In April 2014, the RMP formed a Selenium Strategy Team to evaluate information needs that can be addressed by the Program in the next several years. The charge given to the Team by the RMP Steering Committee was to focus on low-cost, near-term monitoring elements that can provide information that provides high value in support of policy development and decision-making. A TMDL for the North Bay is in development by the Regional Water Board, with a staff report in preparation.

The TMDL will establish a target concentration in white sturgeon muscle tissue as the basis for evaluating impairment. White sturgeon is a bottom-feeding species that is considered to be at substantial risk for selenium exposure in the Bay (Beckon and Mauer 2008). White sturgeon are particularly at risk because their diet consists primarily of the overbite clam (*Corbula amurensis*), which are selenium-rich relative to other prey (Stewart et al. 2004). Other increased risk factors for sturgeon include their longevity (they can live over 100 years), their year-round resident status, and long egg maturation times (several years) (Beckon and Mauer 2008). Green sturgeon are also considered to be vulnerable to selenium but their exposure could be limited. Adults and sub-adults spend a large portion of their lives in coastal marine waters outside of the estuary, and are only briefly exposed to high selenium diet during their infrequent spawning migrations through the Bay. In addition, green sturgeon are a threatened species and fishing for them is prohibited.

White sturgeon have been routinely sampled (in 1997, 2000, 2003, 2006, 2009, and 2014) as part of RMP Status and Trends sport fish monitoring. However, the number of fish collected in

each round of sampling has been small (12 fish per round), and the collections are currently being performed on a five year cycle. The upper end of the distribution of concentrations measured in North Bay sturgeon exceeds the target under consideration for the TMDL, but this determination is based on a relatively small number of samples. Identifying a means to obtain a larger number of white sturgeon muscle samples on a more frequent basis has been identified as a high priority by the Selenium Strategy Team, both to obtain a more precise understanding of impairment and to track inter-annual trends.

In the 2009 RMP sport fish sampling, an effort began to establish a non-lethal and efficient method of collecting sturgeon muscle through the use of plugs. Concentrations in plugs were found to correlate well with concentrations in muscle fillets for the 12 fish sampled. Another round of evaluation of this correlation will occur with the 12 sturgeon to be collected in the 2014 sport fish monitoring. This correlation has opened the door to an opportunity to obtain a larger number of sturgeon muscle samples, non-lethally, through a collaboration with a California Department of Fish and Wildlife (CDFW) annual tagging program that is tracking population trends (DuBois and Harris 2013; more information at http://www.dfg.ca.gov/delta/data/sturgeon/bibliography.asp), and a US Fish and Wildlife Service (USFWS) study on fish movement patterns.

In 2014, RMP staff accompanied CDFW on three sampling dates during their fall sturgeon tagging event. Muscle plugs were successfully collected from nine fish over two days of sampling in Suisun Bay between September and October 2014. All samples were analyzed for selenium, and five samples collected in October will be analyzed for C, N, and S isotopes. This sampling event demonstrated the viability of using muscle plugs to non-lethally sample selenium concentrations in sturgeon tissues. Several improvements to the sample collection and processing methods were identified during the 2014 field season to increase the sample mass collected and optimize sample processing and analysis for low-mass samples. Continued optimization will increase the consistency and reliability of sample results obtained during future studies.

Recent results published in 2015 by Linares-Casenave et al. suggest that selenium concentrations in white sturgeon muscle tissue increases with age, and in particular may be higher in vitellogenic females. Additionally, selenium concentrations in reproductively mature females are the most relevant to understanding the reproductive impacts of selenium in white sturgeon. Sex and sexual maturity data will help both to interpret muscle plug selenium concentrations and to target muscle plug analyses towards reproductively mature females. During future muscle plug sampling events, blood plasma samples can be collected from all fish sampled and tested for testosterone, 17B-estradiol, and calcium to determine the sex and sexual maturity (Webb et al., 2002).

The reliability of blood plasma sex steroid analyses is highest immediately prior to the spawning season and in sexually mature fish, which can be roughly estimated based on fish length. However, this remains the best method for rapidly and non-invasively determining sex and sexual maturity in live fish; alternative methods require substantial technical expertise as well as

field time and equipment costs (ultrasound, endoscopy) or could potentially cause harm to live fish (laparoscopy).

RMP staff originally planned to train CDFW staff to perform sampling independently in 2014; however, due to initially difficulties with the sampling technique and logistical difficulties of freezing and storing the samples, it is not feasible for the CDFW to sample independently. CDFW staff typically do not return to their office between sampling days, crews change daily, and staff rotate between boats on different days, complicating the storage of samples and restocking of ice and other field supplies.

A follow-up muscle plug study was approved for 2015. In 2015, RMP staff is planning to collaborate with USFWS staff to collect muscle plug samples; however, USFWS staff may not be available during future CDFW cruises to assist with sampling. In 2016, RMP staff plans to be present on the CDFW boats in order to collect tissue samples directly, requiring a significant increase in field work costs.

This proposal outlines a scope and budget for collaborative plug sampling in 2016.

Study Objectives and Applicable RMP Management Questions

This objective of this study is to obtain a relatively large number of sturgeon muscle samples (30 white sturgeon) both to obtain a more precise understanding of impairment and to continue to track inter-annual trends.

Selenium Strategy questions addressed:

2. Are the beneficial uses of San Francisco Bay impaired by selenium?
4. How do selenium concentrations and loadings change over time?

RMP Management Questions addressed:

1. Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?
   B. What potential for impacts on humans and aquatic life exists due to contaminants in the Estuary ecosystem?
4. Have the concentrations, masses, and associated impacts of contaminants in the Estuary increased or decreased?
   B. What are the effects of management actions on the potential for adverse impacts on humans and aquatic life due to Bay contamination?

Approach

Up to thirty white sturgeon plugs will be collected and analyzed. Up to another 30 will be collected and archived in case additional samples are needed. Blood plasma samples will be

collected from all fish sampled for muscle plugs, and tested for testosterone, 17B-estradiol, and calcium to determine fish sex and sexual maturity. Muscle plugs chosen for immediate analysis may be informed by the results of the blood plasma analyses, and potentially targeted towards reproductively mature females

This study would be performed in collaboration with CDFW, USGS, and Bozeman Fish Technology Center. RMP staff would plan the study, perform sampling, ship the samples for laboratory analysis, manage the data, and write a brief technical report. CDFW would provide logistical support through the use of their sampling vessels - the sampling would occur during the course of the CDFW cruise in August through October.

USGS (Robin Stewart and her team) will process the plug samples and perform selenium analyses, and subsequently prepare and ship samples to UC Davis to perform C, N, and S stable isotope analyses. The stable isotopes will provide information on diet and habitat use by the sturgeon. The Bozeman Fish Technology Center will perform testosterone, 17B-estradiol, and calcium analyses on blood plasma samples.

Budget

The proposed budget for this Special Study is $42,000.

The increase in the current proposed budget relative to the budgets for the 2014 and 2015 studies primarily reflects an increase in RMP field work for sample collection, as well as an increase in analytical costs to conduct blood plasma analyses to determine fish sex and sexual maturity.

Table 1. Budget for 2016 Selenium in Sturgeon Muscle Plugs Proposal

Task	Estimated Cost
Labor*	
Project Planning & Coordination	$3,000
Field Work	$12,000
Data Management	$8,950
Reporting	$5,500
Subcontracts	
USGS - sample processing, archiving	$500
USGS - 30 selenium analyses @ $165/sample	$4,950
UCD - 30 C, N, S analyses @ $25/sample	$750
Bozeman Fish Technology Lab – 60 T and E2 analyses @ $40/sample each 60 Ca analyses @ $4/sample + $30 for calibration	$5,070
Direct Costs	
Equipment - biopsy plugs, sample containers, plasma sampling equipment, etc.	$400
Shipping – 30-60 samples to labs, 30 samples from USGS to UCD	$430
Travel - 5 days of staff travel to field site	$200
Contingency	$250
Grand Total	$42,000

*Project management, contract management, and archiving costs will be included in the RMP base funding

Reporting

A draft technical report describing the results of the study will be prepared by March 31, 2017. The technical report will be reviewed by the Selenium Strategy Team and the TRC and will be finalized by May 31, 2017.

References

Beckon, W. and T. Mauer. 2008. Species at Risk from Selenium Exposure in San Francisco Estuary. Final report to the USEPA. US Department of the Interior, Fish and Wildlife Service. http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/northsfbayselenium/Species_at_risk_FINAL.pdf

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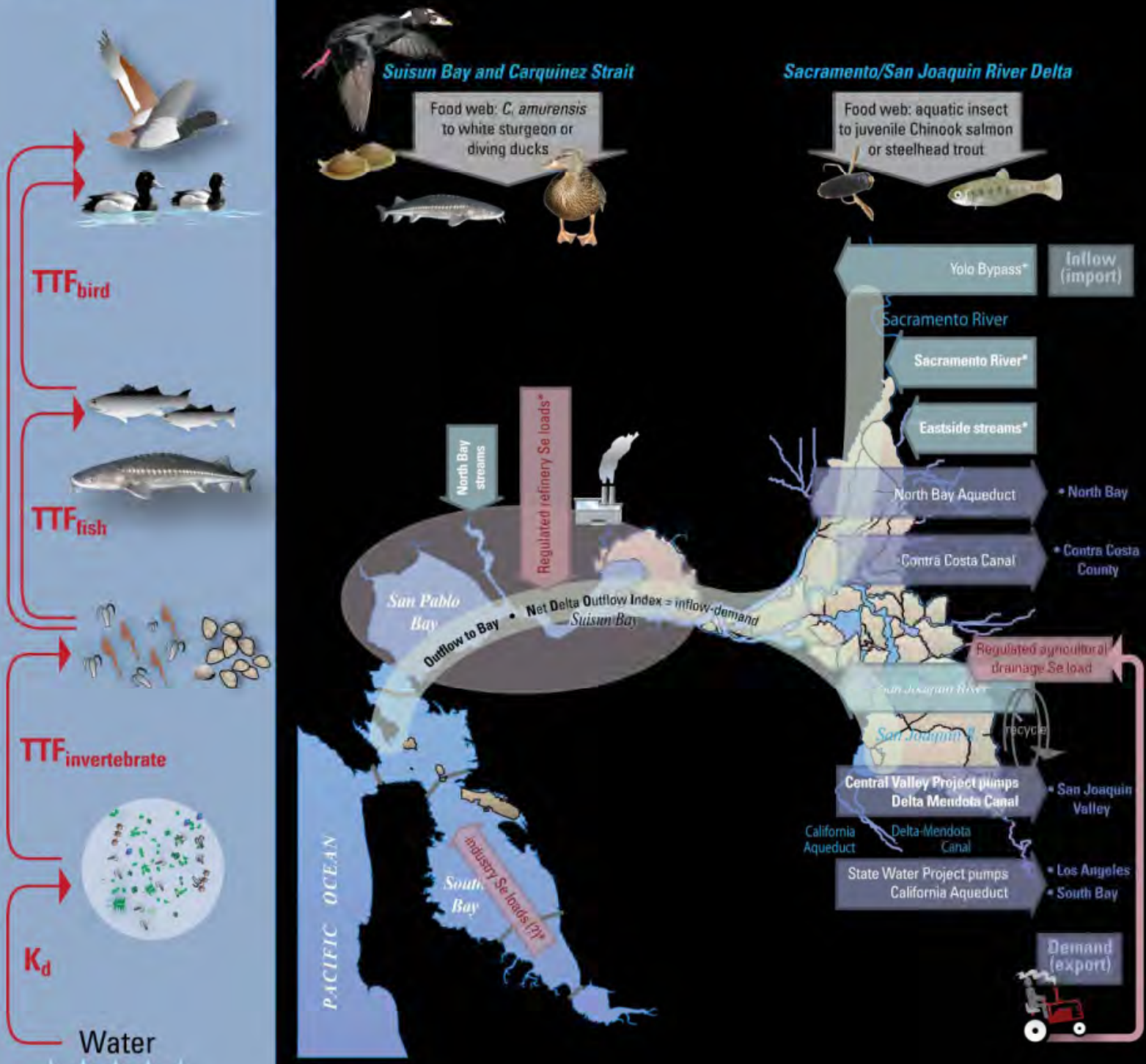
USGS
science for a changing world

Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California



U.S. Department of the Interior
U.S. Geological Survey

Administrative Report

Cover Art and Graphics: Jeanne DiLeo, USGS Office of Communications and Publishing, Menlo Park Publishing Service Center, Menlo Park, California.

Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California

By Theresa S. Presser and Samuel N. Luoma
U.S. Geological Survey, Menlo Park, California

Administrative Report

December, 2010

This work was performed with the support of U.S. Environmental Protection Agency interagency funding agreements.

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

U.S. Geological Survey, Reston, Virginia 2010

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Figures (see separate file)

Tables (see separate file)

Appendices (see separate files)

Note: A review by Coan (2002) concluded that the San Francisco Bay species *Potamocorbula amurensis* is now the genus *Corbula*, but the species name is still unclear. Because of this uncertainty, reference to the bivalve is now suggested as *Corbula* (*Potamocorbula*) *amurensis* (Thompson, 2005). However, we have used *Corbula amurensis* throughout this report.

Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California

By Theresa S. Presser and Samuel N. Luoma

Executive Summary

The San Francisco Bay-Delta Estuary (Bay-Delta) receives selenium (Se) internally from oil refinery effluents and externally through riverine agricultural discharges. Predator species considered at risk from Se (e.g., green and white sturgeon, scoter, scaup) consume the estuary's dominant bivalve, *C. amurensis*, an efficient bioaccumulator of Se. Recently proposed water-quality regulations for protection of the estuary require translating fish and wildlife tissue Se effect guidelines to dissolved Se concentrations. This change in regulatory approach requires consideration of intervening steps that 1) formally document system hydrology, biogeochemistry, biology, ecology, and ecotoxicology; and 2) quantitatively link ecosystem media (water, particulate material, and tissues of different food web species) as Se is processed through site-specific food webs. Such a methodology to predict site-specific ecological risk and derive Se criteria for the Bay-Delta would be the first regulatory action where a bioaccumulative element is managed to protect wildlife in a marine environment. Regulating seaward sites in the estuary also sets in motion consideration of upstream watershed sources.

For regulators and scientists, our approach offers an understanding that 1) diet drives protection and 2) the choice of food web and predator species is critical because the kinetics of bioaccumulation differs widely among invertebrates. Further, adequately characterizing the transformation of dissolved Se to particulate Se and the type and phase of the resulting particulate material quantifies the effect of Se speciation on both Se partitioning and Se exposure to prey through the base of the food web (i.e., particulate material to prey kinetics). Our approach also includes opportunities to analyze alternative modeling choices explicitly throughout the decision-making process.

Site-specific modeling for the Bay-Delta includes derivation of: 1) salinity-specific operationally defined factors for partitioning of Se between water and suspended particulate material (K_ds); 2) dietary biodynamic Trophic Transfer Factors (TTFs) for important food web inhabitants; 3) seasonal scenarios that illustrate hydrologic conditions, life-cycles of predator species, exposure cycles, and habitat use; and 4) species-specific effect guidelines. Effect guidelines for species at risk in the Bay-Delta were provided by the U.S. Fish and Wildlife Service (USFWS). Effect guidelines are explicit to exposure route (e.g., maternal), endpoint (e.g., hatchability) and magnitude of effect realized (EC0, EC05, and EC10) to address regulatory considerations for the U.S. Endangered Species and Migratory Bird Treaty Acts. Knowing the details of an at-risk predator's location during critical life stages for Se effects allows correlating trends in diet and exposure that occur in the estuary. Thus, our approach uses a mechanistic biodynamic basis to quantify transformation and bioaccumulation as a foundation for criteria development and site-specific data for food webs, life cycles, habitat use, and effects to set choices in modeling scenarios.

We employ both a salinity-specific transect approach, encompassing tidally-influenced sites across the Bay-Delta from near Chipps Island to the Golden Gate Bridge, and a geographically focused approach encompassing Suisun Bay and Carquinez Strait. The most recent transect data (i.e., matched datasets for dissolved and suspended particulate material) from 1997-1999 are used for modeling a seaward *C. amurensis*-based food web. Similarly, the most recent transect data from 2003-2004 are used for modeling a landward aquatic insect-based food web. Transect sampling from the 1990s represents wet and above normal years in both low flow and high flow seasons. Transect sampling from the 2000s represents above normal and below normal years in both low flow and high flow seasons.

Profiles across the estuary within a series of specified freshwater residence times (e.g., June, 1998, 11 days; November, 1999, 70 days) show the range of dissolved Se concentrations is narrowly defined as 0.070-0.320 µg/L. The profiles of suspended particulate material Se concentrations show a less narrow definition with a range of 0.15-2.2 µg/g dry weight. In the more restricted approach used for Suisun Bay-Carquinez Strait that eliminates freshwater and ocean interfaces, the range of dissolved Se concentrations is 0.076-0.215 µg/L, with the range of suspended particulate material Se concentrations as 0.15-1.0 µg/g dry weight.

K_ds are the derived ratios of dissolved and suspended particulate material Se concentrations from transect sampling across the estuary. The operational K_ds used here quantify the complex process of transformation to represent exposure and bioavailability at the base of the food web. The profiles of K_ds across the estuary illustrate the range in biogeochemical transformations and their patterns as flow conditions change. Generally, K_ds vary similarly as suspended particulate material Se concentrations across transects because of the narrowly defined range of dissolved Se concentration. Specifically, patterns during high flow conditions in April, 1999 and low flow conditions in November, 1999 are distinctly different. As residence time increases from 16 days in April to 70 days in November, the profile shape moderates and a hydrodynamic span of efficient transformation is identified. The range for the Bay-Delta continuum is 712-26,912, with mean K_ds shown to increase with increasing residence time. K_ds selected for use in modeling scenarios range from 3,198 to 7,614. The K_d range selected when the modeling location is limited to Suisun Bay-Carquinez Strait is 1,180-5,986.

The range of derived $TTF_{C.\ amurensis}$ is 14-26 for local conditions, an increase when compared to a laboratory-derived mean value of 6.25. TTF_{insect} and $TTF_{bird\ egg}$ are not site-specific, but are selected from literature values (TTF_{insect} = 2.8; $TTF_{bird\ egg}$ = 2.6). For TTF_{fish}, both a literature value of 1.1, and in the case of white sturgeon, a field-derived TTF of 0.8 are used.

Validation of the model shows the model is able to generate 1999-2000 seaward conditions for Se concentrations in a *C. amurensis* to white sturgeon food web and 2003 landward conditions for Se concentrations in an aquatic insect to largemouth bass food web. Thus, the model is able to 1) quantify transformation and biodynamics processes for the estuary and its food webs; and 2) predict that food webs dependent on *C. amurensis* are the most sensitive to Se inputs, provide the most Se exposure, and are highly vulnerable.

Modeling to protect sturgeon and clam-eating bird species is based on consumption of the clam *C. amurensis*, an invertebrate that bioaccumulates Se approximately twenty-fold that of the concentration in suspended particulate material (i.e., $TTF_{C.\ amurensis}$ = 17). Modeling to protect juvenile Chinook salmon and steelhead trout is based on consumption of aquatic insects, an invertebrate that bioaccumulates Se approximately three-fold that of the concentration in suspended particulate material (i.e., TTF_{insect} = 2.8). The model also addresses an alternative dietary preference by predators: a mix of invertebrate species (i.e., a 50% *C. amurensis* and 50% amphipod diet generates a TTF_{mixed} of 8.8).

Allowable dissolved, particulate, and prey Se concentration calculated through modeling of a specified predator species are based not only on the dietary TTF for that species (i.e., exposure), but also

on the toxicological sensitivity inherent to the predator (i.e., effects guideline provided by the USFWS for species at risk in the estuary). Hence, bioaccumulation in salmonids will be less than that in sturgeon because of dietary preference, but toxicity guidelines for salmonids are lower due to increased toxicological sensitivity. In this case, the predicted allowable dissolved Se concentration is a value that is a mathematical combination of the influences of the lower dietary TTF and the higher toxicological sensitivity.

Illustrated scenarios using a set of specific guidelines and modeling choices from the range of temporal hydrodynamic conditions, geographic locations, foodwebs, K_d, and TTFs described above, bound allowable dissolved, particulate, and prey Se concentrations. Consideration of compliance with allowed Se concentrations across media (i.e., water, particulate, prey, and predator) harmonizes regulation and is a measure of ecological consistency and relevance of the links among exposure, transfer, and effects. The specificity of these scenarios demonstrates that enough is known about the biotransfer of Se and the interconnectedness of habitats and species to set a range of limits and establish an understanding of the conditions, biological responses, and ecological risks critical to management of the Bay-Delta.

Analysis of dissolved, suspended particulate material and *C. amurensis* Se concentrations and K_ds for Suisun Bay-Carquinez Strait as a function of freshwater residence time (11, 16, 22 and 70 days) shows that critical ecological times are functionally connected to the underlying dynamics and processes of low flow periods. Transformation of dissolved Se to suspended particulate material Se (i.e., dissolved Se decreases as suspended particulate material Se concentrations increases) occurs in the estuary as flow slows down. *C. amurensis* Se concentrations also increase with increasing residence time, as does the presence of a majority of particulate organo-Se within a residence time of 22 days. Given the steepness of these curves, regulation of suspended particulate material Se concentration may be a more sensitive parameter on which to assess change and choice. Defining or conceptualizing a baseline dissolved Se concentration or condition for the estuary is less certain because of the small dynamic range of dissolved Se concentrations.

Predictions from modeling scenarios show that choices of geographic constraints, species, diet, and estuary conditions all are influential in risk management for Se. Thus, the more specificity added to the model, the less uncertainty in predictions. If, for example, the geographic range is narrowed by using data only from Suisun Bay, then freshwater and ocean interfaces are avoided. If the temporal range is narrowed to low flow seasons of dry years, then focus can be on times when the transformative nature of the estuary is elevated. Juxtaposition of times when prey species achieve maximum Se concentrations and critical life stages of species at risk are present allows focus of regulatory considerations on times that govern Se's ecological effects (i.e., *ecological bottleneck*s).

Further refinements to the approach would include consideration of: 1) contributions of Se source riverine end-members; 2) hydrodynamic relationships of riverine and internal Se sources to Se concentrations in the estuary (i.e., an Se budget through the estuary); 3) processes at the interfaces of freshwater/bay/ocean; 4) collection of current temporally and spatially matched Se datasets for water, suspended particulate material, and food web species; and 5) further linkage of ecosystem-scale modeling to fine structure estuary processes. Analysis of Se concentration and speciation for characterized particulate phases are practical measures of the complex water/sediment/particulate *milieu* that forms the base of the food web and is consumed as food by invertebrates. Hence, future monitoring to increase the suspended particulate material database under a suite of flow conditions would enhance our understanding of estuarine transformation. Monitoring invertebrate Se concentrations in food webs also is a practical, informative step in monitoring because the first and second most variable aspect of Se dynamics (i.e., K_d and $TTF_{invertebrate}$) are integrated into invertebrate bioaccumulation.

In particular for modeling of avian species, uncertainties exist around laboratory-derived biodynamic modeling parameters; movement and migration; and links of diet and tissue Se concentrations under site-specific conditions (i.e., field-derived $TTF_{bird\ egg}$). Additionally, modeling of overwintering clam-eating migratory bird species, such as scoter and scaup, based on potential chronic Se effects that may impact staging would assess these species in scenarios relevant to their use of the estuary. Chronic toxicity effects include:

- compromised body condition (low body mass);
- oxidative stress (increased susceptibility to disease as immune system is suppressed);
- decreased winter survival;
- decreased reproductive fitness (decreased breeding propensity, reduced recruitment) and;
- behavioral impairment (missed breeding window, delayed timing of departure).

Predictions from a reference dose methodology for birds also would strengthen outcomes for protection of avian species.

The methodology used here is able to document estuary and ecosystem fine-structure processes and provide the basis and context for future scenario development. The greatest strength of the analytical and modeling processes is that it is an orderly, ecologically harmonized derivation approach for assessing different choices of criteria for protection of fish and birds. Collection of modern data and additional modeling in collaboration with the final development of criteria would test if identified mechanisms and derived factors are applicable to the Bay-Delta of today. Further modeling also would provide decision-makers with additional choices based on specific questions that arise during collaborative discussions.

Introduction

Aquatic-dependant wildlife are unprotected under national aquatic life water quality criteria for Se, but these criteria are currently being revised [U.S. Environmental Protection Agency (USEPA), 1992; 2004]. National freshwater water quality Se criteria (5 µg/L chronic and 20 µg/L acute) for the protection of aquatic life are directed at protection of fish and are based on field data for effects in fish at Belews Lake (USEPA, 1987). National water quality Se criteria for the protection of marine aquatic life allow a maximum concentration of 290 µg/L and a continuous concentration of 71 µg/L, concentrations approximately an order of magnitude higher than freshwater criteria. What evidence is available from estuarine environments suggests that these guidelines are seriously under-protective for at least some predator species (Luoma et al., 1992; Presser and Luoma, 2006; Luoma and Presser, 2009).

Consideration of development of Se criteria specific to wildlife began in 1989 as an outcome of the ecological disaster at Kesterson National Wildlife Refuge, California, where aquatic birds experienced death and deformity (Presser and Ohlendorf, 1987; USEPA, 1989). The U.S. Clean Water Act (1972) provides the legal authority for deriving water quality criteria for the protection of aquatic life, wildlife, and human health. USEPA in 1985 developed methodologies for deriving water quality criteria that included protection of wildlife under determination of a Final Residue Value (FRV) (USEPA, 1985). A USEPA revision of criteria for the Great Lakes System [Great Lakes Initiative (GLI), USEPA, 1995] deleted the FRV method and applied a new methodology for contaminants and wildlife. Since that time, the GLI methodology has been applied to DDT, PCBs, and mercury on a Great Lakes-specific basis for piscivorous birds and mammals. As an outgrowth of the GLI methodology, Petersen and Nebeker (1992) proposed a freshwater waterborne Se threshold estimate for protection of aquatic-dependent birds and mammals. Skorupa and Ohlendorf (1991) proposed a range of waterborne

Se concentrations for the protection of nesting aquatic birds through use of field-derived regressions of food web and avian uptake.

Adjustments to the development of Se criteria specifically for California were called for by 1) the USEPA through the National Toxics Rule (NTR) and the California Toxics Rule (CTR) (USEPA, 1992; 2000); and 2) the USFWS and National Marine Fisheries Service (NMFS) through their Biological Opinion (USFWS and NMFS, 1998 and amended, 2000). In general, these adjustments were necessary to consider 1) the bioaccumulative nature of Se in aquatic systems; 2) Se's long-term persistence in aquatic sediments and food webs; 3) the importance of dietary pathways in determining toxicity; and 4) protection of threatened and endangered species.

Specifically, pursuant to section 7(a) of the U.S. Endangered Species Act (ESA) (1973), the USEPA consulted with the USFWS and NMFS concerning USEPA's rulemaking action for California. USEPA submitted a Biological Evaluation for their review as part of the consultation process in 1994. This evaluation found that the proposed CTR was not likely to jeopardize the continued existence of any federally listed species or result in the destruction or adverse modification of designated critical habitat. In April of 1998, the Services sent USEPA a draft Biological Opinion that found that USEPA's proposed rule would jeopardize federally listed species. After discussions with the USFWS and NMFS, the USEPA agreed to several changes in the final rule and USFWS and NMFS, in turn, issued a final Biological Opinion finding that USEPA's action would not likely jeopardize the continued existence of federally listed species. The agencies agreed that federally listed fish and wildlife species that are aquatic system foragers would be protected under future criteria and procedures for site-specific adjustments.

To achieve these goals and as part of the remedy for these problems, the USEPA initiated an interagency project with the USFWS and the U.S. Geological Survey (USGS) to address issues of 1) a methodology for translation of a tissue guidelines to protective site-specific dissolved Se concentrations (implementation of tissue criteria); 2) inclusion of protection of wildlife species (i.e., federally listed species) in regulatory methodologies; and 3) site-specific criteria development for the Bay-Delta (USEPA, 1999).

A methodology for ecosystem-scale modeling of Se is now available (see **Appendices A and B**, Luoma and Presser, 2009; Presser and Luoma, 2010). Analysis from this biodynamically-based methodology showed, in general, that:

- a crucial factor ultimately defining Se toxicity is the link between dissolved and particulate phases at the base of the food web (i.e., K_d);
- collection of particulate material phases and analysis of their Se concentrations are key to representing the dynamics of the system;
- bioaccumulation in invertebrates is a major source of variability in Se exposure of predators within an ecosystem, although that variability can be explained by invertebrate physiology (i.e., $TTF_{invertebrate}$);
- TTF_{fish} is relatively constant over the range of species considered here; and
- Se concentrations are at least conserved and usually magnified at every step in a food web.

Here, we specifically adapt this methodology to the conditions and food webs of the Bay-Delta and present ecosystem-scale Se modeling in support of fish and wildlife criteria development for the estuary.

San Francisco Bay-Delta Estuary

Regulation

Habitats in California important to consider for site-specific Se criteria development include the Bay-Delta and its watersheds (Presser and Luoma, 2006) (**Figure 1**). In 1992, USEPA found that the utilization of the saltwater Se criteria for the Bay-Delta would be inappropriate and promulgated the current national chronic freshwater selenium criteria for the Bay-Delta (USEPA, 1992; 2000). USEPA also reserved the acute freshwater aquatic life criterion for Se (USEPA, 2000). In doing so, USEPA disapproved the statewide Se objective for the Bay-Delta on the basis that there was clear evidence that the objective would not protect the designated fish and wildlife uses (USEPA, 2000). For example, the California Department of Health Services had issued waterfowl Se consumption advisories and scientific studies had documented Se toxicity to fish and wildlife (USEPA, 2000; Presser and Luoma, 2006). The USEPA also re-stated its commitment to object to National Pollutant Discharge Elimination System (NPDES) permits issued for the estuary that contained effluent limits based on objectives greater than the freshwater criteria of 5 µg/L (four day average) and 20 µg/L (1 hour average).

Setting

The Bay-Delta, the largest estuary on the west coast, has been described as the *urbanized* estuary because of the extensive modification of its marshlands and the hydrologic systems that feed it (Conomos et al., 1979; 1985; Nichols et al., 1986). Two major rivers, the southward flowing Sacramento and the northward flowing San Joaquin, join at the Delta, with seawater entering through the Golden Gate Bridge (**Figure 1**). The generalized schematic of the estuary (**Figure 1**) shows the locations of:

- Sacramento River;
- San Joaquin River;
- Delta (nominally upstream of Chipps Island);
- North Bay (Suisun Bay, Carquinez Strait, San Pablo Bay);
- Central Bay;
- Pacific Ocean at the Golden Gate Bridge; and
- South Bay.

The major portion of the estuary from the rivers to the Golden Gate Bridge is termed the Northern Reach. The North Bay and the Delta are emphasized here as areas for criteria development. The South Bay is not a focus here. Although similar concepts apply, the South Bay can be modeled separately because it receives source inputs from a different watershed than the Northern Reach (**Figure 1**). However, waters do exchange and similar estuarine processes, habitats, and inhabitants do occur within all segments of the estuary.

Selenium Sources

Current major sources of Se to the Bay-Delta (**Figure 2)** are:

- irrigation drainage from seleniferous agricultural lands of the western San Joaquin Valley conveyed through the San Joaquin River; and
- oil refinery wastewaters from processing of seleniferous crude oils at North Bay refineries.

Regulation of Se for oil refiners is occurring through water quality Se criteria promulgated by USEPA for the Bay-Delta (USEPA, 1992; 2000) and limits on loads and concentrations enacted by the state in

1992 [San Francisco Bay Regional Water Quality Control (San Francisco Bay Board), 1992 a,b; 1993; 2010] (**Figure 3**). The five refineries located in the North Bay and their discharge locations are: Chevron Refinery at Richmond, discharge to San Pablo Bay; Martinez (Shell) Refinery at Martinez, discharge to Carquinez Strait; Tosco (Conoco Phillips) Refinery at Rodeo, discharge to San Pablo Bay; Tesoro Golden Eagle Refinery at Martinez, discharge to Suisun Bay; Valero Refinery at Benicia, discharge to Suisun Bay. A compilation of refinery Se loads from 1986-2009 is shown in **Table 1** (San Francisco Bay Board, 1992a,b; 1993; Lila Tang and Johnson Lam, San Francisco Bay Board, personal communication, 1999-2006; USEPA, 2010) and recent Se data are displayed in **Appendix C, Figures C1-C5**. Previous refinery mass emissions were reduced by 75% (cumulative reduction from baseline of 4,936 lbs during 1989-1991) (San Francisco Bay Board 1992a,b; 1993). Proposed load reductions were achieved in 1998 and since then, the combined Se load from the refiners has remained at approximately 1,200 pounds (lbs)/year. The target of 1,234 lbs/year was a balance between ecological, technological, and economic considerations. An iterative mass emissions strategy was used in lieu of site-specific water quality objectives because water-column Se concentrations were considered not predictive of Se bioaccumulation (San Francisco Bay Board, 1993). Daily water-column Se concentrations in effluents were as elevated as 300 µg/L before 1998, but allowed daily maximum effluent limits now are within the range of 34-50 µg/L. Discharger's outflows are designed to achieve a minimum initial dilution of 10:1, but the range of estimated initial dilutions is 15:1-200:1 (San Francisco Bay Board, 2009; 2010). Dilution credits of 8:1 and 10:1 are in-place, with an average daily flow range of 1.9-7.4 million gallons/day. The range of allowed average effluent Se limits is 0.85-2.0 lbs/day.

Regulation of Se for the agricultural community of the Grassland Drainage Area is occurring through the Grassland Bypass Project (**Figures 3 and 4**). The project was initiated in 1996 and is for use of the San Luis Drain and the tributaries of the San Joaquin River for discharge of agricultural drainage from approximately 100,000 acres of land [U.S. Bureau of Reclamation (USBR), 1995; 2001]. As noted below, the amount of agricultural Se load discharged to the Bay-Delta depends on the amount of San Joaquin River flow that is allowed to enter the Bay-Delta and how much is recycled back to the south (Presser and Luoma, 2006) (**Figure 2**).

Historical and current Se loads from the Grassland Bypass Project measured where the San Luis Drain discharges into a tributary of the San Joaquin River (i.e., Mud Slough) are shown in **Figure 3**. The use agreement for the project was re-negotiated in 2001 and was to end in 2010 with zero discharge. However, the project did not meet its goals and is now being re-negotiated to continue through 2020 (USBR and San Luis and Delta-Mendota Water Authority, 2009). Although dependent on water-year type, compliance with Se load targets gradually reduces the amount of Se allowed for discharge into the San Joaquin River (**Figure 4**). For example, the Se load measured at the compliance point (i.e., the San Luis Drain at Mud Slough) was 7,096 lbs in 1998; 5,023 lbs in 2003; 4,286 lbs in 2005; 3,301 lbs in 2008; and 1,239 lbs in 2009 (**Figure 4**). Imposition of more restrictive Se targets for the San Joaquin River is balanced by shifting a percentage of the generated annual drainage Se load to storage in groundwater aquifers and lands designated for disposal (San Francisco Estuary Institute, 2004-2005). For example, drainage control activities resulted in storage of 4,200 lbs Se within the Grassland Drainage Area in 2005. For proposed targets from 2009-2019, wetter years allow greater discharge (e.g., 4,480 lbs Se/year during 2009-2014) than drier years (**Figure 4**). Proposed targets continue to ramp down in the coming years with ultimate goals ranging from 150-600 lbs/year by 2019 (**Figure 4**). The long-term ecological consequences of such a shift in environmental compartments and increased storage of Se within the existing Se reservoir in the San Joaquin Valley is currently under debate (Presser and Schwarzbach, 2008). However, data for the Grassland Bypass Project area show Se is accumulating to levels in bird eggs of black-necked stilt, American avocet, and killdeer that far

exceed threshold Se concentrations for impairment of reproduction (San Francisco Estuary Institute, 2004-2005; H.T. Harvey and Associates, 2004-2009).

Restoration of the San Joaquin River is proceeding under a comprehensive program with many environmental goals such as increasing flows in the upper reaches of the river to re-establish salmon runs in the river (Natural Resources Defense Council and others, 1988, 1989, 1992, 1999; San Joaquin River Group, 2010). Also, regulation of salinity for the San Joaquin River is taking place at Vernalis and three locations interior to the southern Delta (California State Water Resources Control Board, 1999). Few data are available to quantify a San Joaquin River end-member Se concentration at the head of the estuary. Dissolved Se concentrations for the San Joaquin River averaged 0.71 µg/L (range 0.40-1.07 µg/L) at Vernalis during wet year and above normal conditions in 1998-1999 (Cutter and Cutter, 2004).

Discharge of Se to the Sacramento River is unregulated. Again, few data are available to quantify a Sacramento end-member Se concentration at the head of the estuary. Dissolved Se concentrations in the Sacramento River averaged 0.07 µg/L (range 0.05-0.11 µg/L) at Freeport during wet year and above normal conditions in 1998-1999 (Cutter and Cutter, 2004). Other unregulated sources of Se include 1) effluents from wastewater treatment plants and industries other than refineries; and 2) discharges from watersheds that drain directly into the estuary.

Restoration of the estuary also is underway. The Delta Regional Ecosystem Restoration Implementation Plan (DRERIP) is focusing on construction of conceptual models that describe the processes, habitats, species, and stressors of aquatic environments of the estuary (http://www.dfg.ca.gov/delta/erpdeltaplan/). The models will be interconnected and used to help evaluate future restoration actions.

Hydrodynamic Connections

A current detailed Se budget or mass balance of Se as a function of source and conveyance is not available for the Bay-Delta. Riverine inputs as they mix with seawater and internal Se sources determine Se concentrations in the Bay. Seasonal and year-to-year variations in discharges from rivers, streams, and anthropogenic sources influence dissolved Se concentrations in the Delta and estuary (Presser and Luoma, 2006). The Sacramento and San Joaquin Rivers are the main sources of inflow, with the Sacramento River being the dominant inflow under current management conditions. The Sacramento River dilutes the more concentrated Se inputs from other sources.

Parameters critical in determining the balance of water and Se inputs for the Bay-Delta are:

- total river (Sacramento River and San Joaquin River) inflow;
- water diversions or exports (i.e., pumping at Tracy and Clifton Court Forebay south to the Delta-Mendota Canal and the California Aqueduct);
- proportion of the San Joaquin River directly recycled south before entering the estuary; and
- total outflow of the estuary to the Pacific Ocean or Net Delta Outflow Index (NDOI).

NDOI is essentially inflow minus demand (USBR, 2010) (**Figure 2**). NDOI is related to residence time for freshwater in the Bay-Delta (Cutter and Cutter, 2004) and, hence, to processes that affect Se transformations within flow seasons of a water year and within types of water years (Presser and Luoma, 2006). Water years begin on October 1st and are classified here based on Sacramento Valley unimpaired runoff (http://cdec.water.ca.gov/cgi-progs/iodir/WSIHIST). Maximum discharge from the rivers is during January-February and minimum discharge is during July through August (Conomos et al., 1979; 1985; Peterson et al., 1985; Presser and Luoma, 2006).

Flow, and thus freshwater residence time, vary dramatically during the year as water management and diversions take place (http://www.usbr.gov/mp/cvo/; Enright and Culberson, 2010)

(**Figure 3**). Processes such as phase transformation and uptake by prey depend on, to some extent, the hydrodynamics of the estuary (Meseck and Cutter, 2006; Presser and Luoma, 2006; Tetra Tech Incorporated, 2010). Residence time, seasonal period (low flow and high flow), and water year type (critically dry, dry, below normal, normal, above normal and wet) can be used to categorize modeling scenarios (see later discussion).

Overview of Modeling

Used optimally, the modeling approach provided here is a tool to frame a site-specific ecological occurrence of Se exposure; quantify exposure within that ecosystem; and narrow uncertainties about how to protect it by understanding the specifics of the underlying system ecology, biogeochemistry, and hydrology (Luoma and Rainbow, 2005; Luoma and Presser, 2009; Presser and Luoma, 2010). With this approach, it is possible to differentiate consumer species and their food webs in terms of bioaccumulative potential and predict overall ecological risk. Specifically, modeling in support of development of wildlife Se criteria for the Bay-Delta is through adaptation of the San Francisco Bay-Delta Selenium Model (Luoma and Presser, 2000; Presser and Luoma, 2006) (**Figure 5**) and the Ecosystem-Scale Selenium Model (Luoma and Presser, 2009; Presser and Luoma, 2010) (**Figure 6**).

The linked factors that determine the effects of Se in ecosystems and the data needs for modeling and understanding these linkages are shown in **Figure 6**. The organizing principle for the methodology is the progressive solution of a set of equations or models, each of which quantifies a process important in Se exposure (**Figure 7**). **Table 2** compiles the generalized steps used to translate a predator tissue Se concentration guideline to a dissolved Se concentration. The ecotoxicology of Se and the specific effects of Se on fish and birds are shown in **Figure 8.** Reproductive effects are key in Se's actions, but chronic effects also are expressed. Modeling and prediction thus enables quantifying Se toxicity under different management or regulatory proposals.

Modeling is used to quantify the environmental concentrations and conditions that would result from a pre-determined Se concentration in the tissues of a predator. Assuming the tissue guideline is generic for all fish or birds, the choice of the predator species in which to assess that concentration is still important because it determines the food web invertebrate species (**Figure 6**). That specific predator's feeding habits drive the choice of invertebrate, for which a species-specific transfer factor (i.e., TTF) connects an invertebrate Se concentration to a suspended particulate material Se concentration that is the source of food for the invertebrate. An environmental partitioning factor (or a range of factors) for partitioning of Se between water and suspended particulate material (K_d) feasible for that ecosystem is then used to determine the *allowable* water-column concentration, which is ultimately the concentration in that specific type of environment and food web that would result in the specified Se concentration in the predator (i.e., the applied criterion). Thus, the *allowable* water column concentration can differ among environments; an outcome that reflects the realities of nature. This biologically explicit approach also forces consideration of the desired uses and benefits in a watershed (i.e., which species of birds and fish are the most threatened by Se or are the most important to protect). To translate exposure into toxicity here, we employ species-at-risk for the Bay-Delta (e.g., sturgeon and salmonids) and their effect guidelines provided by the USFWS (see later discussion).

Figure 2 illustrates some of the complexities that need to be addressed in developing a site-specific approach for an estuary affected by several Se sources (i.e., internal oil refinery and watershed agricultural drainage) and supporting different food webs associated with a gradient of salinities. For example, agricultural Se loading is through the San Joaquin River into the Delta where food webs are modeled as aquatic insect-based. Yet, Se loading through the Delta affects the Bay and adds to oil refinery Se loads where food webs are modeled as *C. amurensis*-based. The North Bay, where *C.*

amurensis is the dominant bivalve species and is a strong Se bioaccumulator, is the most affected by Se loading (Stewart et al., 2004; Presser and Luoma, 2006) (**Figure 2**). Hence, overall, tracking and differentiation of Se sources is an important component of management for the estuary, especially as changes to the hydrologic configuration of the Delta (e.g., the amount of Sacramento River and San Joaquin River allowed to enter the Bay) are considered in the future.

Figure 9 shows site-specific processes and parameters for the Bay-Delta and acts as a roadmap through the modeling process detailed in the sections below. The approach for the estuary is through specified food webs, locations, and flow seasons in modeling scenarios. Detailed model steps, parameters, and derivations are illustrated for a seaward *C. amurensis* food web and a landward aquatic insect food web (**Figure 9)**. A spatial component for modeling is based on a salinity gradient across the estuary or on a particular portion of the estuary (i.e., Suisun Bay). A temporal component for modeling addresses the effect of water-year type and within that type, a flow season (low flow, nominally June through November; high flow, December through May). Addition of a temporal component based on residence time further delineates a fine-scale approach, as do the additions of details of species life cycles and habitat use. The more detailed the modeling choices or approach, the less uncertainty there is in the forecasts. As illustrated (**Figure 9**), the main considerations used here for a site-specific Bay-Delta approach are:

- species-specific effects guidelines to quantify regulatory concerns;
- food webs to define the choice of prey and predator pairs (i.e., TTFs);
- salinity to constrain locations and thus potential pathways for loading, transformation, and exposure;
- flow seasons to connect to hydrology, predator life cycles, and habitat use; and
- residence time to further constrain transformation and biodynamic processes.

Thus, a formalized approach captures both mathematical components and exposure gradients over time. A focused area approach would enable regulatory consideration of sources or impacted downstream areas.

Fish and Wildlife

Species at Risk

The USFWS (2008) provided a comprehensive list of species for evaluation of Se exposure risk in the Bay-Delta (**Table 3**). They stated that 1) aquatic dependent species feeding directly in the benthic food web of the Bay-Delta were considered at greater risk to Se exposures than those feeding in the pelagic/planktonic food web; and 2) exposure assessment was based on a) dependence on a benthic food web, b) population status, and c) sensitivity to Se. The list included 27 bird species, 15 fish species, the salt marsh harvest mouse, the giant garter snake, and the Dungeness crab. The species listed in **Table 3** then were narrowed to provide a list of species considered most at risk (**Table 4**). Species most at risk from Se in the Bay-Delta and their status (federal/state) include:

- bald eagle (*Haliaeetus leucocephalus*): delisted, U.S. Migratory Bird Treaty Act (MBTA), Bald and Golden Eagle Protection Act (BGEPA)/protected, endangered;
- California clapper rail (*Rallus longirostris obsoletus*): endangered/protected, endangered;
- greater scaup (*Aythya marila*): MBTA/none;
- lesser scaup (*Aythya affinis*): MBTA/none;
- white-winged scoter (*Melanitta fusca*): MBTA/none;
- surf scoter (*Melanitta perspicillata*): MBTA/none;

- black scoter (*Melanitta nigra*): MBTA/none;
- Chinook salmon (*Oncorhynchus tshawytscha*): endangered, threatened/endangered, threatened;
- steelhead (*Oncorhynchus mykiss*): threatened/none;
- green sturgeon (*Acipenser medirostris*): threatened/concern, fishing prohibited;
- white sturgeon (*Acipenser transmontanus*): none/limited fishing;
- Sacramento splittail (*Pogonichthys macrolepidotus*): concern/threatened; and
- giant garter snake (*Thamnophis gigas*): threatened/threatened.

Although its diet does not include bivalves, Delta smelt (*Hypomesus transpacificus*) is a threatened species that is endemic to the estuary and, hence, is considered by the USFWS (2008) as threatened overall. A reptile species (USFWS, 2006, 2009a) and an invertebrate species USFWS (2008) also are documented as important inhabitants of the estuary. The threatened giant garter snake (*Thamnophis gigas*) inhabits the Delta Basin and watershed valleys (USFWS and NMFS, 1998; amended 2000; USFWS, 2006). This species is an aquatic predator that feeds on small fish and larval/sub-adult frogs (USFWS, 2009a). The estuary is a nursery for the ocean-breeding, bottom-feeding Dungeness crab (*Cancer magister*). This species consumes *C. amurensis*, but invertebrates, in general, are known to have lower toxicological sensitivity (Presser and Luoma, 2006). However, Dungeness crab may serve to further biomagnify Se by providing an additional trophic transfer step (i.e., *C. amurensis* to Dungeness crab to large predator fish or mammals).

Effects and Effect Levels

Effects of concern for Se in fish and wildlife (**Figure 8**) are:

- reproductive effects
  - birds: hatchability, teratogenesis, chick survival and growth; and
  - fish: deformity, larva and fry survival and growth
- chronic effects.

Species-specific effect models developed as part of the DRERIP process are shown for diving ducks, sturgeon, and salmonids inhabiting the Bay-Delta (**Figure 10**, adapted from DRERIP Selenium Model, Presser, et al., in review). These effects can lead to changes within ecosystems including population reductions, loss of species or individuals, and community changes.

The USFWS (2009b) provided Se effect guidelines and associated levels of protection (e.g., EC10 for birds is the Se concentration in eggs associated with a 10% reduction in hatchability) for predator species at risk in the estuary based on several different toxicity endpoints (**Table 5**). [Note: Technically, the term EC10 does not apply to quantitative reproductive performance endpoints. The proper term to apply to quantitative reproductive performance endpoints such as 10% reduction in egg hatchability is IC10 (or 10% Inhibition Concentration). However, the subtle conceptual distinction between these two technical terms has not been recognized in the avian toxicology literature for Se; therefore, we conform with the common use of the term EC10 with reference to avian egg hatchability and simply note here that we are aware of this issue (see Environment Canada, 2005)]. Data from the study of toxicity in mallards is used when modeling clam-eating bird species in the estuary because these are the most comprehensive studies available. The effect guideline ranges derived for tissue and diet in dry weight (dw) are:

- mallard (egg 2.8-7.7; diet 2.3-5.3 µg/g dw);
- adult female white sturgeon (whole-body 7.0-8.1 µg/g dw; diet 26-32 µg/g dw);
- juvenile white sturgeon (diet 0.95-1.6 µg/g dw);
- juvenile Chinook salmon (whole-body 1.0-1.8 µg/g dw; diet 1.5-2.7 µg/g dw);

- juvenile rainbow trout (whole-body 1.3-2.2 µg/g dw; diet 2.4-5.0 µg/g dw); and
- larval rainbow trout (diet 0.31-1.6 µg/g dw).

Table 6 gives generic guidelines for Se effect concentrations also developed by the USFWS (USFWS, 2005; 2009b; Skorupa, et al., 2004; Skorupa, 2008). A subset of the effects guidelines and associated levels of protection shown in **Tables 5 and 6** are used in modeling to predict toxicity under different regulatory proposals. Emphasis here is on illustration of Se exposure for juvenile white sturgeon, diving ducks as represented by the mallard, and juvenile Chinook salmon.

Estuary Food Web and Exposure Models

Conceptual models for the estuary show clam-based food webs for seaward sites and aquatic insect-based food webs for landward sites (**Figures 2 and 11**). The *C. amurensis*-based food web has been of major importance to the estuary since the clam's invasion in 1986 (Nichols et al., 1990). Fish and bird species that consume *C.* amurensis are shown (**Figure 11**). A Dungeness crab food web also is shown because the diet of the crab includes *C. amurensis*. However, little Se-specific information is known for this crab. The bald eagle food web shows the complexity of a high order trophic level predator. USFWS suggested that the bald eagle would be representative of a resident high order predator for the purposes of modeling (USFWS, 2008). Chinook salmon and steelhead, along with the California black rail, are modeled for landward sites. Invertebrate prey items, in addition to aquatic insects, that may be of importance at landward sites also are listed. Environmental partitioning factors (K_ds) and Trophic Transfer Factors (TTFs) used to quantify the biotransfer of Se through food webs of the estuary also are shown in **Figure 11**. The development of these factors is shown in detail later (see *Derivation of Site-Specific Model Components* section).

A diagram across flow seasons illustrates exposure media (water, suspended particulate material, and clams) and the potential for exposure based on the life cycles and habitat-use of predators in the estuary (**Figure 12)**. Migratory and resident bird and fish species are illustrated. Knowing the details of a predator's location during critical life stages for Se effects allows correlating trends in diet and exposure that occur in the estuary. This knowledge, in turn, sets choices in modeling scenarios. Combining food web, life cycle, habitat use, and effects data (**Figures 10, 11, and 12**) results in Bay-Delta specific information for criteria development.

The probable critical life stages of predators most at risk for Se effects as given in USFWS (2008) are:

- bald eagle and California clapper rail: adult female (egg laying);
- scoter and scaup: adult male and female (migration);
- Chinook salmon and steelhead: migrating/rearing juvenile; and
- green and white sturgeon and Sacramento splittail: juvenile or adult female.

The estimated maximum percentage of diet that is clam-based for each predator most at risk (USFWS, 2008) (**Figure 11**) is:

- lesser scaup 96%;
- surf scoter 86%;
- greater scaup 81%;
- black scoter 80%;
- white-winged scoter 75%;
- California clapper rail 64%;
- white sturgeon and assumed for green sturgeon 41%;
- Sacramento splittail 34%; and

- bald eagle 23%.

Specifically, migratory bird species such as surf scoter and greater and lesser scaup are at risk based on their consumption of a clam-based diet (75-96%) (**Figure 11**). Overwintering populations of diving ducks in the estuary can reach 50-92% of migrating populations (Wainwright-De La Cruz et al., 2008; Poulton et al., 2002) (**Figure 11**). Diving ducks arrive in the estuary when Se concentrations are elevated (**Figure 11**). The ducks eat voraciously as they stage for migration in the spring, which puts them at risk from chronic effects that influence many facets of their migratory and breeding behavior (**Figures 7 and 10**). Surf scoters during overwintering move throughout the North Bay and thus can be exposed to different clam species (i.e., *V. philippinarum* in the Central Bay) (Wainwright-De La Cruz, 2008). Food webs for clapper rails with an estimated 64% clam-based diet present opportunities for modeling of reproductive effects for resident species (**Figures 4 and 5**).

White and green sturgeon consume a diet that is approximately 41% clams (USFWS, 2008). Green sturgeon is a federally listed endangered species that spends more time migrating than white sturgeon. Although white sturgeon migrate upstream to spawn, they are described as semi-anadromous because they spend a substantial amount of their life in the estuary. White and green sturgeon are very long-lived (50-100 years) and have a two year internal egg maturation that is conducive to Se loading of eggs (**Figure 12**) (Linville, 2006).

Sacramento splittail is a federally listed species of concern that consumes a diet of approximately 34% clams (USFWS, 2008). This species spawns both in the upper Delta and the estuary and is known to inhabit Suisun Bay.

The USFWS (2008) stated that although the diets of salmon and steelhead trout are not known to be clam-based, these species may still be at risk from Se because of their greater toxicological sensitivity to Se. Migratory salmon and trout are known to be in the Delta during migration upstream and emigration to the ocean (**Figure 12**). Steelhead trout may be best described as nearly year-around spawners (i.e., juveniles may hold over for many months to a year and may not even emigrate to the ocean at all) (USFWS, 2008). Population numbers for the Delta smelt are alarmingly low, and thus the USFWS concluded that this species is particularly vulnerable to any adverse effect.

The giant garter snake is a federally listed species that is known to inhabit the Delta (USFWS and NMFS, 1998; amended 2000; USFWS, 2006; 2009a). The species is an aquatic predator that feeds on small fish and larval/sub-adult frogs. Modeling for this species of reptile is not included here, but future modeling could include a food web specific to the giant garter snake.

Ecosystem-Scale Model Components

Partitioning and Transformation

Profiles of dissolved and suspended particulate material Se concentrations across the Bay-Delta (Cutter and Cutter, 2004, Doblin et al., 2006; Lucas and Stewart, 2007) initiate ecosystem-scale modeling by developing a detailed understanding of the relationship of dissolved and particulate Se concentrations at specific landward and seaward locations (**Figure 2**). Consideration of the transformations of dissolved Se phases to particulate Se phases is critical to quantifying the entrance of Se into food webs (**Figure 13**). The environmental partitioning factor K_d is used here to operationally characterize the bioconcentration of dissolved Se into the base of the food web (**Figures 7 and 13**). K_d is environment specific and is the ratio of the particulate material Se concentration to the dissolved Se concentration. The specific equation is

$$K_d = (C_{\text{particulate material}}, \mu g/kg\ dw) \div (C_{\text{water}}, \mu g/L) \quad (1)$$

Note that particulate Se concentrations are usually expressed as µg/g dw. These units must be converted to µg/kg dw to make the particulate concentration comparable to the water concentration.

Dissolved Se is the preferred parameter to measure and model, although total water column Se (i.e., unfiltered Se) can be specified in the derivation of K_d for modeling to accommodate using existing datasets. Measurement of a total water column Se concentration would include a fraction attributable to digested suspended material Se. Specifically for Bay-Delta profiles or transects, dissolved Se samples were collected and dissolved Se concentrations are available (Cutter and Cutter, 2004).

A particulate material Se concentration is the other component of K_d to measure and model (**Figure 13**). The base of the food web, as sampled in the environment, can include phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment and/or attached vascular plants (Presser and Luoma, 2010). For simplicity in our discussion here, we define this mixture of living and non-living entities as *particulate material*. Specifically for Bay-Delta profiles and transects, suspended particulate material samples were collected and suspended particulate material Se concentrations are available (Doblin et al., 2006).

As illustrated in **Figure 13**, K_d represents phase transformation in the system (i.e., the efficiency with which dissolved Se is converted to particulate material Se). Phase transformation reactions from dissolved to particulate material Se are of toxicological significance because particulate material Se is the primary form through which Se enters food webs (Luoma et al, 1992; Presser and Luoma, 2010; Stewart et al., 2010). The different biogeochemical transformation reactions result in different forms of Se in particulate material: organo-Se, elemental Se, or adsorbed Se (**Figure 13**). The resulting particulate Se speciation, in turn, affects the bioavailability of Se to invertebrates depending upon how an invertebrate "samples" the complex water/sediment/particulate *milieu* that composes its environment. Collection of a complete dataset of particulate phases and their Se concentrations and speciation can greatly aid in quantifying the biogeochemical dynamics of an estuarine system and, hence, the prediction of prey and predator Se concentrations.

Dissolved Se species that are present will influence the type of phase transformation reaction that creates particulate Se. Examples of types of reactions and the particulate species they produce (**Figure 13**) include: 1) uptake by plants and phytoplankton of selenate, selenite or dissolved organo-Se and reduction to particulate organo-Se by assimilatory reduction (e.g., Sandholm et al., 1973; Riedel et al., 1996; Wang and Dei, 1999; Fournier et al., 2006); 2) sequestration of selenate into sediments as particulate elemental Se by dissimilatory biogeochemical reduction (e.g., Oremland et al., 1989); 3) adsorption as co-precipitated selenate or selenite through reactions with particle surfaces; and 4) recycling of particulate phases back into water as detritus after organisms die and decay (e.g., Velinsky and Cutter, 1991; Reinfelder and Fisher, 1991; Zhang and Moore, 1996). Selenate is the least reactive of the three forms of Se and its uptake by plants is slow. If all other conditions are the same, K_d will increase as selenite and dissolved organo-Se concentrations increase (even if that increase is small). Experimental data support this conclusion. Calculations using data from laboratory microcosms and experimental ponds show speciation-specific K_ds of 140-493 where selenate is the dominant form; 720-2,800 when an elevated proportion of selenite exists; and 12,197-36,300 for 100% dissolved seleno-methionine uptake into algae or periphyton (Besser et al., 1989; Graham et al., 1992; Kiffney and Knight, 1990).

Measurement of suspended particulate material Se concentrations in the Bay-Delta, therefore, is important for initiating modeling, understanding the extent of biological transformations, and developing accuracy within the model. Data collection in site-specific field situations for particulate phases can include benthic or suspended phytoplankton, microbial biomass, detritus, biofilms, and nonliving organic materials associated with fine-grained (<100 µm) surficial sediment (Luoma et al.,

1992). Analysis of particulate Se and particulate Se speciation of each phase collected would account for partitioning of Se in different media and elucidate how K_d may be best defined to represent the dynamic conditions present in the estuary. If few data are available to characterize particulate phases or data are inconsistent as to a particle type that can be compared among locations, the greater the uncertainty in any predictions. Further information on choice of particulate material type, sample collection in aquatic systems, and modeling limitations are given in Presser and Luoma (2010). For example, K_d can be influenced by the type of particulate material collected where a hierarchy of Se concentrations exist within an ecosystem (e.g., 2.4 µg/g in sediment; 3.2 µg/g biofilm, and 5.5 µg/g for filamentous algae). Using these concentrations with a field-measured dissolved Se concentration would yield a range of K_ds that reflects the complexities of the system. In this regard, collection of one consistent type of material is an option, with bed sediments (especially if the sediments vary from sand to fine-grained) among the samples being the least desirable choice for calculating K_d,.

Biodynamics: Invertebrates, Fish, and Birds

Kinetic bioaccumulation models (i.e., biodynamic models, Luoma and Fisher, 1997; Luoma and Rainbow, 2005) account for the now well-established principle that Se bioaccumulates in food webs principally through dietary exposure. Tissue Se attributable to dissolved exposure makes up less than 5% of overall tissue Se in almost all circumstances (Fowler and Benayoun, 1976; Luoma et al., 1992; Roditi and Fisher, 1999; Wang and Fisher, 1999; Wang 2002; Schlekat et al., 2004; Lee et al., 2006). Biodynamic modeling (**Figures 6 and 8**) shows that the extent of Se bioaccumulation (the concentration achieved by the organism) is driven by physiological processes specific to each species (Reinfelder et al., 1998; Wang 2002; Baines et al., 2002; Stewart et al., 2004). Biodynamic models have the further advantage of providing a basis for deriving a simplified measure of the linkage between trophic levels: TTFs (**Figure 7)**. For each species, a TTF can be derived from either experimental studies or field observations, where the TTF defines the relationship between Se concentrations in an animal and in its food (**Figure 7**).

Experimental derivation of TTFs is based upon the capability of a species to accumulate Se from dietary exposure as expressed in the biodynamic equation (Luoma and Rainbow, 2005):

$$dC_{species}/dt = [(AE)\ (IR)\ (C_{food})] - (k_e + k_g)(C_{species}) \quad (2)$$

where C is the contaminant concentration in the animal (µg/g dw), t is the time of exposure in days (d); AE is the assimilation efficiency from ingested particles (%); IR is the ingestion rate of particles (g/g/d); C_{food} is the contaminant concentration in ingested particles (µg/g dw); k_e is the efflux rate constant (/d) that describes Se excretion or loss from the animal; and k_g is the growth rate constant (/d). The equation shows that key determinants of Se bioaccumulation are the ingestion rate of the animal, the efficiency with which Se is assimilated from food, and the rate constant describing Se turnover or loss from the tissues of the animal (Luoma and Rainbow, 2005). Experimental protocols for measuring such parameters as AE, IR, k_e are now well developed (Wang et al., 1996; Luoma and Rainbow, 2005).

In the absence of rapid growth, a simplified, resolved biodynamic exposure equation for calculating a Se concentration in an invertebrate is

$$C_{invertebrate} = [(AE)\ (IR)(C_{particulate})] \div [k_e] \quad (3)$$

where C_{food} is defined as $C_{particulate}$.

For modeling, these physiological parameters can be combined to calculate a $TTF_{invertebrate}$, which characterizes the potential for each invertebrate species to bioaccumulate Se. $TTF_{invertebrate}$ is defined as

$$TTF_{invertebrate} = [(AE)\ (IR)] \div k_e \quad (4)$$

Similarly, foodweb biodynamic equations for fish and birds are

$$C_{fish\ or\ bird} = [(AE)\ (IR)\ (C_{invertebrate})] \div k_e \text{ and } TTF_{fish\ or\ bird} = [(AE)\ (IR)] \div k_e \quad (5) \text{ and } (6)$$

When laboratory data are not available, a field $TTF_{invertebrate}$ can be defined from matched datasets (in dw or converted to dw) of particulate and invertebrate Se concentrations as

$$TTF_{invertebrate} = C_{invertebrate} \div C_{particulate} \quad (7)$$

A field derived species-specific TTF_{fish} is defined as

$$TTF_{fish} = C_{fish} \div C_{invertebrate} \quad (8)$$

where $C_{invertebrate}$ is for a known prey species, C_{fish} is reported as muscle or whole-body tissue, and both Se concentrations are reported in µg/g dw. If necessary, the modeling approach can represent a diet that includes a mixed proportion of prey in the diet through use of the equation

$$C_{fish} = (TTF_{fish}) [(C_{invertebrate\ a}) (\text{prey fraction}) + (C_{invertebrate\ b}) (\text{prey fraction}) + (C_{invertebrate\ c}) (\text{prey fraction})] \quad (9)$$

Once TTFs are know, invertebrate Se concentrations are calculated from particulate material Se concentrations through use of the equation

$$C_{invertebrate} = (TTF_{invertebrate}) (C_{particulate}) \quad (10)$$

Equations are combined to represent step-wise bioaccumulation from particulate material through invertebrate to fish as

$$C_{fish} = (TTF_{invertebrate}) (C_{particulate}) (TTF_{fish}) \quad (11)$$

Similarly for birds, the combined equation is

$$C_{bird} = (TTF_{invertebrate}) (C_{particulate}) (TTF_{bird}) \quad (12)$$

Modeling can accommodate longer food webs that contain more than one higher trophic level consumer (e.g., forage fish being eaten by predatory fish) by incorporating additional TTFs. One equation for this type of example is

$$C_{predator\ fish} = (TTF_{invertebrate}) (C_{particulate}) (TTF_{forage\ fish}) (TTF_{predator\ fish}) \quad (13)$$

Modeling for bird tissue also can represent Se transfer through longer or more complex food webs (e.g., TTFs for invertebrate to fish and fish to birds) as

$$C_{bird} = (TTF_{invertebrate}) (C_{particulate}) (TTF_{fish}) (TTF_{bird}) \quad (14)$$

Variability or uncertainty in processes that determine AEs or IRs can be directly accounted for in sensitivity analysis (Wang et al, 1996). That is accomplished by considering the range in the experimental observations for the specific animal in the model. Field derived factors require some knowledge of feeding habits and depend upon available data for that species. Laboratory and field factors for a species can be compared and refined to improve levels of certainty in modeling. Hence, physiological TTFs derived from kinetic experiments for a species and ecological TTFs derived either from data for a species across different field sites (global) or from one site (site-specific) are of value in modeling and understanding an ecosystem.

TTFs are species-specific because of the influence of the physiology of the animal. They may vary to some extent as a function of the concentration in food or if AE or IR vary (Besser et al., 1993; Luoma and Rainbow, 2005). The approach here leads to consideration of a single TTF to quantify trophic transfer from diet to tissue for each species illustrated in modeling. If enough data are available to develop diet-tissue concentration regressions specific to inhabitants of an estuary or watershed, then use of those regressions would provide more detailed relationships than single determinations. Additionally, in nature, if it is assumed that organisms regulate a constant minimum concentration of Se, then the observed TTF will increase when the concentration in food is insufficient to maintain the regulated concentration (Beckon et al., 2008). Datasets from which non-site-specific TTFs were derived for use in modeling here were collected from sites exposed to Se contamination and identified as problematic because of Se bioaccumulation (Presser and Luoma, 2010). However, discretion was used when considering datasets from extremely contaminated sites (e.g., Kesterson). The relatively small

variation of TTF within taxonomically similar animals is evidence that these potential sources of uncertainty may be minimal in terms of biodynamic kinetics variations (Presser and Luoma, 2010).

Available Data

Table 7 lists available data for the Bay-Delta. Comprehensive data collection to evaluate Se concentrations in the Bay-Delta began in 1986. Transects of the Bay-Delta from November 1997 to November 1999 provide spatially and temporally matched datasets for samples collected at one meter below the surface (Cutter and Cutter, 2004; Doblin et al., 2006). The parameters measured for these datasets were:

- salinity;
- dissolved Se concentration;
- dissolved Se speciation;
- suspended particulate material Se concentration;
- suspended particulate material Se speciation;
- amount of total suspended material; and
- particulate carbon (C) concentration.

Transects during July, 2000 to January, 2004 characterize the area mainly from Rio Vista and Stockton to Benicia near the Carquinez Strait (Lucas and Stewart, 2007) (**Figure 1**). These more landward transects were limited to:

- dissolved Se;
- dissolved Se speciation; and
- suspended particulate material Se concentration.

Not all datasets are complete, so graphed profiles shown later may vary somewhat because matched pairs for each combination of data (e.g., dissolved Se and suspended particulate material Se in comparison to percentage of suspended particulate organo-Se) across the salinity gradient were not always available.

The matched data pairs for dissolved Se concentrations and suspended particulate material Se concentrations used here are for tidally-influenced sites. Doblin et al. (2006) hydrodynamically categorized (i.e., binned), for the conditions of each transect, the most landward suspended particulate material Se samples as *the Delta*. These Delta sites are nominally upstream of Chipps Island (Doblin et al., 2006) and, thus, these sites are tidally influenced (**Figure 1**). Therefore, our site-specific derivation does not address Se concentrations in end-members such as the Sacramento and San Joaquin Rivers (i.e., Sacramento River at Freeport and the San Joaquin River at Vernalis).

The methodology for collection and analysis of dissolved and suspended particulate material Se samples is described in Doblin et al. (2006). Methods for determining particulate Se can result in presentation of data either as µg/L or µg/g. For work here, direct determination of particulate Se concentrations as µg/g dw is preferable. However, a particulate Se concentration in µg/L can be converted to µg/g dw through division by the available matched data on amount of total suspended material (in mg/L). Because of the limited data available for characterization of the Bay-Delta and the data needs of modeling for criteria development, all necessary conversions were made in order to make full use of available data. Future monitoring of the Bay-Delta should consider collection of suspended particulate material Se concentration data as µg/g dw. All solids are expressed in dry weight (dw).

Other types of datasets are available for the Bay-Delta (**Table 7**). Meseck (2002) collected sedimentary Se samples from box-cores and extracted pore waters from Bay-Delta locations from 1997-1999. Sedimentary Se samples (sediment cores at 2-4 meter-depth of water) also were collected in 1998

from six locations in the Delta (M. Doblin, personal communication March, 2009) and in 2000 from three locations in the Delta (Lucas and Stewart, 2007) (**Table 7**).

Datasets for Se concentrations in specific predators and food webs (e.g., the clam, *C. amurensis*, white sturgeon, surf scoter) also are listed (**Table 7**), but few current, matched datasets are available to provide comprehensive documentation of food webs. Fifteen years of monitoring data in the northern estuary for Se in *C. amurensis* was recently published (Kleckner et al., 2010) and is illustrated later in the report. **Appendix D** (**Tables D1-D5)** gives a compilation of some of the available food web Se data including for invertebrates, fish, and birds. Because there are minimal data available, data are generalized in model validations; however, data used in validation scenarios and illustrations are as closely matched as possible.

Application of Ecosystem-Scale Methodology

Estuarine Approaches

A methodology based on a salinity gradient across the Bay-Delta, from the tidally-influenced landward sites above Chipps Island to seaward sites near the Pacific Ocean at the Golden Gate Bridge (Cutter and Cutter, 2004; Doblin et al., 2006; Lucas and Stewart, 2007) is used here to provide location-specific modeling for the estuary (Presser and Luoma, 2006). Given a specific food web and Se tissue guideline, the approach uses salinity-specific data to derive K_ds and TTFs and to predict allowable dissolved Se concentrations at each salinity measured across an estuary profile. This gradient modeling approach illustrates the variability across the estuary in terms of transformations, bioaccumulative potential, and protective dissolved allowable Se concentrations (**Figures 2 and 9**). A generalized approach (i.e., using a mean K_d from a transect) would add uncertainty to the derivations and predictions because of, for example, inclusion of samples from freshwater and ocean interfaces. Mean Se concentrations for transects can be used as a way to compare datasets through time, but that approach may be of limited applicability. Other statistical parameters or analysis techniques also could be used (i.e., median, 75^{th} percentile value) for comparison of estuarine conditions.

A second modeling approach, a focused location approach, uses compartmentalized data for Suisun Bay and Carquinez Strait (Doblin et al., 2006) to illustrate how the Bay-Delta can be divided into segments for explicit regulatory consideration (**Figure 14**). Doblin et al. (2006) grouped particulate material Se samples as a function of salinity into four embayments: 1) Central Bay; 4) San Pablo Bay; 3) Carquinez Strait-Suisun Bay; and 4) Delta. **Figure 14** shows the range of suspended particulate material Se concentrations within the compartmentalized segments and the patterns within the range of illustrated flow conditions. Focusing on transect samples that specifically represent Carquinez Strait-Suisun Bay allows modeling and prediction for the localized area most affected by internal oil refinery Se sources and for time periods of specified flow conditions. Again, a mean or other statistical measure for each transect, but within the Suisun Bay-Carquinez Strait segment, can be used to characterize conditions through time, but thus at a more narrowly defined site.

Modeling that specifies 1) water-year type and flow season; or 2) freshwater residence time further narrows uncertainties within the estuarine approaches by addition of a temporal component. Modeling of the Bay-Delta based on hydrologic season or residence time also enables connection to hydrodynamic cycles, prey/predator exposure, and habitat-use (**Figure 12**) in developing site-specific allowable Se concentrations. Specific dates, freshwater residence times, water-year types, and flow seasons for transects of the Bay-Delta (Cutter and Cutter, 2004; Doblin et al., 2006) are:

- November 5-6, 1997, 68 days, wet year, low flow season;
- June 16-17, 1998, 11 days, wet year (El Niño), high flow season;

- October 7-8, 1998, 22 days, wet year, low flow season;
- April 13-14, 1999, 16 days, wet year, and high flow season; and
- November 4-5, 1999, 70 days, above normal year, low flow season.

The conditions in the estuary during these transects and the proportion of the recent historical record represented by these five transects are given context by showing the sampling dates within the variability afforded by NDOI for the period 1996-2009 (**Figure 15**). During an 11-day residence time in June, 1998, NDOI is 73,732 cfs as a daily average/month, but during a 70-day residence time in November, 1999, NDOI is 6,951 cfs as a daily average/month. Thus, consideration of a temporal component in modeling may be imperative for applying predictions here to conditions in the estuary in the future.

Dissolved and Suspended Particulate Material Selenium Profiles for Modeling

Modeling and predictions for criteria development for a *C. amurensis* food web uses Se data from the Bay-Delta transects listed above (November, 1997; June and October, 1998; April and November, 1999) (**Figures 16 and 17**). Transect sampling for the Bay-Delta included 19 to 20 sites per transect, except for the June 1998 transect, which included 13 sites. Conditions represented are all wet or above normal years, with sampling in June, 1998 and April, 1999 being during high flow seasons and October, 1998, November, 1997, and November, 1999 being during low flow seasons (**Figure 15**).

Salinity at the Golden Gate Bridge varies from 24.8 to 32.5 psu for the five transects. Distinctive profiles for dissolved Se concentrations from June 1998 shows conditions in the Bay-Delta when flows were exceptionally high because of extremely wet conditions related to El Niño (**Figures 16**). Approximately 70% of the data for this transect was obtained at sites with salinities < 5 psu. In contrast, profiles for residence times of 68 to 70 days in November, 1997 and 1999 show a span of salinities up to approximately 32 psu.

Specifically, **Figure 16** shows dissolved Se concentrations across the estuary during a progression of residence times (11-70 days) from November, 1997 to November, 1999. The transect for November, 1997 is separated out from the main analysis here because of 1) decreasing refinery Se loads as proposed reductions took place (**Table 1; Figure 3**); and 2) a noticeably higher dissolved Se concentration-profile across the estuary. The range of dissolved Se concentrations is narrowly defined as 0.070-0.320 µg/L for all Bay-Delta transects (**Table 8**).

The range of suspended particulate Se concentrations (0.15-2.2 µg/g dw) for all Bay-Delta transects is not as narrowly defined as that for dissolved Se (**Figure 17; Table 8)**. The patterns of particulate enrichment vary with specified flow condition (e.g., April, 1999; November, 1999). The variation at freshwater and ocean interfaces would contribute differently (or may contribute substantially) to a calculated overall mean condition. Also depicted is the variation in calculated K_ds across the estuary. These K_ds will be used later as critical location-specific inputs for ecosystem modeling.

A subset of dissolved and suspended particulate material Se concentrations is developed using the samples defined as Suisun Bay-Carquinez Strait in **Figure 14** (Cutter and Cutter, 2004; Doblin et al., 2006) (**Table 9**). The range of dissolved Se concentrations is from 0.076-0.215 µg/L and the range of suspended particulate material Se concentrations is 0.15-1.0 µg/g dw.

Profiles of dissolved and suspended particulate Se concentrations also are derived from more limited transects of the estuary from Rio Vista and Stockton to Benicia during 2003 and 2004 (**Figure 18**). Four transects (January, April, and October, 2003; January, 2004) are used to model an aquatic insect food web. Specific dates, water-year types, and flow seasons for transects (Lucas and Stewart, 2007) are:

- January 22, 2003, above normal year, high flow season;
- April 22-23, 2003, above normal year, high flow season;
- October 10, 2003, below normal year, low flow season; and
- January 15, 2004 below normal year, high flow season.

As previously noted, samples for these transects were taken as part of work defining processes in the Delta (Lucas and Stewart, 2007), but sampling was extended to some seaward locations in the estuary (i.e., near Benicia). NDOI (daily average per month) varies from to 4,350 to 50,847 cfs over the range of transects, with October, 2003 representing a below normal year-low flow condition. The range of dissolved Se concentrations is 0.068-1.01 µg/L and the range of suspended particulate material Se concentrations is 0.23-1.5 µg/g dw (**Table 10**).

Dissolved and Suspended Particulate Material Selenium Speciation

Selenium speciation in source discharges and within the gradient of the estuary itself are important in quantifying the efficiency of transformations from dissolved Se to particulate Se (**Figure 2**). Profiles of dissolved Se speciation across the salinity gradient for September, 1986 and November, 1997 show that the percentages of dissolved selenite generally have decreased over time (Cutter, 1989; Cutter and Cutter, 2004) (**Figure 19**). During the period 1992-1998, new treatment technologies were put into place that were designed to reduce the amount of dissolved selenite in the effluent (San Francisco Bay Board, 1992a,b; 1993). Other factors to consider in broad comparisons such as these, are that the salinity for Carquinez Strait near the refineries during November, 1997 ranged from approximately 12 to 19 psu (Doblin et al., 2006) and that the residence time was 24 days during the 1986 transect and 70 days during the 1997 transect.

Figure 20 shows profiles across the Bay-Delta of suspended particulate material organo-Se concentrations as the percentage of the total of the three suspended particulate material Se species analyzed [(i.e., organo-Se, elemental Se, and inorganic Se (adsorbed selenate and selenite), Doblin et al., 2006]. The patterns of organo-Se particulate enrichment identified here serve as the basis for quantifying the effects of transformations to particulate material Se (i.e., K_d) and the assimilation efficiency of Se in the particulate material by prey (i.e., understanding the particulate material to prey kinetics of bioaccumulation).

Bioaccumulated Selenium in Prey

Central to the seaward ecosystem is the *C. amurensis* food web (Nichols et al., 1990; Linville et al., 2002; Presser and Luoma, 2006). **Figure 21** shows monthly mean Se concentrations for *C. amurensis* from several USGS monitoring stations for the time periods encompassed by the Bay-Delta transects (see inset). Mean observed *C. amurensis* Se (Kleckner et al., 2010) for each transect (Cutter and Cutter, 2004; Doblin et al., 2006) are shown in order of high flow seasons (June, 5.4 µg/g dw and April, 7.3 µg/g dw) to low flow seasons (October, 10.8 µg/g dw and November, 11.3; 14.3 µg/g dw) during wet or above normal years (**Figure 21**) (see additional discussion in *Choices, Limitations, and Reduction of Uncertainty* section). Data here illustrate the connection of bivalve Se concentrations to the cumulative productivity of the estuary in terms of Se transformation, uptake, and exposure during low flow periods. The variability within the available 15-year monthly *C. amurensis* Se concentration dataset is illustrated to give context to means for 1997-1999 (grand mean, 12.1 µg/g dw).

Less data are available for landward insect-based food webs (**Table 7; Appendix D, Table D5**). Data for invertebrate Se concentrations are from 2001 and 2002, with means ranging from 0.6-4.8 µg/g

dw. With limited invertebrate data, patterns and connections to hydrodynamic and ecological cycles are difficult to assess.

Derivation of Site-Specific Model Components

Environmental Partitioning Factors (K_ds)

Location-specific K_ds based on salinity across the Bay-Delta are calculated from spatially and temporally matched datasets for dissolved and suspended particulate material Se (**Figures 17 and18; Tables 8, 9, and 10**). Statistical evaluations of dissolved and suspended particulate material Se concentrations for complete transects or focused Suisun Bay-Carquinez Strait transect yield a set of mean, 75th percentile, median, and 25th percentile K_ds (**Tables 11 and 12**). The location-specific K_ds and set of statistical K_ds are then used to represent conditions in the estuary for modeling a seaward clam-based food web and predicting an allowable dissolved Se concentration. The set of K_ds used to represent conditions in the estuary for modeling a landward insect-based food web and predicting an allowable dissolved Se concentration is shown in **Table 10**.

Location-specific K_ds show the variation that can be expected across the estuary in the recent past (**Figures 17 and 18**). K_ds vary similarly as suspended particulate material Se concentrations do across transects because of the narrowly defined range of dissolved Se concentration. For Bay-Delta transects, K_ds range from 712 to 26,912 (**Figure 17; Table 8)**. For Suisun Bay-Carquinez Strait transects, K_ds range from 712 to 7,725 (**Table 9)**. For Rio Vista and Stockton to Benicia transects, K_ds range from 554 to 12,650 **(Table 10)**. As noted previously, these latter transects also extend to seaward locations and, hence, calculated means include combinations of data from both landward and seaward locations. These means and ranges for K_ds agree well with compiled field datasets for K_ds for estuaries and choices used in previous Bay-Delta modeling scenarios (i.e., 3,000 to 10,000) (Presser and Luoma, 2006; Presser and Luoma, 2009; Presser and Luoma, 2010).

Trophic Transfer Factors (TTFs)

Clam (*C. amurensis*)

The choice of food web is critical to modeling success because the particulate material to prey kinetics of bioaccumulation differs widely among invertebrates (Presser and Luoma, 2010). $TTF_{C. amurensis}$ derived from laboratory experiments averaged 6.25 over a range of assimilation efficiencies, ingestion rates, and efflux rate constants (Presser and Luoma, 2010). This average is within a range of 0.6 to 23 for invertebrate species, with TTFs for species of bivalves being the highest (Presser and Luoma, 2010).

Experimental physiological biodynamic parameters and rates are derived under idealized conditions in the laboratory. These biodynamic equations can be adjusted for a specific ecosystem by incorporating data from that system (Presser and Luoma, 2010). $TTF_{C. amurensis}$ is developed here for the estuary from a mechanistic equation for quantifying the biodynamics of *C. amurensis* and estuary-specific data for suspended particulate material (i.e., the food for clams). Selenium bioaccumulated at steady state by *C. amurensis* is calculated using a site-specific modification of equation (3)

$$C_{C. amurensis} = [(AE)\ (IR)\ (C_{\text{suspended particulate material}}] \div (k_e) \quad (14)$$

where $(AE)\ (IR)/k_e$ is defined as $TTF_{C. amurensis}$ and C_{food} is defined as the Se concentration in estuary suspended particulate material ($C_{\text{suspended particulate material}}$). Among field data available to quantify site-specific biodynamics of *C. amurensis* are spatially and temporally matched datasets from estuary transects (Doblin et al., 2006) for:

- suspended particulate material Se concentration;
- suspended particulate material C concentration;
- percentage of C in suspended particulate material; and
- percentages of suspended particulate elemental Se, adsorbed Se, and ogano-Se.

Our site-specific approach here differs from broader approaches where 1) laboratory data for biodynamic parameters such as AE and IR of particulate material may be generalized; 2) particulate Se concentrations may be an average of several phases of material (i.e., particulate Se_{total}); or 3) field data may be sparse and thus applied across an entire watershed (Presser and Luoma, 2009).

In general, for the purposes of a Bay-Delta location and estuarine processes, the suspended particulate material Se concentration carries with it assumptions about Se being associated primarily with organic material (detritus and living organisms). This allows us to determine IR on the same organic material basis (assuming clams seek organic material in the suspended particulate material) and to refine AE to account for suspended particulate material speciation (i.e., divide AE into three components of Se in suspended particulate material and their individual bioavailabilities). These assumptions are all rooted in well established biological understanding of bivalve feeding (Cammen, 1980; Lopez and Levinton, 1987). We ignore the possibility of uptake directly from water by the clams because that has been shown in a large body of work to be trivial (Luoma and Rainbow, 2005).

Justifications for values used in each parameter of the equation for a site-specific approach are:

1. We can either assume that Se is associated with carbonaceous materials or Se is spread across all suspended particulate material. For the former, the concentration of Se is expressed as µg Se/g C. We obtain µg Se/g C by dividing the suspended particulate material Se concentration (µg Se/g suspended particulate material) by mg C/mg suspended particulate material. For the present calculations we employ suspended particulate material Se concentrations as justified below.
2. IR is determined by filtration rate (125 L/g clam/d, Cole et al., 1992) multiplied by C (median = 0.4 mg C/L) to achieve the units (g C/g clam/d) in the suspended particulate material at each sampling. In the average condition in the estuary, clams ingest 5% of their body weight per day in C across all days for which data is available. At an average of 2% C in suspended particulate material (again, the average across all data) they ingest 2.5 times their body weight per day in total suspended particulate material. If IR is calculated at each of three low river discharge months where data is available, the average is 1.7 g suspended particulate material/g clam/d. Experience has indicated that the ingestion model is more accurate when actual outcomes are used (or averaged) for the generic situation (i.e., 1.7 g suspended particulate material/g clam/d) as compared to taking the average of each component of the outcome and calculating a generic average. Therefore, we recommend using 1.7 g suspended particulate material/g clam/d for modeling.
3. The derivation of a refined site-specific AE based on individualized bioavailabilities of Se in suspended particulate material uses observed fractions of particulate organo-Se, adsorbed Se, or elemental Se found in the estuary (Doblin et al., 2006) combined with individual AEs for those particulate Se species from the literature (living phytoplankton, AE = 60%; adsorbed on seston, AE = 40%; elemental, AE = 0%; Schlekat et al., 2004; Wang et al., 1996). The equation is:

   $$AE = (\text{fraction organic particulate Se})(AE_{\text{organic particulate Se}}) + (\text{fraction adsorbed particulate Se})(AE_{\text{adsorbed particulate Se}}) + (\text{fraction elemental particulate Se})(AE_{\text{elemental particulate Se}}) \quad (15)$$

   For example, if a site-specific sample of suspended particulate material collected in the estuary contains 45% Se in phytoplankton at an assumed AE of 60%; 30% Se adsorbed on seston at an

assumed AE of 40%; and 25% elemental Se in sediment at an assumed AE of 0%, then the composite AE = (0.45 x 0.6) + (0.30 x 0.40) + (0.25 x 0) = 0.39 or 39% AE.

4. We apply the efflux rate constant derived experimentally (Lee et al., 2006): $k_e = 0.03/d$.
5. When we model for times when all data are available from the estuary, we use all data from that sampling date. When we model generically we employ mean parameters.

Given the above protocol and assumptions, we can directly calculate *C. amurensis* Se concentrations for comparison to observed Se concentrations to validate predictions or calculate a $TTF_{C. amurensis}$ for use in modeling. If the data and assumptions given above are used in a site-specific modification of equation (4)

$$(IR)\ (AE) \div k_e = TTF_{clam} \qquad (16)$$

then

$$TTF_{clam} = (1.7\ g\ suspended\ particulate\ material/g\ clam/d)\ (0.39) \div 0.03 = 22.1$$

Or, in terms of a *C. amurensis* Se concentration, if a 0.84 µg/g dw suspended particulate material Se concentration is assumed, then

$$C_{C. amurensis} = (0.84\ \mu g\ Se/g)\ (1.7\ g/g/d)\ (0.39) \div 0.03/d = 18.6\ \mu g\ Se/g$$

Salinity-specific or transect specific Se concentrations and TTFs for *C. amurensis* can be calculated using the same protocol as above, but with percentages of C and suspended particulate material Se species observed in that transect. Thus, an individual *C. amurensis* Se concentration and $TTF_{C. amurensis}$ can be calculated from each matched set of data from the five suspended particulate material transects for the estuary (Doblin et al., 2006), making the predictions and derivations as detailed as the data permit. This data-intensive approach yields a mean $TTF_{C. amurensis}$ of 17.1 excluding April, 1999 transects data as out of the norm (i.e., El Niño condition in the estuary) or 18.1 using the focused approach for Suisun Bay-Carquinez Strait. We assume a $TTF_{C.amurensis}$ of 17 in modeling scenarios here. The range of TTFs across all estuarine conditions was 14-26. These values are higher than laboratory-derived values primarily because ingestion rates are higher in these field systems than in experiments. This is the first calculation of a field-derived TTF for a marine bivalve species.

Aquatic Insect and Other Invertebrates

A Se TTF_{insect} of 2.8 is used here for modeling a landward aquatic insect food web based on a compilation of insect TTFs by Presser and Luoma (2010) (**Figure 11**). This value represents a mean TTF derived from matched field datasets for particulate Se and insect Se concentrations in freshwater environments for several species of aquatic insects including mayfly, caddisfly, dragonfly, midge and waterboatman. TTFs for other potential invertebrates in landward food webs (range is 0.6 to 2.8) are shown in **Figure 11** (Presser and Luoma, 2010).

Bird Egg

Selenium TTFs for aquatic bird eggs are derived from data listed in USFWS (2009b) that is compiled from Heinz et al. (1989). TTFs calculated from matched data pairs for diet and bird egg tissue show a range of $TTF_{bird\ egg}$ from 0.87 to 4.7. The mean $TTF_{bird\ egg}$ is 2.7. If dietary Se concentrations that are unrealistic for estuary food webs are eliminated (< 1 µg/g dw and >18 µg/g dw), then a similar mean for $TTF_{bird\ egg}$ or 2.6 is calculated. A $TTF_{bird\ egg}$ of 2.6 is used here for modeling (**Figure 11**). A regression equation for diet and egg Se concentrations could be used in future modeling if scenario choices are specific enough in terms of dietary Se concentrations for birds and enough laboratory or field data are available. Modeling by Presser and Luoma (2010) showed a similar range for $TTF_{bird\ egg}$, but a somewhat lower TTF of 1.8 was chosen for modeling, which was near the lower limit for the captive mallard studies.

Fish Whole-Body or Muscle

A Se TTF_{fish} of 1.1 is used here for modeling based on a compilation of fish TTFs by Presser Luoma (2010) (**Figure 11**). This value represents a mean TTF derived from laboratory experiments and from matched field datasets for invertebrate and fish Se concentrations in saltwaters and freshwater environments (Presser and Luoma, 2010). TTFs derived from laboratory data from biodynamic experiments range from 0.51- 1.8. TTFs for different fish species derived from field studies range from 0.6 to 1.7. TTFs derived specifically for white sturgeon range from 0.6 to 1.7, with a mean of 1.3. Selenium TTFs for fish also can be derived from data given in USFWS (2009b) (**Table 5**). If data provided for laboratory dietary Se concentrations are limited to a range of 1 to 20 µg/g dw and the corresponding fish tissue Se concentrations, then TTFs calculated from the USFWS data range from 0.32 to 5.6, with a mean of 1.07. Again, as for modeling for birds, a regression equation for diet and fish whole-body or muscle Se concentrations could be used in future modeling if scenario choices are specific enough in terms of dietary Se concentrations for fish and enough laboratory or field data are available.

Validation

Prediction of Selenium Concentrations in *C. amurensis*

In general, biodynamic modeling is validated for a site location or food web by comparing predicted Se concentrations to observed Se concentrations. Monthly mean observed clam Se concentrations from USGS monitoring station 8.1 near Carquinez Strait from 1996-2009 (Linville et al., 2002; Kleckner et al., 2010) show the range of Se concentrations in *C. amurensis* (**Figure 21**). **Figure 21** also shows the time period (see inset) and compiled observed Se concentrations for *C. amurensis* from all monitoring stations during the transect collection period from November, 1997 to November, 1999. Each transect time period was two days, but reported clam data are several monthly averages near the transect collection.

Observed *C. amurensis* Se concentrations compare well with predicted Se concentrations using the biodynamic methodology described above (**Table 13**). Specific illustrated examples from the November, 1999 and June, 1998 estuary transects predict the variability seen in clams during the low flow season with a residence time of 70 days (12.6 µg/g dw observed versus 14.1 µg/g dw predicted) and a high flow season with a residence time of 11 days (4.4 µg/g dw observed versus 6.6 µg/g dw predicted), respectively (**Figure 22**).

Prediction of Existing Conditions Across Media

Comprehensive validation of Bay-Delta ecosystem-scale modeling (**Figure 9**) is through prediction of Se concentrations in water, suspended particulate material, and tissues of food-web species during times when observed datasets are available. The generalized equation for translation of a fish tissue Se concentration to dissolved or water-column Se concentration is shown in **Table 2** and **Figure 7**. Simulations here include conditions for 1) the estuary during November, 1999 for a clam-based food web (**Table 14)**; 2) Suisun Bay-Carquinez Strait during November, 1999 for a clam-based food web (**Table 15)**; and 3) the estuary during 2003-2004 for a landward insect-based food web (**Tables 16)**. Datasets are matched as much as possible given the scarcity of available data across all media. Several choices for $TTF_{sturgeon}$, $TTF_{C.\ amurensis}$, and K_d that are based on the ranges derived for the estuary are illustrated.

Using existing Se concentrations in seaward white sturgeon, landward white sturgeon, and largemouth bass in the Delta (Stewart et al., 2004; Foe, 2010) as the starting points for modeling, predicted prey, suspended particulate material, and dissolved Se concentrations are comparable to the range of observed conditions and most are within the range of observed Se concentrations (**Tables 14-16**). Simulations across the gradient of the Bay-Delta for a clam-based food web are calculated using both a seaward and a landward observed sturgeon Se concentration to test the uncertainty within a continuum approach (**Table 14**). The more focused Suisun Bay-Carquinez Strait simulations better narrow the range of suspended particulate material Se concentrations (**Table 15**). Simulations for an insect-based food web are all within observed dissolved Se concentrations (**Table 16**).

Modeling Scenarios and Predictions

Bay-Delta Continuum

Site-specific model parameters and methodology steps are illustrated in **Figure 9;** exemplified food webs are shown in **Figure 11;** and life cycles for critical phases and habitat are shown in **Figure 12**. Tissue Se concentrations and specified EC levels used as regulatory guidelines are from **Tables 5 and 6.** Species, modeled tissue guidelines, and associated ECs include:

- adult female white sturgeon (whole-body) at EC10 and 05 (8.1 and 7.0 µg/g dw);
- generic fish (whole-body) (5.0 µg/g dw);
- juvenile white sturgeon (diet) EC10 and 05 (1.6 and 0.95 µg/g dw);
- scoter or scaup (egg) at EC10, 05, and 0 (7.7, 5.9, 2.8 µg/g dw);
- scoter or scaup (diet) at EC10, 05, and 0 (5.3, 4.4, 2.3 µg/g dw);
- generic bird (egg) (same as above for EC10 egg of 7.7 µg/g dw);
- juvenile salmon (whole-body) at EC10, 05 and 0 (1.8, 1.5, 1.0 µg/g dw); and
- juvenile salmon (diet) at EC10, 05, and 0 (2.7, 2.2, 1.5 µg/g dw).

Targets for trout inhabiting the Delta are encompassed within those for salmon with the exception of extremely low targets for diet of 0.31 µg/g dw (EC0) and 1.0 µg/g dw (EC05).

Once choices for modeling scenarios are made, the generalized equation for translation of a fish tissue Se concentration to water-column Se concentration (**Table 2 and Figure 7**) is

$$C_{water} = (C_{fish}) \div (TTF_{fish})\,(TTF_{invertebrate})\,K_d \quad (17)$$

where $(K_d)\,(C_{water})$ is substituted for $C_{particulate}$ and the equation is solved for C_{water}. An analogous equation for translation of a bird egg Se concentration is

$$C_{water} = (C_{bird\ egg}) \div (TTF_{bird})\,(TTF_{invertebrate})\,K_d \quad (18)$$

Model scenarios and predicted allowable dissolved, suspended particulate material, and dietary Se concentrations for *C. amurensis*-based food webs are compiled in **Tables 17-18** and for aquatic insect-based food webs are compiled in **Table 19**. Food webs assume exposure of predators through a 100% clam diet or a 100% insect diet (see following section for mixed diet scenarios). K_ds are transect specific and TTFs are those listed above (TTF_{clam} for *C. amurensis* = 17.1; TTF_{insect} = 2.8; $TTF_{bird\ egg}$ = 2.6; TTF_{fish} = 1.1).

Hydrologic conditions (residence time, water-year type, flow season, and NDOI, **Tables 17-19**) are listed because of their importance in determining processes that affect Se transformations between dissolved and suspended particulate material Se concentrations and the bioavailability of organic matter and Se to food webs (see additional discussion in *Choices, Limitations, and Reduction of Uncertainty* section). Modeling for a clam-based food web is limited to wet and above normal years because transects are not available for below normal, dry, or critically dry conditions. Landward modeling is

limited to above normal (January, 2003 and April, 2003) and below normal (October, 2003 and January, 2004) water years because of data availability. Modeling exposure for low flow seasons is emphasized here in illustrated scenarios. Low flow seasons (and especially low flow seasons during dry years) are considered critical times (i.e., *ecological bottlenecks*) that mainly will determine the ecological effects of Se on the estuary (Presser and Luoma, 2006). As discussed previously, **Figure 12** illustrates the importance of the low flow season in terms of cycles of prey Se contamination and habitat-use by species important to the Bay-Delta.

Modeling here predicts allowable Se concentrations that are linked to calculated K_ds across the estuary for individual transects **(Figures 23-25)**. Thus, a Bay-Delta continuum approach can be used to generate a set of salinity-specific predictions. The theoretical constructs of predicted allowable dissolved Se concentrations illustrated in **Figures 23-25** are compared to observed dissolved Se concentrations in order to quantify the amount of reduction at a salinity-specific location, if needed, to meet assumed tissue guidelines for fish and birds. In a broader application, the approach generates means and ranges for dissolved and suspended particulate material Se concentrations across the estuary that can serve as an indicator to compare across time (**Tables 17-19; Figures 23-25**). As noted previously, use of a continuum mean may increase modeling uncertainty, but use of a continuum approach for modeling can give context for overall regulatory and management considerations by addressing salinity-specific locations.

Protection of fish for a seaward location is illustrated by specific exposure scenarios for an adult female white sturgeon (EC05 whole-body), a generic fish species (EC10 whole-body), and a juvenile white sturgeon (EC05 diet) under above normal water year and low flow season conditions (**Table 17; Figure 23**). Shown are: guidelines for whole-body fish; observed K_ds for November, 1999; and modeled dissolved, diet, and suspended particulate material Se concentrations (**Table 17**). Predicted allowed dissolved Se concentrations are shown across the salinity gradient and observed dissolved Se concentrations from the November 4-5, 1999 transect are given for comparison. All observed dissolved Se concentrations in November, 1999 exceed predicted allowable dissolved Se concentrations across the salinity gradient (**Table 17; Figure 23**).

Protection of aquatic birds at a seaward location is illustrated by specific exposure scenarios for a clam-eating bird species (EC05 diet and EC05 egg) and a generic bird species (EC10 egg) under above normal water year and low flow season conditions (**Table 18; Figure 24**). Both sets of scenarios are referenced to guidelines based on effects to mallards. As above, shown are: guidelines for bird eggs; observed K_ds for November, 1999; and modeled dissolved, diet, and suspended particulate material Se concentrations (**Table 18**). Predicted allowed dissolved Se concentrations are shown across the salinity gradient and observed dissolved Se concentrations from the November 4-5, 1999 transect are given for comparison. All observed dissolved Se concentrations in November, 1999 exceed predicted allowable dissolved Se concentrations (**Table 18; Figure 24)**.

Protection of fish for a landward location is illustrated by specific exposure scenarios for a juvenile Chinook salmon (EC05 diet and EC05 whole-body) under two different transect conditions (below normal, low flow season; above normal, high flow season) (**Table 19; Figure 25**). As above, shown are: guidelines for whole-body fish; observed K_ds for October 10, 2003 and April 22-23, 2003; and modeled dissolved, diet, and suspended particulate material Se concentrations (**Table 19**). Predicted allowed dissolved Se concentrations are shown across the salinity gradient from Rio Vista and Stockton to Benicia and observed dissolved Se concentrations are given for comparison. Interpretation across these transects is complex given the interface with freshwater and the variation in K_d. For landward sites (categorized as Delta, **Figure 25;** see discussion below) during conditions in the low flow season of October, 2003, observed dissolved Se concentrations exceed predicted allowable dissolved Se

concentrations for fish whole-body targets of 1.5 and 2.4 µg/g dw (**Figure 25**). For the furtherest landward sites during conditions in the high flow season of April, 2003, observed dissolved Se concentrations are less than predicted allowable dissolved Se concentrations for these targets (**Figure 25**).

Noted on **Figure 25** is a nominal division of Delta and Bay at Antioch, which is above Chipps Island. Data analysis and modeling for these transects assumes that an aquatic insect diet is consumed by fish even in habitats of higher salinity, a scenario that is unlikely. Additional data are needed to resolve food web questions such as this, along with monitoring at freshwater interfaces to better quantify and interpret the variation in location-specific K_ds. However, a broader point is proven by the results given in **Figure 25**: if the Bay supported an aquatic insect-based food web rather than a clam-based food web, then observed dissolved Se concentrations in the Bay would not be above predicted allowable dissolved Se concentrations during times and locations modeled here for the Bay.

Because of the importance of particulate material in determining food-web bioaccumulation, **Figure 26** shows observed and predicted suspended particulate material Se concentrations for the previously modeled exposure scenarios and set of guidelines (**Figures 23-25**). In addition, an exposure scenario for the estuary during June, 1998 (wet year, high flow season) is modeled (**Tables 17 and 18**). Patterns and ranges of particulate enrichment during a low flow season and high flow season are distinctly different and underlie the outcomes of overall exposure in modeling (also see *Choices, Limitations, and Reduction of Uncertainty* section). For seaward clam-based food webs during the low flow season in November, 1999, observed suspended particulate material Se concentrations exceed predicted allowable suspended particulate material Se concentrations (**Figure 26A**). For a seaward clam-based food webs during the high flow season in June, 1998 (an El Niño event), outcomes are varied for low salinity sites (**Figure 26B**). However, observed suspended particulate material Se concentrations exceed predicted allowable suspended particulate material Se concentrations at higher salinities (**Figure 26B**). For landward aquatic insect-based food webs (Delta) during October, 2003 (low flow season) and April, 2003 (high flow season), observed mean suspended particulate material Se concentrations exceed predicted allowable suspended particulate material Se concentrations for juvenile salmon, except at two low salinity locations (**Figure 26C**).

Suisun Bay-Carquinez Strait

As previously described, a focused approach for Suisun Bay-Carquinez Strait uses compartmentalized data to narrow modeling to a specific location (**Figure 14**). Additionally, this site is especially impacted by oil refinery effluents. This narrowing of modeling eliminates some of the uncertainties associated with end-member processes (i.e., the variability at ocean-influenced and freshwater-influenced sites) that are part of the spectrum of the Bay-Delta. Landward sites can show the influence of elevated Se in allochthonous suspended particulate material and seaward sites can show the influence of amplified Se processing, a pattern seen in other estuaries (LeBlanc and Schroeder, 2008; Presser and Luoma, 2009) (**Figures 16, 17, 20**).

For modeling, a focused approach for Suisun Bay-Carquinez Strait lends itself mathematically to representation by a bounded range of parameter choices for regulatory consideration. Hence, modeling scenarios and predictions for *C. amurensis*-based food webs generated here illustrate the effect of a limited set of choices for Se effect guidelines, K_ds, and TTFs (**Tables 20 and 21**). As discussed previously, model choices can be altered to illustrate sensitivity to model parameters and uncertainties in model predictions under a range of regulatory or management actions. Comparative scenarios thus develop a range of predictions and identify data gaps and monitoring needs.

Tables 20 and 21 show comparative prediction scenarios using a general set of Se effect guidelines for whole-body fish (8, 5, and 1.5 ppm dw) and for bird eggs (12, 7.7, 5.9 ppm dw) suggested through discussion with USEPA and USFWS. For Suisun Bay-Carquinez Strait, four choices for K_d are illustrated (mean K_ds of 1,180; 2,666; 3,435; and 5,986 during increasing residence times in low and high flow transects in 1998 and 1999) (**Tables 20 and 21**). Choices for TTF_{fish} are 0.8 and 1.1 and the choice for $TTF_{bird\ egg}$ is 2.6. Choices for TTF_{prey} are:

- *C. amurensis*, TTF = 17;
- mixed diet composite, TTF = 8.8 (50% *C. amurensis*, TTF = 17; 50% amphipod, TTF = 0.6);
- aquatic insect (TTF = 2.8).

If a mixed diet composite TTF is used in modeling, then predicted prey Se concentrations also are composites that would need to be separated into individual components to assess allowable *C. amurensis* and amphipod Se concentrations. For example, if the predicted particulate Se concentration of 0.826 µg/g is derived using a $TTF_{C.\ amurensis\ +\ amphipod}$ of 8.8, then allowable individual prey Se concentrations are

(0.826 µg/g) (17) (0.5) = 7.02 µg/g for *C. amurensis*, and

(0.826 µg/g) (0.6) (0.5) = 0.25 µg/g for a generic amphipod

for a sum of 7.27 µg/g as a composite prey Se concentration. Therefore, *C. amurensis* could not exceed 7.02 µg/g in this mixed diet composite scenario ($TTF_{C.\ amurensis\ +\ amphipod}$) as compared to 7.72 µg/g in a scenario using a 100% clam diet (TTF= 17). However, the predicted allowed particulate Se concentrations would be affected more significantly, with 0.428 µg/g allowed in the single species scenario and 0.826 µg/g in the mixed diet scenario. Overall though, the effect of this theoretical construct is to reduce the bioaccumulative potential of the modeled invertebrate species.

Modeling for the area of Suisun Bay-Carquinez Strait within the specified set of parameters listed above, gives ranges of predicted dissolved, suspended particulate material, and prey Se concentrations that can serve as the basis for regulatory consideration (**Tables 20 and 21**). Choices by regulatory agencies of necessary and sufficient combinations of model parameters will set the outcomes for criteria development and regulatory action in the future.

Landward Sites

Comparative prediction scenarios also are generated from transects that focus on landward sites (Lucas and Stewart, 2007). Comparative outcomes from scenarios for aquatic insect-based food webs are illustrated in **Tables 22 and 23**. For a landward aquatic insect-based food web four choices for K_d are illustrated (means K_ds of 2,268, 2,981, 2,684, and 5,855 during low and high flow transects in 2003 and 2004) (**Tables 22 and 23**). Choices for predator TTFs are TTF_{fish} = 1.1 and $TTF_{bird\ eggs}$ = 2.6. As above, ranges of predicted dissolved, suspended particulate material, and prey Se concentrations can serve as the basis for regulatory consideration.

Choices, Limitations, and Reduction of Uncertainty

Several figures throughout the report illustrate processes and outcomes important to the site-specific modeling approach used here for the Bay-Delta. These figures represent the fine-scale information that defines and quantifies the ecological, hydrodynamic, and biodynamic processes of the estuary that underlie and enable modeling. These figures include details of: sources and food webs (**Figure 2**); site-specific modeling approach (**Figure 9**); transformation and partitioning reactions **(**K_d) (**Figure 13**); species and effects (**Figures 8, 10, 11, and 12**); and hydrodynamics during sampling of the estuary **(**e.g., **Figure 14**).

Presser and Luoma (2010) discuss the limitations of an ecosystem-scale modeling approach in general, but also note how models provide insights that advance understanding of value both to science and management. For the Bay-Delta, combining modeling with knowledge of fine structure estuary processes is important for reducing uncertainty and fortifying a mechanistic basis for modeling applications and predictions in the future. For example, **Figure 17** shows the effect of estuary processes on suspended particulate material Se concentrations during a low and a high flow season (April, 1999; November, 1999) across the Bay-Delta continuum. In further analysis of data for Suisun Bay-Carquinez Strait, **Figure 27** shows mean observed dissolved and suspended particulate material Se concentrations and K_ds as a function of residence time. Dissolved Se concentration decreases as residence time increases, but suspended particulate material Se concentrations increase sharply with increasing residence time. Including suspended particulate material Se concentrations and residence time as variables in **Figure 27** illustrates that transformation of dissolved Se to particulate Se (i.e., dissolved Se decreases as suspended particulate Se concentrations increases) occurs in the estuary as flow slows down (i.e., during increased residence time) as expected from theoretical considerations of Se phase dynamics (see previous discussion and Presser and Luoma, 2010). Given the steepness of the curve, regulation of suspended particulate material Se concentration may be a more sensitive parameter on which to assess change and choice. Defining or conceptualizing a baseline dissolved Se concentration or condition for the estuary is less certain because of the small dynamic range of dissolved Se concentrations.

If mean observed *C. amurensis* Se concentrations measured in samples from Suisun Bay-Carquinez Strait during the months surrounding the transect sampling are added to **Figure 27** to complete linkages of dissolved, particulate, and prey phases, then it is seen that *C. amurensis* Se concentrations also increase with increasing residence time (**Figure 27**). To further elucidate the efficiency of Se assimilation in this food web, **Figure 28** shows that the percentage of suspended particulate material organo-Se reaches 50% in both plots at a residence time of 22 days. Hence, the presence of a majority of organo-Se leads to efficient uptake into *C. amurensis* at increased residence times.

Thus, **Figures 27 and 28** inform the model as to 1) the fundamental underlying mechanistic linkage between hydrodynamics and Se dynamics in the estuary and 2) why scenarios should be tied to specific transformation and flow conditions (see also **Figure 9** for linked mechanistic components of model approach). Further, **Figure 27** helps establish the benefits of a K_d-approach in reducing uncertainties otherwise associated with modeling the complex processes of transformation and speciation, and of a biodynamic approach that incorporates the assimilation efficiency of particulate material.

Data Collection, Model Updates, and Refinements

Current Data and Additional Modeling: Current data for dissolved, suspended particulate material, invertebrate, and predator Se concentrations (i.e., spatially and temporally matched datasets) are needed to update model predictions. Sampling and analysis would include Se concentrations for the dissolved phase; suspended particulate material; seaward bivalves and amphipods (or other seaward invertebrate species); aquatic insects (or other landward invertebrate species); sturgeon, salmon, steelhead (or other fish species); and eggs and tissue from avian species (see complete list in **Figure 11**). A designated set of methods for collection and analysis of samples used in modeling of the Bay-Delta are needed to add consistency to model inputs. Further documentation of a predator's dietary preference also would be desirable because food webs may change as criteria development goes forward. Follow-

up modeling can be done in response to collection of additional monitoring data and consideration of the pending USEPA national fish tissue guidance.

Representation of Hydrologic Conditions: Analysis of flow conditions to give context to the environmental partitioning and foodweb biodynamic processes described here is fundamental to modeling for the Bay-Delta. For example, transect data for wet and above normal water years illustrate how Se concentration, Se speciation, and K_d profiles vary during conditions in April, 1999 (a high flow season) as compared to November, 1999 (a low flow season) (**Figures 17 and 20)**.

Below Normal, Dry, and Critically Dry-Year Low-Flow Conditions: Available seaward datasets do not include data from a below normal, dry, or critically dry year to model a clam-based food web. Hence, modeling here could not assess effects in the North Bay during times of low flow in a dry year (i.e., the *ecological bottleneck*) and locations where oil refinery Se effluents may exert their maximum effect. Available landward datasets do not include data from a dry year to model an insect-based food web. Comparing model predictions for scenarios based on a range of hydrologic conditions will help develop a more complete basis for regulatory guidance. The estuarine system is highly variable in terms of flow (**Figure 15**) because of management demands and the natural variability induced by climate.

Hydrodynamic Tracking of Se: A Se budget through the estuary is needed to differentiate sources and develop relationships to internal refinery sources and upstream river sources. For example, quantifying end-member Se concentrations for the Sacramento River and San Joaquin River would define the influence of riverine sources on Se concentrations in the estuary. Spatial and temporal definition in such a study should be such to resolve questions as future management strategies are implemented (**Figure 2**).

Chronic Effects in Birds: Modeling of clam-eating migratory bird species, such as scoter and scaup, in reference to potential chronic Se effects that may impact staging of diving ducks overwintering in the estuary (**Figures 8, 10 and 12**) would assess these species in scenarios relevant to the estuary use by these bird species.

Changes in Population Dynamics and Species Diversity: Monitoring and comprehensive compilation of data for community change, introduction of species, loss of species, and loss of individuals that are threatened or endangered would document changes to ecological pathways important to the sustainability and restoration of the estuary.

Site-Specific TTFs: Updated Se TTFs for *C. amurensis* could be calculated from modern matched datasets for suspended particulate material and bivalve Se concentrations. Biodynamic parameters could be investigated to further define bivalve kinetics. Modeling for *C. amurensis* also could be location-specific to add more specificity to modeling. Modeling could utilize TTF_{fish} of up to 1.9. Important site-specific Se TTFs to be updated include those for aquatic insects and other invertebrates that serve as food for landward food chains. Matched datasets for suspended particulate material and invertebrate Se concentrations would be needed.

Field-derived TTFs for bird species: Field-derived TTFs for bird species (and other predators) would encompass habitat use and other factors that influence exposure.

Particulate Material Se Concentrations: In modeling, derivation of a particulate Se concentration can be very site-specific as defined by the monitoring data available for modeling. This type of refinement to model parameters is discussed in Presser and Luoma (2010). For example, a concentration of Se in food can be calculated that takes into account site-specific bioavailability of particulate material to invertebrates. The generalized equation is

$$C_{particulate} = (AE)\ (C_{particulate\ a})\ (\text{sediment fraction}) + (AE)\ (C_{particulate\ b})\ (\text{detritus fraction}) + (AE)\ (C_{particulate\ c})\ (\text{algae fraction})] \quad (19)$$

In terms of suspended particulate material as used for Bay-Delta modeling, a composite assimilation efficiency can be derived (see equation 15) to adequately represent food for clams.

Mixed Diet: Rather than assuming a 100% clam-diet for predators, allowable dissolved Se concentrations could be calculated using the equation for a mixed invertebrate diet

$$C_{water} = (C_{fish}) \div (TTF_{fish}) (K_d) [(TTF_{invertebrate\ a}) (\text{prey fraction})] + [(TTF_{invertebrate\ b}) (\text{prey fraction})] + [(TTF_{invertebrate\ c}) (\text{prey fraction})] \quad (20)$$

The percentage of clam in the diet of species at risk (**Figure 11)** could be used specifically. A choice as to the percentages of other types of invertebrates in the diet of each predator and a $TTF_{invertebrate}$ would need to be developed or assumed from literature sources for each additional invertebrate modeled.

Longer Food Webs: For fish-eating birds or the bald eagle food webs, model scenarios could incorporate sequential bioaccumulation in longer food webs

$$C_{water} = (C_{fish}) \div (TTF_{fish}) K_d (TTF_{invertebrate}) (TTF_{forage\ fish}) \quad (21)$$

$$C_{water} = (C_{fish}) \div (TTF_{fish}) K_d (TTF_{TL2\ invertebrate}) (TTF_{TL3\ invertebrate}) (TTF_{TL3\ fish}) \quad (22)$$

For example, modeling a Dungeness crab food web would constitute an additional bioaccumulative step when juveniles are consumed by large predator fish or adults are consumed by mammals (**Figure 11**).

Specificity for Low-Salinity Locations: As noted previously, low-salinity locations were not sampled on a consistent basis for the Bay-Delta during the analysis periods reported on here. Designation of specific sampling locations would greatly improve predictions for landward sites. Data analysis that compares dissolved and suspended particulate material Se concentrations and calculated K_ds at specific locations across time also would be helpful to regulatory guidance. Datasets specific to Se concentrations in landward food webs (e.g., invertebrates and salmonids) need to be collected because the current record is inadequate.

Reference Dose Methodology Comparison: Ecosystem-scale modeling here is applicable to using a dietary Se concentration as a regulatory guideline. The USFWS provided, in some cases, both tissue and diet Se concentrations as effects levels. An alternative approach would be to calculate a dietary Se concentration or dose for aquatic wildlife based on a protective reference dose and specific body weights of predators (USFWS, 2003; Presser and Luoma, 2010). Validation would be important; uncertainties in the relationship of body weight and ingestion rate, for example, would need to be considered. Results of this analysis could be compared to those outcomes of modeling scenarios shown here to add weight to the conclusions drawn for the protection of predators in the Bay-Delta estuary. Steps like this in the methodology could also serve to harmonize regulation, a goal long sought in obtaining consensus and understanding (Reiley et al., 2003).

Data Analysis: Ecosystem-scale modeling is more than mathematical correlations. Its success, in part, depends on formalization and conceptualization of existing data for food web ecology, system hydrology, and the biogeochemistry of partitioning. Thus, ultimately a comprehensive Bay-Delta model (i.e., addressing interconnection of estuarine processes, habitats, species, and stressors) as originally conceived by CALFED, would help with details of species, habitat use, competing contaminants, and estuary hydrodynamics.

Conclusions

Analysis from the biodynamically-based methodology for ecosystem-scale modeling as presented in Presser and Luoma (2010) showed, in general, that:

- a crucial factor ultimately defining Se toxicity is the link between dissolved and particulate phases at the base of the food web (i.e., K_d);
- collection of particulate material phases and analysis of their Se concentrations are key to representing the dynamics of the system;

- bioaccumulation in invertebrates is a major source of variability in Se exposure of predators within an ecosystem, although that variability can be explained by invertebrate physiology (i.e., $TTF_{invertebrate}$);
- TTF_{fish} is relatively constant over the range of species considered here; and
- Se concentrations are at least conserved and usually magnified at every step in a food web.

In addition, an ecosystem-scale approach: 1) clearly documents pathways that connect dissolved Se to bioaccumulated Se in species of concern; 2) provides a record of supporting data on which to base decisions; 3) uses site-specific ecology, biogeochemistry, and hydrology; 4) includes choices explicitly throughout the decision-making process; 5) addresses uncertainties by showing outcomes of different choices in modeling scenarios; and 6) validates outcomes through comparison to field data.

A site-specific methodology for development of Se criteria for the Bay-Delta includes the following steps:

- identification of predators at risk and their critical life stages;
- development of conceptual food-web models for predators at risk that include dietary preferences (i.e., percentages of species of invertebrate consumed);
- development of seasonal-cycle and habitat-use diagrams for prey and predators at risk;
- derivation of tissue guidelines for species at risk specific to exposure route, effect endpoint, and magnitude of effect (EC0, EC05, and EC10);
- analysis of spatially and temporally matched datasets for dissolved and suspended particulate material Se concentrations across the salinity gradient;
- derivation of salinity-specific or location-specific K_ds;
- derivation of site-specific $TTF_{C.\ amurensis}$;
- selection or development of TTF_{fish}, TTF_{bird}, and TTFs for other invertebrates;
- validation of modeling through comparison of predictions to observed Se concentrations;
- development of exposure scenarios specific to location and season or residence time; and
- prediction of allowable dissolved, suspended particulate material, and prey Se concentrations.

Consideration of compliance with allowed Se concentrations across media (i.e., water, particulate, prey and predator) harmonizes regulation and is a measure of ecological consistency and relevance of the links among exposure, transfer, and effects.

Modeling here for a seaward *C. amurensis*-based food web is referenced to data from transects from November, 1997 to November, 1999. Modeling for a landward aquatic insect-based food web is referenced to data from transects from January, 2003 to January, 2004 from Rio Vista and Stockton to Benicia. USFWS effect guidelines and associated levels of protection are used in modeling to predict toxicity under different regulatory proposals. Validation of the model shows the model is able to generate 1999-2000 seaward conditions for Se concentrations in a *C. amurensis* to white sturgeon food web and 2003 landward conditions for Se concentrations in an aquatic insect to largemouth bass food web.

Site-specific analysis and modeling show that:

- estuarine approaches that focus on seaward, landward, and Suisun Bay-Carquinez Strait locations can illustrate influences of site, time, and flow-specific partitioning conditions;
- choices of geographic constraints, species, diet, and estuary conditions all are influential in risk management for Se;
- the field-derived $TTF_{C.\ amurensis}$ that is derived here is the first instance of a field-derived TTF for a marine bivalve species; the value is appreciably higher than laboratory-derived values;
- modeling of species at risk takes into account both inherent sensitivity and potential exposure;

- a *C. amurensis*-based food web in the estuary is highly vulnerable to Se inputs because of high potential exposure;
- regulation of suspended particulate material Se concentration may be a more sensitive parameter on which to assess change and choice because of the small dynamic range of dissolved Se concentrations in the estuary; and
- critical ecological times are functionally connected to the underlying dynamics and processes of low flow periods in Suisun Bay-Carquinez Strait thus allowing modeling and prediction as changes occur in management and regulations.

The approach could be refined by:

- collecting modern matched datasets for water, suspended particulate material, invertebrates, fish, and birds as illustrated in **Figure 11**;
- determining contributions of specific sources;
- quantifying end-member Se concentrations and their hydrodynamic connection to estuary Se concentration;
- further limiting geographic (e.g., Suisun Bay) and temporal constraints (dry year, low flow season);
- analyzing processes at interfaces of freshwater/bay/ocean;
- addressing biodynamics of Se and chronic toxicity in avain species; and
- further linking ecosystem-scale modeling to fine structure estuary processes.

Analysis of Se concentration and speciation for characterized particulate phases are practical measures of the complex water/sediment/particulate *milieu* that forms the base of the food web and is consumed as food by invertebrates. Future monitoring to increase the suspended particulate material database under a suite of flow conditions would enhance our understanding of estuarine transformation. Monitoring invertebrate Se concentrations in food webs also is a practical, informative step in monitoring because the first and second most variable aspect of Se dynamics (i.e., K_d and $TTF_{invertebrate}$) are integrated into invertebrate bioaccumulation.

Expressly for modeling of avian species, uncertainties exist around biodynamic modeling parameters **(**$TTF_{bird\ egg}$); movement and migration; and links of bioaccumulation, exposure, and toxicity under site-specific conditions. Additionally, modeling of overwintering clam-eating migratory bird species, such as scoter and scaup, based on potential chronic Se effects that may impact staging would assess these species in scenarios relevant to their use of the estuary. Chronic toxicity effects include:

- compromised body condition (low body mass);
- oxidative stress (increased susceptibility to disease as immune system is suppressed);
- decreased winter survival;
- decreased reproductive fitness (decreased breeding propensity, reduced recruitment) and;
- behavioral impairment (missed breeding window, delayed timing of departure).

Predictions from a reference dose methodology for birds also would strengthen outcomes for protection of avian species.

In sum, the amount of available data for the Bay-Delta may be limited, especially under below normal, dry, and criticallydry year conditions, but given the specificity of Se processes and food web species that is documented and modeled here, enough is known about the biotransfer of Se and the interconnectedness of habitats and species to set a range of limits and establish an understanding of the relevant conditions, biological responses, and ecological risks critical to management of the Bay-Delta. Site-specific modeling here bounds predictions within spatial and temporal components and quantifies key characteristics of the system that can influence exposure and uptake of Se by fish and birds. The

uncertainty that stems from the variability in these processes reflects the complexity of the estuary. Nevertheless, the methodology used here is able to document fine-structure processes in different habitats and provide context for future scenario development. The greatest strength of the analytical and modeling processes is that it is an orderly, harmonized derivation approach across media for assessing different choices of Se criteria for protection of fish and birds.

Acknowledgments

This work was supported by U.S. Environmental Protection Agency (San Francisco, California, Region 9) and the U.S. Geological Survey National Research and Toxics Substances Hydrology Programs. Thanks to Diane Fleck and Eugenia McNaughton of USEPA for support and insight throughout this process. Thanks also to the U.S. Fish and Wildlife Service, Division of Environmental Quality, Sacramento, California, for provision of effects levels for predators at risk in the San Francisco Bay-Delta Estuary.

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Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California

Administrative Report

Tables 1 through 22

U.S. Department of the Interior
U.S. Geological Survey
December, 2010

Find the full report and other attachments at http://www.epa.gov/region9/water/ctr

Table 1. Oil refinery Se loads discharged to the Bay-Delta during 1986-2009. [San Francisco Bay Board, 1992a,b; 1993; Lila Tang and Johnson Lam, San Francisco Bay Board, personal communication, 1999-2006; USEPA, 2010].

year	Chevron Refinery (Richmond, CA; discharge to San Pablo Bay) lbs Se/year	Martinez (Shell) Refinery (Martinez, CA; discharge to Carquinez Strait) lbs Se/year	Tosco (Conoco Phillips) Refinery (Rodeo, CA; discharge to San Pablo Bay) lbs Se/year	Tesoro Golden Eagle Refinery (Martinez, CA; discharge to Suisun Bay) lbs Se/year	Valero Refinery (Benicia, CA; discharge to Suisun Bay) lbs Se/year	refinery total lbs Se/year	proposed permitted load[d] lbs Se/yr
1986	-	-	-	-	-	5783	-
1987	-	-	-	-	-	4419	-
1988	-	-	-	-	-	4417	-
1989	-	-	-	-	-	3953	-
1990	-	-	-	-	-	5222	-
1991	-	-	-	-	-	5634	-
1992	-	-	-	-	-	5592	-
1993	-	-	-	-	-	-	2666
1994	-	-	-	-	-	-	2222
1995	-	-	-	-	-	-	1727
1996	-	-	-	-	-	-	1234
1997	-	-	-	-	-	-	1234
1998	-	-	-	-	-	-	1234
1999	314	441	107	129	133	1124	1234
2000	174	368	114	130	126	912	1234
2001	282	451	123	100	144	1100	1234
2002	197	455	145	145	153	1095	1234
2003	239	464	90	144	175	1112	1234
2004	204	472	115	149	159	1099	1234
2005	276	490	154	154	177	1251	1234
2006	278	542	159	193	195	1367	1234
2007[a]	-	-	-	-	-	-	1234
2008	221	709	187	193[b]	160	1470[c]	1234
2009	210	515	209	193[b]	160	1287[c]	1234

[a]Data not available from USEPA (2010); [b]Data not available from USEPA (2010), therefore estimated as 2006 Se load ; [c]Includes estimated Se load for Tesoro Refinery; [d]baseline for reductions defined as 1989-1991 average annual loading of 4,935 lbs Se/year.

Table 2. Generalized steps in ecosystem-scale methodology for translation of a tissue Se concentration to a water-column Se concentration for protection of fish and aquatic-dependent wildlife. [Adapted from Table 5, Presser and Luoma, 2010.]

Translation of Tissue Criterion to Water-Column Concentration

• Develop a conceptual model of food webs in watershed.
• Choose toxicity guideline for fish or aquatic bird species in estuary.
• Choose fish or bird species to be protected in watershed.
• For fish, choose species-specific TTF_{fish} or use default TTF_{fish} of 1.1; for birds, choose species-specific TTF_{ibird} or use default TTF_{bird} of 2.0.
• Identify appropriate food web(s) for selected fish or bird species based on species-specific diet.
• Choose site-specific $TTF_{invertebrate}$ for invertebrates in selected food web(s) or use default $TTF_{invertebrate}$ for species of invertebrate (see list in Presser and Luoma, 2010).
• Choose site-specific K_d or use K_d indicative of a) generalized source of Se and receiving water conditions or b) site-specific hydrologic type and speciation; or a default K_d of 1000 (see list in Presser and Luoma, 2010).
• Solve equation(s) for allowable water-column concentration for protection of fish or birds (i.e., predator)
If assume single invertebrate diet, then
o $C_{water} = (C_{predator}) \div (TTF_{predator})\ K_d\ (TTF_{invertebrate})$
If assume a mixed diet of invertebrates, then
o $C_{water} = (C_{predator}) \div (TTF_{predator})\ (K_d)\ [(TTF_{invertebrate\ a})\ (\text{prey fraction})] + [(TTF_{invertebrate\ b})\ (\text{prey fraction})] + [(TTF_{invertebrate\ c})\ (\text{prey fraction})]$
If assume sequential bioaccumulation in longer food webs, then
o $C_{water} = (C_{predator}) \div (TTF_{predator})\ K_d\ (TTF_{invertebrate\ a})\ (TTF_{forage\ fish})$
o $C_{water} = (C_{predator}) \div (TTF_{predator})\ K_d\ (TTF_{TL2\ invertebrate})\ (TTF_{TL3\ invertebrate})\ (TTF_{TF3\ fish})$
where TL = trophic level

Table 3. List of species considered for evaluation of Se exposure risk in the San Francisco Bay/Delta. [Reproduced from USFWS, 2008, Table 1. Updates, personal communication, S. Detwiler, USFWS, Sacramento, California, 11/17/10).

Common Name	Scientific Name	Federal Status	California State Status	Potential to be adversely affected by selenium in Bay/Delta*
Mammals				
salt marsh harvest mouse	*Reithrodontomys raviventris*	endangered	protected	As a terrestrial herbivorous mammal, unlikely to be among the most exposed and sensitive of wildlife species; therefore not likely to be a "species most at risk."
Birds				
American white pelican	*Pelecanus erythrorhynchos*	MBTA	concern	SF Bay is North end of West Coast distribution of non-breeders. Preys on some bottom-feeding fish as well as schooling fish, but not likely to be a "species most at risk.".
California brown pelican	*Pelecanus occidentalis californicus*	endangered (delisted 11/2009, MBTA)	protected, endangered (protected 2/09)	SF Bay is North end of W Coast distribution. Feeds mainly on surface-schooling fish; therefore, not part of benthic-based food chain and not likely to be a "species most at risk."
white-faced ibis	*Plegadis chihi*	concern	concern	Breeds and winters in San Joaquin Valley. Inhabits mainly freshwater wetlands, but also estuarine wetlands. Eats aquatic and moist soil invertebrates. At some risk but not likely to be a "species most at risk."
double-crested cormorant	*Phalacrocorax auritus*	MBTA	concern	Winters in Central Valley and SF Bay/Delta. Feeds on bottom-dwelling fish and invertebrates as well as schooling fish. At some risk but not likely to be a "species most at risk."
American bittern	*Botaurus lentiginosus*	concern	none	Feeds mainly in freshwater marshes, eating mainly insects and small vertebrates; therefore not likely to be a "species most at risk."
western least bittern	*Ixobrychus exilis hesperis*	concern	concern	Breeds in SF Delta. Feeds in fresh and brackish water marshes, eating mainly small fish and insects; therefore not likely to be a "species most at risk.".
Aleutian Canada goose	*Branta canadensis leucopareia*	delisted, MBTA	none	Winters in California, feeding primarily in upland crops and fallow fields. Sensitive to selenium but unlikely to be exposed in estuary; therefore not likely to be a "species most at risk."
greater scaup	*Aythya marila*	MBTA	none	SF Bay is one of 2 major wintering areas on W coast of N America. Feeds on benthic mollusks that efficiently bioaccumulate selenium in the SF Bay/estuary, therefore likely to be a "species most at risk."
lesser scaup	*Aythya affinis*	MBTA	none	SF Bay is an important wintering area; feeds on clams; therefore likely to be a "species most at risk."
black scoter	*Melanitta nigra*	MBTA	none	Winters along California coast, diving mainly for mollusks; therefore likely to be a "species most at risk."
white-winged scoter	*Melanitta fusca*	MBTA	none	Winters along California coast and estuaries, diving mainly for mollusks; therefore likely to be a "species most at risk."
surf scoter	*Melanitta perspicillata*	MBTA	none	Winters along California coast, diving mainly for mollusks; therefore likely to be a "species most at risk."
osprey	*Pandion haliaetus*	MBTA	concern	High trophic level piscivore; not at risk overall and exposure well represented by bald eagle. Therefore not treated here as a "species most at risk."
bald eagle	*Haliaeetus leucocephalus*	delisted, MBTA,BGEPA	protected, endangered	High trophic level piscivore; at risk overall and exposed to aquatic food chain in the SF Bay/Delta; therefore likely to be a "species most at risk."

Common Name	Scientific Name	Federal Status	California State Status	Potential to be adversely affected by selenium in Bay/Delta*
northern harrier	*Circus cyaneus*	MBTA	concern	High trophic level but less exposed to aquatic food chain than bald eagle; therefore not likely to be a "species most at risk."
white-tailed kite	*Elanus leucurus*	concern	protected	Feeds mainly on terrestrial mammals; minimal exposure to aquatic selenium; therefore not likely to be a "species most at risk."
American peregrine falcon	*Falco peregrinus anatum*	delisted, MBTA	protected, concern	Delisted but monitored for population status and contaminants. Exposed to selenium in aquatic food chain as predator on piscivorous birds, but exposure generally diluted by terrestrial component of diet; therefore not likely to be a "species most at risk."
prairie falcon	*Falco mexicanus*	MBTA	concern	Winters along California coast; high trophic level but in mainly terrestrial food chain; therefore not likely to be a "species most at risk."
California black rail	*Laterallus jamaicensis coturniculus*	MBTA	protected, concern	Inhabits tidal marsh in SF Bay estuary. Feeds on invertebrates, including snails, but also seeds; therefore not likely to be a "species most at risk."
California clapper rail	*Rallus longirostris obsoletus*	endangered	protected, endangered	Subspecies endangered and endemic to SF estuary; feeds on benthic invertebrates, including filter-feeders that bioaccumulate selenium; therefore likely to be a "species most at risk."
marbled murrelet	*Brachyramphus marmoratus*	threatened	endangered	Forages in bays along Pacific coast in summer, but not recorded in SF Bay/Delta. Dives for pelagic food: schooling fish and euphausiids (krill). Therefore not likely to be a "species most at risk."
California least tern	*Sterna antillarum browni*	endangered	protected, endangered	Breeds primarily in Central San Francisco Bay but can nest throughout estuary. Feeds throughout estuary, mainly on surface fish, not part of the benthic mollusk-based food chain; therefore not likely to be a "species most at risk."
black tern	*Chlidonias niger*	concern	concern	Breeds in C Valley including SF Delta. Feeds on marine and freshwater surface fish and insects; therefore not likely to be a "species most at risk."
Caspian tern	*Sterna caspia*	MBTA	none	Preys heavily on juvenile salmonids, but not endangered overall; therefore not likely to be a "species most at risk."
western snowy plover	*Charadrius alexandrines*	threatened	concern	Terrestrial component of diet likely provides dietary dilution of aquatic system selenium exposures; have been shown to be very tolerant of selenium exposure; therefore not likely to be a "species most at risk."
mountain plover	*Charadrius montanus*	concern	concern	Winters in agricultural fields of Sacramento/San Joaquin Valley. Diet mainly terrestrial; therefore not likely to be a "species most at risk."
tricolored blackbird	*Agelaius tricolor*	concern	concern	Nests colonially, mainly in freshwater marshes. Feeds on terrestrial as well as freshwater insects; therefore not likely to be a "species most at risk."
Reptiles				
giant garter snake	*Thamnophis gigas*	threatened	threatened	Aquatic predator, but not known to inhabit the estuary; therefore not likely to be a "species most at risk" in the estuary.
Fish				
Chinook salmon	*Oncorhynchus tshawytscha*	endangered/ threatened	endangered/ threatened	Sensitive to selenium; most sensitive life stages occur in rivers and estuary; therefore likely to be a "species most at risk."
steelhead	*Oncorhynchus mykiss*	threatened	none (in Central Valley)	Sensitive to selenium; most sensitive life stages occur in rivers and estuary; therefore likely to be a "species most at risk."
delta smelt	*Hypomesus transpacificus*	threatened	threatened	Endemic to the Bay/Delta estuary. Feeds on zooplankton, not a pathway of greatest exposure, but threatened overall, so included as a "species most at risk."

Common Name	Scientific Name	Federal Status	California State Status	Potential to be adversely affected by selenium in Bay/Delta*
longfin smelt	*Spirinchus thaleichthys*	concern	endangered	SF Bay/estuary is S end of distribution. Prefers more saline water than delta smelt. Overall less threatened and probably less exposed than delta smelt so adequately represented by that species. Therefore not treated here as a "species most at risk."
green sturgeon	*Acipenser medirostris*	threatened	concern; fishing prohibited	Threatened overall, and vulnerable to selenium as a clam-eating bottom feeder in the SF estuary; therefore likely to be a "species most at risk." Emergency regulations issued by CDFG March 2006--Zero (0) bag limit for green sturgeon year-round in all areas.
white sturgeon	*Acipenser transmontanus*	none	limited fishing	Population in the SF estuary not federally listed, but vulnerable to selenium as a clam-eating bottom feeder. Therefore, treated here as a "species most at risk." The daily bag and possession limit established by CDFG is one fish that must be between 46 inches and 72 inches total length. The yearly limit is three.
river lamprey	*Lampetra ayresi*	none	watch list	Anadromous; feeds on young salmon. Recorded from lower Sacramento and San Joaquin Rivers. Not federally listed; therefore not considered to be a "species most at risk."
Sacramento perch	*Archoplites interruptus*	concern	concern	Fry feed primarily on bottom-dwelling crustaceans, insect larvae, snails, and fish. One captured in the Delta in 1992, not likely to represent an established population there. Therefore not considered to be a "species most at risk" in the Delta. Update: However, plans for possible future reintroduction of this species in the Delta should take into account possible risk to individuals of a recovering population segment (pers. comm.., Victoria Poage, Delta Native Fishes Recovery Coordinator, Bay Delta Fish and Wildlife Office, USFWS.
Sacramento splittail	*Pogonichthys macrolepidotus*	concern	threatened	Vulnerable to selenium as clam-eating bottom feeder in the SF estuary; therefore likely to be a "species most at risk."
striped bass	*Morone saxatilis*	none	none	Introduced sport fish in California. Population in Delta declined sharply in early 2000s, but species overall not threatened. Therefore not considered to be a "species most at risk."
threadfin shad	*Dorosoma pretenense*	none	none	Introduced in California as food for game fish. Population in Delta declined sharply in early 2000s, but species overall not threatened. Therefore not considered to be a "species most at risk."
tidewater goby	*Eucyclogobius newberryi*	endangered	endangered	Bottom-dwelling carnivore. Prefers semi-closed estuaries. Potentially exposed, but not found recently (since 1984) in the Bay area; therefore not considered to be a "species most at risk" in the SF Bay/Delta.
California halibut	*Paralichthys californicus*	none	none	Bottom dweller inhabiting the SF Bay, but overall not threatened; therefore not likely to be a "species most at risk."
leopard shark	*Triakis semifasciata*	none	none	Bottom dweller inhabiting the SF Bay, but overall not threatened; therefore not likely to be a "species most at risk."
starry flounder	*Platichthys stellatus*	none	none	Bottom dweller inhabiting the SF Bay. Population in bay declined sharply since 1980, but overall not threatened; therefore not likely to be a "species most at risk."
Invertebrates				
Dungeness crab	*Cancer magister*	none	none	Estuary is nursery for this ocean-breeding bottom feeder, but overall not threatened; therefore not likely to be a "species most at risk."

Federal Status: Endangered: listed as endangered under the Federal Endangered Species Act; Threatened: listed as threatened under the Federal Endangered Species Act; Proposed threatened: proposed as threatened under the Federal Endangered Species Act; Concern: designated a species of concern; Delisted: removed from the list of endangered and threatened species under the Federal ESA; MBTA: protected under Migratory Bird Treaty Act; BGEPA protected under the Bald and Golden Eagle Protection Act.
California State Status: Endangered: listed as endangered under the California Endangered Species Act; Threatened: listed as threatened under the California Endangered Species Act; Concern: designated by the California Department of Fish and Game as a species of concern; Protected: Fully protected under the Fish and Game Code of California predating the California Endangered Species Act
* Assessment based upon population status, dependence upon benthic food web, and sensitivity to selenium. Aquatic dependent species feeding directly in the benthic food web of the San Francisco Estuary were considered to be at greater risk to selenium exposure than those species feeding in a pelagic/planktonic food web. This assumption is based upon the work of Stewart et al. (2004).

Table 4. Species most at risk from Se exposure in the San Francisco Estuary: summary data. [Reproduced from USFWS, 2008 Table 2].

Common Name	Scientific Name	Probable critical life stage for Se effects[1]	Food ingestion rate at critical life stage (g ww/day) [2]	Food ingestion rate at critical life stage (g dw/kg body weight/day) [3]	Body weight at critical life stage (g) [4]	Diet	Mainly clam-based food chain?[5]	Percent of diet that is clam-based (worst case)
bald eagle	*Haliaeetus leucocephalus*	Adult female (egg laying)	644	249	5275 (female)	fish, birds, mammals	no	22.8[6]
California clapper rail	*Rallus longirostris obsoletus*	Adult female (egg laying)	172	46.8	346	mussels, spiders, clams, crabs, snails, marsh cordgrass seeds	yes	64.1
greater scaup	*Aythya marila*	Adult male and female (migration)	313	85.8	1054 (male)	clams, snails, other mollusks, crustaceans, algae	yes	80.7
lesser scaup	*Aythya affinis*	Adult male and female (migration)	246	67.5	734 (male)	clams, other mollusks, aquatic insects, crustaceans, plants	yes	96
white-winged scoter	*Melanitta fusca*	Adult male and female (migration)	465	127.3	1917 (male)	clams, other mollusks, crustaceans, aquatic insects	yes	75[7]
surf scoter	*Melanitta perspicillata*	Adult male and female (migration)	314	86.0	1059 (male)	mussels, other mollusks, plants, crustaceans	yes	86[8]
black scoter	*Melanitta nigra*	Adult male and female (migration)	325	89.1	1117 (male)	mussels, clams, snails, barnacles	yes	80[9]
Chinook salmon	*Oncorhynchus tshawytscha*	Migrating/rearing juvenile		23.3	0.5-18	insects, crustacea, juvenile fish	no	0[10]
steelhead	*Oncorhynchus mykiss*	Migrating/rearing juvenile		19.9	31-105	insects, annelids, *Daphnia*	no	0[10]
green sturgeon	*Acipenser medirostris*	Juvenile or adult female		20	1300 (average caught)	benthic crustacea, mollusks and fish	probably substantially	See white sturgeon
white sturgeon	*Acipenser transmontanus*	Juvenile or adult female		15-20	6280 (mode)	benthic mollusks and crustacea	substantially	41.1[11]
delta smelt	*Hypomesus transpacificus*	Juvenile or adult female		114	0.32 (average Jun-Aug)	copepods, cladocerans, amphipods, insect larvae	no	0
Sacramento splittail	*Pogonichthys macrolepidotus*	Juvenile or adult female		33.7	121 (mode)	benthic detritus, clams, other mollusks, mysids	substantially	34

1. For most species it is premature and speculative to designate a critical life stage at this time. Such designation prejudges the outcome of a thorough search of the toxicology literature.
2. Food ingestion rates based on wet weight can be calculated from available parameters (Nagy 2001) for birds, mammals, reptiles and amphibians, but not, in general for fish. [Note: food ingestion rate for fish are available elsewhere (e.g., Baines et al., 2002); see text for further discussion].
3. For birds, the food ingestion rate as dry weight is calculated from the regression parameters for dry matter intake per day from Table 3 in Nagy (2001), using categories of birds used to calculate food ingestion rate in terms of wet weight as described in the text below.
4. See note 1 above. For anadromous species, a range of body weights is given corresponding to the period spent rearing in the estuary.
5. We interpret "clam-based" broadly to mean filter-feeding benthic mollusk-based.
6. For the worst case, we assume that all birds consumed are those waterfowl (scaups and scoters) that primarily feed on benthic mollusks (clams, etc.).

7. Percent of mollusks in gizzards of 819 adults and 4 juveniles collected in coastal Maine and Washington (Cottam C. *U.S. Dep. Agric. Tech. Bull.* 643).
8. Wet weight percents of summer and winter gizzard contents, British Columbia salt water (Vermeer K. 1981 *Wildfowl* 32:107-116; Vermeer and Bourne 1984 as summarized in Appendix 1 of Savard *et al*. 1998).
9. Percent mussels, winter, coastal New England (reviewed in Bordage and Savard 1995).
10. Although the diets of salmon, steelhead and delta smelt are not known to be clam-based, these species may still be at risk from selenium because of greater sensitivity to selenium. The sensitivity of salmon and steelhead is documented below. The sensitivity of delta smelt to selenium is unknown; population numbers are alarmingly low, so this species is particularly vulnerable to any adverse effect.
11. Percentage clams by volume, fall, Suisun Bay and Carquinez Strait (Table 10 below).

Table 5. Selenium effect levels derived for protection of species at risk in the San Francisco Estuary. [Reproduced from USFWS, 2009b, Table 1).

	Concentration of selenium (µg/g dry wt.) corresponding to effect level:										
	Se in diet			Se in target species (whole body or egg)							
Species	0%	5%	10%	0%	5%	10%	Effect	Exposure duration (days)	Form of selenium	Model	Data source
Mallard	2.30	4.36	5.29	2.77	5.86	7.73	hatchability	>40 (parental)	seleno-DL-methionine	Beckon *et al*. 2008	Heinz *et al*. 1989
White sturgeon				na	7.03	8.13	larval edema and skeletal defects	up to 6 months	selenized yeast	log-logistic	Linville 2006
adult[a]	na	25.5	32.5				assimilation	6 months	selenized yeast	power	Linville 2006
juvenile[a]	na	0.95	1.57				assimilation	56	seleno-L-methionine	power	Tashjian *et al* 2006
Chinook salmon	1.54	2.25	2.67	1.01	1.53	1.84	mortality	90	assimilated or seleno-DL-methionine	Brain and Cousens 1989	Hamilton et al. 1990
Rainbow trout	2.41	4.22	5.04	1.27	1.89	2.19	reduction in growth	140	sodium selenite	Beckon *et al*. 2008	Hilton *et al*. 1980
	0.31	1.01	1.56				assimilation	90	seleno-L-methionine	power	Vidal *et al*. 2005

[a] Adult and juvenile white sturgeon effect guidelines are being revised; [b] Revision, personal communication, USFWS, William Beckon, 10/27/10: EC05 = 3.8; EC10 = 8.2.

Table 6. Generic selenium effect levels for fish and birds.

	Se (µg/g dw)	Se (µg/g dw)	Se (µg/g dw)
bird (egg)	5.5 (NEC) (Skorupa, 2008)	7.7 (EC10) (USFWS, 2009b; Skorupa, 2008)	12 (>EC20)
fish (wb)	-	5.0 (EC10) (USFWS, 2005; Skorupa et al., 2004)	8.0 (EC40)
diet (fish and birds)	3.6	<4.9 (Skorupa et al., 2004)	5.7

Table 7. Available data for the Bay-Delta including transects and biota studies. [Water year classification based on precipitation in the Sacramento Valley. A high flow season is defined from December through May; a low flow season is defined as June through November.]

study date	water year/flow season	residence time (days)/ salinity at Golden Gate Bridge (psu)	reference	Se data
Northern Reach from Sacramento/ San Joaquin Rivers to Golden Gate Bridge				
April 1986	wet/high	9.8/--	Cutter 1989; Meseck, 2002	dissolved; dissolved speciation; particulate
September 1986	wet/low	24.4/-	Cutter 1989; Meseck, 2002	dissolved; dissolved speciation; particulate
October 1987	critical/low	73.5/-	Cutter and San Diego-McGlone, 1990	dissolved; dissolved speciation
December 1987	critical/high	8.0/-	Cutter and San Diego-McGlone, 1990	dissolved; dissolved speciation
March 1988	critical/high	35.5/-	Cutter and San Diego-McGlone, 1990	dissolved; dissolved speciation
May 1988	critical/high	25/-	Cutter and San Diego-McGlone, 1990	dissolved; dissolved speciation
1989-1990	critical	-	Urquhart and Regalado, 1991; Kroll and Doroshov, 1991	white sturgeon: flesh; ovary; egg yolk components; plasma
1986-1990	wet 1986; dry 1987; 1988 critical; 1989 dry; 1990 critical	-	White et al., 1987, 1988, 1989; Urquhart and Regalado, 1991	surf scoter, greater and lesser scaup liver and flesh: Suisun and San Pablo Bays
1975, 1986, 1987	wet 1975; wet 1986; dry, 1987	-	Lonzarich et al., 1992	California clapper rail eggs from the northern and southern reaches of Bay
1982; 1985	wet 1982; dry 1985	-	Ohlendorf et al., 1986; 1991	surf scoter, greater scaup liver (southern and northern Bay)
December 1986-1987 (early winter); March 1986-1987(late winter)	wet 1986; dry 1987	-	Takekawa et al., 2002	canvasbacks (n = 29), greater scaup, lesser scaup (n =30) liver and kidney from North, Central, and South Bays
1989	dry	-	Hoffman et al., 1998	surf scoter, greater scaup, ruddy duck liver (Suisun Bay; Tomales Bay)
1985-1986	dry 1985; wet 1986	-	White et al., 1987, 1988, 1989; Urquhart and Regalado, 1991; Johns et al., 1988	sediment and clam
1991, 1992, 1998, 1999 breeding seasons	critical 1991, 1992; wet 1998, 1999	-	Schwarzbach et al., 2006	California clapper rail egg from six tidal marshes in northern and southern reaches of Bay
1994, 1995, 1997, 1999, 2000, 2001	critical 1994; wet 1995-1999; above normal 2000; dry 2001	-	CH2M HILL, 1994; 1995; 1998; 2000; 2001; 2002; Ohlendorf and Gala, 2000; Skorupa, 1998	shorebird eggs from Chevron Richmond Refinery Water Enhancement Wetland
November 1997	wet/low	68/32.5	Cutter and Cutter, 2004; Meseck, 2002; Doblin et al., 2006	Bay-Delta transects: dissolved; dissolved speciation; particulate; particulate speciation
June 1998	wet (El Niño) /high	11/24.8	Cutter and Cutter, 2004; Doblin et al., 2006	Bay-Delta transects: dissolved; dissolved speciation, particulate; particulate speciation
October 1998	wet/low	22/30.2	Cutter and Cutter, 2004; Doblin et al., 2006	Bay-Delta transects: dissolved; dissolved speciation, particulate; particulate speciation

study date	water year/flow season	residence time (days)/ salinity at Golden Gate Bridge (psu)	reference	Se data
April 1999	wet/high	16/28.5	Cutter and Cutter, 2004; Doblin et al., 2006	Bay-Delta transects: dissolved; dissolved speciation, particulate; particulate speciation
November 1999	above normal/ low	70/32.2	Cutter and Cutter, 2004; Doblin et al., 2006	Bay-Delta transects: dissolved; dissolved speciation,particulate; particulate speciation
Nov 97, Jun 98, Oct 98, Nov 99	see above for Cutter and Cutter, 2004	-	Meseck, 2002	sedimentary Se and speciation; pore-water Se: San Pablo Bay: Suisun Bay, Delta, mudflat marsh near Martinez
1995-1997	all wet years	-	Linville et al., 2002 (see Presser and Luoma, 2006, Fig 15)	clams from 21 locations
1997-2000	1997-1999 wet; 2000 above normal	-	Greenfield et al., 2005	sport fish at 6 locations including San Pablo Bay
1999-2000	1999 wet; 2000 above normal	-	Stewart et al., 2004	fall and early winter food webs
1998-1999	wet 1998-1999	-	Purkerson et al., 2003	zooplankton from stations in northern, central and southern reaches of Bay
March to July, 2000; 2001	above normal 2000; dry 2001	-	Schwarzbach and Adelsbach, 2003	aquatic bird eggs including California clapper rail eggs from San Francisco Bay, Suisun Bay, and the Delta
March, 2002	dry	-	Hunt et al., 2003	surf scoter and greater scaup muscle: Suisun and San Pablo Bays:
May, 1995-February, 2010		-	Kleckner et al., 2010	USGS clam database: monthly *C. amurensis*: at seven USGS stations
2004-2006 winter	below normal 2004; above normal 2005; wet 2006	-	Wainwright-De La Cruz, et al., 2008	surf scoter liver :San Pablo, Suisun, and Central Bays
Mar-Apr, 2005	above normal	-	Ackerman and Eagles-Smith, 2009	avocet, stilt, tern liver: north and south Bay, prebreeding season
2003-2005	above normal 2003; 2005; below normal 2004	-	Linares-Casenave et al., 2010	white sturgeon tissues (muscle, gonad, kidney, liver): six locations from Chipps Island to San Pablo Bay
Rio Vista and Stockton to Benicia/Carquinez Strait				
October 7-8,1998	wet/low	-	Personal communication M. Doblin, March 2009	sediment cores from six Delta locations
July 12-13, 2000	above normal/low	-	Lucas and Stewart, 2007	dissolved; dissolved speciation; particulate
January 22, 2003	above normal/high	-	Lucas and Stewart, 2007	dissolved; dissolved speciation; particulate
April 22-23, 2003	above normal/high	-	Lucas and Stewart, 2007	dissolved; dissolved speciation; particulate
June 17, 2003	above normal	-	Lucas and Stewart, 2007	dissolved; dissolved speciation; particulate
October 10, 2003	below normal/low	-	Lucas and Stewart, 2007	dissolved; dissolved speciation; particulate
January 15, 2004	below normal/high	-	Lucas and Stewart, 2007	dissolved; dissolved speciation; particulate
2002	dry	-	Lucas and Stewart, 2007	sediment cores from three Delta locations

Table 8. Bay-Delta hydrologic conditions, Net Delta Outflow Index, salinity, observed dissolved Se concentrations, observed suspended particulate material Se concentrations, and calculated K_ds. [Arranged by increasing residence time of transect, except for November, 1997. See text for additional discussion.]

hydrologic condition (transect, residence time, water year/flow season)	Net Delta Outflow Index daily average per month (cfs)	salinity mean and range (psu)	observed dissolved Se mean and range (µg/L)	observed particulate Se mean and range (µg/g dw)	calculated K_d mean and range[a]
June 16-17, 1998 11 day residence; wet/high	73,732	5.8 (0.01-24.5)	0.181 (0.101-0.303)	0.518 (0.150-1.59)	3,198 (712-11,054)
April 13-14, 1999 16 day residence; wet/high	35,034	11.4 (0-28.9)	0.116 (0.076-0.165)	0.636 (0.190-1.41)	5,824 (1,151-13,317)
October 7-8, 1998 22 day residence; wet/low	12,251	14.6 (0-30.1)	0.120 (0.077-0.164)	0.713 (0.289-2.21)	6,501 (2,202-26,912)
November 4-5, 1999 70 day residence; above normal/low	6,951	15.0 (0-32.2)	0.102 (0.070-0.137)	0.746 (0.428-1.66)	7,614 (3,496-19,785)
November 5-6, 1997 68 day residence; wet/low	9,632	17.2 (0.56-32.0)	0.192 (0.101-0.320)	0.842 (0.470-1.58)	4,652 (2,333-8,349)

[a] K_d grand mean for 1998-1999 transects = 5,784

Table 9. Suisun Bay-Carquinez Strait hydrologic conditions, Net Delta Outflow Index, salinity, observed dissolved Se concentrations, observed suspended particulate material Se concentrations, and calculated K_ds. [Arranged by increasing residence time of transect, except for November, 1997. See text for additional discussion. See Doblin et al., 2006 and Figure 14 for division into subset.]

hydrologic condition (transect, residence time, water year/flow season)	Net Delta Outflow Index (daily average per month cfs)	salinity mean and range (psu)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	calculated K_d mean and range[a]
June 16-17, 1998 11 day residence; wet/high	73,732	0.76 (0.44-1.08)	0.213 (0.211--0.215)	0.252 (0.150-0.354)	1,180 (712-1,647)
April 13-14, 1999 16 day residence; wet/high	35,034	5.82 (4.9-7.3)	0.118 (0.076-0.154)	0.303 (0.240-0.350)	2,666 (2,274-3,168)
October 7-8, 1998 22 day residence; wet/low	12,251	7.0 (2.5-11.6)	0.135 (0.128-0.151)	0.462 (0.289-0.667)	3,435 (2,202-5,212)
November 4-5, 1999 70 day residence; above normal/low	6,951	17.5 (11.4-23.1)	0.123 (0.104-0.132)	0.740 (0.428-1.03)	5,986 (3,496-7,725)
November 5-6, 1997 68 day residence wet/low	9,632	16.1 (12.7-19.2)	0.210 (0.192-0.236)	0.710 (0.572-0.809)	3,381 (2,722-4,078)

[a] K_d grand mean for 1998-1999 transects = 3,317.

Table 10. Landward hydrologic conditions, Net Delta Outflow Index, salinity, observed dissolved Se concentrations, observed suspended particulate material Se concentrations, and calculated K_ds.

hydrologic condition (transect, residence time, water year/flow season)	Net Delta Outflow Index (daily average per month cfs)	salinity[a] range (psu)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	calculated K_d mean and range
January 22, 2003 above normal/high	50,847	0.011-8.45	0.245 (0.111-0.599)	0.411 (0.27-0.58)	2,268 (554-3,503)
January 15, 2004 below normal/high	30,924	0.012-8.105	0.215 (0.114-0.523)	0.519 (0.23-1.0)	2,981 (1,256-6,398)
April 22-23, 2003 above normal/high	21,218	0.013-3.99	0.356 (0.115-1.008)	0.614 (0.28-1.31)	2,684 (927-4,351)
October 10, 2003 below normal/low	4,350	0.019-12.68	0.174 (0.068-0.532)	0.751 (0.37-1.53)	5,855 (1,628-12,650)

[a]Calculated from chlorinity.

Table 11. Bay-Delta mean, median, 75th percentile, and 25th percentile for observed dissolved Se concentrations, observed suspended particulate material Se concentrations, and K_ds. [Arranged by increasing residence time of transect, except for November, 1997. See text for additional discussion.]

	Jun-1998 (11 day residence)	Apr-1999 (16 day residence)	Oct-1998 (22 day residence)	Nov-1999 (70 day residence)	Nov-1997 (68 day residence)
dissolved Se µg/L					
mean	0.181	0.116	0.122	0.102	0.192
75th percentile	0.204	0.128	0.134	0.122	0.215
median	0.183	0.121	0.128	0.099	0.200
25th percentile	0.148	0.093	0.105	0.085	0.163
particulate Se µg/g dw					
mean	0.518	0.636	0.712	0.746	0.842
75th percentile	0.456	0.829	0.807	0.854	1.005
median	0.392	0.528	0.627	0.725	0.783
25th percentile	0.357	0.391	0.516	0.570	0.609
K_d					
mean	3198	5824	6501	7614	4652
75th percentile	2491	7151	6525	8114	6060
median	2330	5252	4963	6569	3970
25th percentile	2059	3253	3782	5893	3173

Table 12. Suisun Bay-Carquinez Strait mean, median, 75th percentile, and 25th percentile for observed dissolved Se concentrations, observed suspended particulate material Se concentrations, and K_ds. [Arranged by increasing residence time of transect, except for November, 1997. See text for additional discussion. See Doblin et al., 2006 and Figure 14 for division into subset.]

	Jun-1998 (11 day residence)	Apr-1999 (16 day residence)	Oct-1998 (22 day residence)	Nov-1999 (70 day residence)	Nov-1997 (68 day residence)
dissolved Se µg/L					
mean	0.213	0.118	0.135	0.123	0.210
75th percentile	0.214	0.139	0.137	0.128	0.217
median	0.213	0.125	0.131	0.125	0.208
25th percentile	0.212	0.100	0.129	0.120	0.200
particulate Se µg/g dw					
mean	0.252	0.303	0.462	0.740	0.710
75th percentile	0.303	0.335	0.606	0.892	0.780
median	0.252	0.319	0.447	0.738	0.740
25th percentile	0.201	0.280	0.308	0.597	0.637
K_d					
mean	1180	2666	3435	5986	3381
75th percentile	1414	2861	4498	7089	3647
median	1180	2555	3111	6142	3378
25th percentile	946	2414	2286	5019	3091

Table 13. Comparison of predicted and observed *C. amurensis* Se concentrations during Bay-Delta transects.

transect	mean predicted clam Se µg/g dw	mean observed clam Se (all stations) (µg/g dw)	field location (station number)	mean observed clam Se by station and month (µg/g dw)
June 16-17, 1998	4.4 all salinities[a] 1.6 Carquinez Strait/Suisun Bay salinities[b]	5.4	Suisun Bay (6.1) San Pablo Bay (12.5)	Jun 5.1 Jun 5.8
April 13-14, 1999	9.5 all salinities[a] 8.7 Carquinez Strait/Suisun Bay salinities[b]	7.3	Suisun Bay (6.1) Carquinez Strait (8.1)	Mar 7.4; Apr 7.5; May 5.7; Jun 6.8 Jun 9.2
October 7-8, 1998	13.1 all salinities[a] 11.2 Carquinez Strait/Suisun Bay salinities[b]	10.8	Chipps Island (4.1) Suisun Bay (6.1) Carquinez Strait (8.1) San Pablo Bay (12.5)	Oct 5.6 Oct 12.3 Sep 15.5; Oct 13; Nov 14; Dec 14 Sep 10.5; Oct 9.6
November 4-5, 1999	12.6 all salinities[a] 12.0 Carquinez Strait/Suisun Bay salinities[b]	11.3 (12.8 Carquinez Strait data only)	Suisun Bay (6.1) Grizzly Bay (415) Grizzly Bay (411) Suisun Bay (405.1) Carquinez Strait (8.1) San Pablo Bay (12.5)	Sep 9.4; Oct 12.7; Nov 12.5 Sep 8.3; Oct 9.5; Nov 7.9 Sep 8.4; Oct 11.3; Nov 11.7; Dec 13.3 Sep 10.4; Oct 16.7; Nov 15.3 Sep 8.3; Oct 15.3; Nov 14.7 Sep 7.2; Oct 10.2; Nov 11
November 5-6, 1997	16.6 all salinities[a] 11.7 Carquinez Strait/Suisun Bay salinities[b]	14.3	Chipps Island (4.1) Suisun Bay (6.1) Carquinez Strait (8.1) San Pablo Bay (12.5)	Nov 11.6 Nov 14.0 Oct 15.5; Nov 15.3 Nov 14.9

[a] Predicted clam Se concentrations calculated with outliers deleted (TTFs>35).

[b] Table 1, Doblin et al. (2006) estuarine stations grouped into embayments: Delta; Carquinez Strait-Suisun Bay; San Pablo Bay; and Central Bay.

Table 14. Validation for existing conditions at a seaward estuary location for November, 1999 or a generalized mean condition using observed Se concentrations in seaward and landward white sturgeon; derived K_ds and TTFs; and a food web for suspended particulate material>*C. amurensis* >white sturgeon.

observed sturgeon muscle Se[a] µg/g	site-specific $TTF_{sturgeon}$	predicted *C. amurensis* Se µg/g	mean observed *C. amurensis* Se[b] µg/g	biodynamic site-specific $TTF_{C.\ amurensis}$	predicted particulate Se µg/g	observed particulate Se[c] µg/g	calculated K_d	predicted dissolved Se µg/L	observed dissolved Se[d] µg/L
10.2	1.1	9.3	12.8	17	0.545	0.428-1.66	7614(Nov 99 mean)	0.072	0.070-0.137
10.2	1.1	9.3	12.8	17	0.545	0.150-2.21	5784 (grand mean)	0.094	0.070-0.320
6.9	1.1	6.3	12.8	17	0.369	0.428-1.66	7614(Nov 99 mean)	0.048	0.070-0.137
6.9	1.1	6.3	12.8	17	0.369	0.150-2.21	5784 (grand mean)	0.064	0.070-0.320
10.2	0.8	12.8	12.8	17	0.753	0.428-1.66	7614(Nov 99 mean)	0.099	0.070-0.137
10.2	0.8	12.8	12.8	17	0.753	0.150-2.21	5784 (grand mean)	0.130	0.070-0.320
6.9	0.8	8.6	12.8	17	0.506	0.428-1.66	7614(Nov 99 mean)	0.066	0.070-0.137
6.9	0.8	8.6	12.8	17	0.506	0.150-2.21	5784 (grand mean)	0.088	0.070-0.320

[a]1998-2001 data; seaward, 10.2 µg/g; landward, 6.9 µg/g (Stewart et al., 2004);[b]Carquinez Strait (USGS station 8.1): mean observed fall 1999; note also station 405 clams, 14.6 µg/g dw Se (Kleckner et al., 2010) (see also **Table 13**); [c]1998-1999 data (Doblin et al., 2006); [d]1998-1999 data (Cutter and Cutter, 2004).

Table 15. Validation for existing conditions in Suisun Bay-Carquinez Strait for November, 1999 or a generalized mean condition using observed Se concentrations in seaward white sturgeon; derived K_ds and TTFs; and a food web for suspended particulate material>*C. amurensis* >white sturgeon.

observed sturgeon muscle Se[a] µg/g	site-specific $TTF_{sturgeon}$	predicted *C. amurensis* Se µg/g	mean observed *C. amurensis* Se[b] µg/g	biodynamic site-specific $TTF_{C.\ amurensis}$	predicted particulate Se µg/g	observed particulate Se[c] µg/g	calculated K_d	predicted dissolved Se µg/L	observed dissolved Se[d] µg/L
10.2	1.1	9.3	12.8	17	0.545	0.428-1.03	5986 (Nov 99 mean)	0.091	0.104-0.132
10.2	1.1	9.3	12.8	17	0.545	0.150-1.03	3317 (grand mean)	0.164	0.076-0.215
10.2	0.8	12.8	12.8	17	0.753	0.428-1.03	5986 (Nov 99 mean)	0.126	0.104-0.132
10.2	0.8	12.8	12.8	17	0.753	0.150-1.03	3317 (grand mean)	0.227	0.076-0.215

[a]1998-2001 data; seaward, 10.2 µg/g; landward, 6.9 µg/g (Stewart et al., 2004);[b]Carquinez Strait (USGS station 8): mean observed fall 1999; note also station 405 clams, 14.6 µg/g dw Se (Kleckner et al., 2010) (see also **Table 13**). [c]1998-1999 data (Doblin et al., 2006); [d]1998-1999 data (Cutter and Cutter, 2004).

Table 16. Validation for existing conditions at a landward estuary location for 2003-2004 using observed Se concentrations in landward largemouth bass; derived K_ds and TTFs; and a food web for suspended particulate material>aquatic insect>largemouth bass food web.

observed bass wb Se[a] µg/g	generic TTF_{fish}	predicted insect Se µg/g	mean observed chironomid Se[b] µg/g	generic TTF_{insect}	predicted particulate Se µg/g	observed particulate Se[b] µg/g	calculated K_d	predicted dissolved Se µg/L	observed dissolved Se[b] µg/L
2.9	1.1	2.6	2.7	2.8	0.942	0.27-0.58	2268 (Jan 2003 mean)	0.415	0.111-0.599
2.9	1.1	2.6	2.7	2.8	0.942	0.23-1.0	2981 (Jan 2004 mean)	0.316	0.114-0.523
2.9	1.1	2.6	2.7	2.8	0.942	0.37-1.5	5855 (Oct 2003 mean)	0.161	0.068-0.532

[a] 2007 data (Foe et al., 2010); [b]2002-2004 data (Lucas and Stewart, 2007) (see also **Appendix D, Table D5**).

Table 17. Predicted allowed dissolved Se concentrations for Bay-Delta transects at different effect guidelines and associated levels of protection (USFWS, 2009b) for a suspended particulate material>*C. amurensis*>sturgeon food web. Also shown are 1) observed dissolved Se concentrations, suspended particulate material Se concentrations, and calculated K_ds; and 2) hydrologic conditions including water-year type, flow season, residence time, and NDOI. [Assumptions: TTF_{clam} = 17.1; TTF_{fish} = 1.1. Transect data and predictions for 1998 through 1999 are arranged by increasing residence time; transect data and predictions for November, 1997 are delineated separately (see text for explanation). Means and K_ds are based on individual data points, not composites. Further studies are needed to consider sensitivity of green sturgeon].

calculated K_d mean and range	food web: particulate material > *C. amurensis* >fish	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, residence time, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	Net Delta Outflow Index (daily average per month cfs)
FISH (WHOLE-BODY)											
3,198 (712-11,054)	adult female white sturgeon	whole-body	8.1	10	0.208 (0.039-0.605)	0.43	7.4	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.110 (0.032-0.374)	0.43	7.4	April 13-14, 1999 16 day residence wetl/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.096 (0.016-0.196)	0.43	7.4	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.064 (0.022-0.123)	0.43	7.4	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.108 (0.052-0.185)	0.43	7.4	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
3,198 (712-11,054)	adult female white sturgeon	whole-body	7.0	05	0.180 (0.034-0.523)	0.37	6.4	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.095 (0.028-0.323)	0.37	6.4	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.083 (0.014-0.169)	0.37	6.4	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.055 (0.019-0.106)	0.37	6.4	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652					0.093	0.37	6.4	November 5-6, 1997	0.192	0.842	9,632

calculated K_d mean and range	food web: particulate material > *C. amurensis* >fish	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, residence time, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	Net Delta Outflow Index (daily average per month cfs)
(2,333-8,349)					(0.045-0.160)			68 day residence wet/low	(0.101-0.320)	(0.470-1.58)	
3,198 (712-11,054)	clam-eating fish	whole-body	5.0 generic		0.128 (0.024-0.373)	0.27	4.5	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.068 (0.020-0.231)	0.27	4.5	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.059 (0.010-0.121)	0.27	4.5	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.040 (0.013-0.076)	0.27	4.5	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.066 (0.032-0.114)	0.27	4.5	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
FISH (DIET)											
3,198 (712-11,054)	juvenile white sturgeon	diet	1.6 (=1.8 wb)	10	0.0452 (0.0085-0.1314)	0.094	1.6	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0247 (0.0070-0.0813)	0.094	1.6	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0211 (0.0035-0.0425)	0.094	1.6	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0139 (0.0047-0.0268)	0.094	1.6	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0234 (0.0112-0.0401)	0.094	1.6	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
3,198 (712-11,054)	juvenile white sturgeon	diet	0.95 (=1.0 wb)	05	0.0268 (0.0050-0.0780)	0.056	0.95	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732

calculated K_d mean and range	food web: particulate material *> C. amurensis* >fish	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, residence time, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	Net Delta Outflow Index (daily average per month cfs)
5,824 (1,151-13,317)					0.0147 (0.0042-0.0483)	0.056	0.95	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0126 (0.0021-0.0252)	0.056	0.95	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0082 (0.0028-0.0159)	0.056	0.95	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0139 (0.0066-0.0238)	0.056	0.95	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632

Table 18. Predicted allowed dissolved Se concentrations for Bay-Delta transects at different effect guidelines and associated levels of protection (USFWS, 2009b) for a suspended particulate material>*C. amurensis*>clam-eating bird species food web. Also shown are 1) observed dissolved Se concentrations, suspended particulate material Se concentrations, and calculated K_ds; and 2) hydrologic conditions including water-year type, flow season, residence time, and NDOI. [Assumptions: TTF_{clam} = 17.1; TTF_{bird} = 2.6. Transect data and predictions for 1998 through 1999 are arranged by increasing residence time; transect data and predictions for November, 1997 are delineated separately (see text for explanation). Means and K_ds are based on individual data points, not composites.]

calculated K_d mean and range	food web: particulate> *C. amurensis* >bird	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, residence time, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	Net Delta Outflow Index (daily average per month cfs)
BIRD (EGG)											
3,198 (712-11,054)	scoter and scaup	egg	7.7 generic	10	0.0837 (0.0157-0.243)	0.17	3.0	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0440 (0.0130-0.1505)	0.17	3.0	April 13-14, 1999 16 day residence wet/ high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0404 (0.0064-0.0786)	0.17	3.0	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0258 (0.0088-0.0495)	0.17	3.0	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0432 (0.0207-0.0742)	0.17	3.0	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
3,198 (712-11,054	scoter and scaup	egg	5.9	05	0.0641 (0.0120-0.1864)	0.13	2.3	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0337 (0.0100-0.1153)	0.13	2.3	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0310 (0.0049-0.0603)	0.13	2.3	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0197 (0.0067-0.0380)	0.13	2.3	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0331 (0.0159-0.0596)	0.13	2.3	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632

calculated K_d mean and range	food web: particulate> *C. amurensis* >bird	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, residence time, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	Net Delta Outflow Index (daily average per month cfs)
3,198 (712-11,054	scoter and scaup	egg	2.8	0	0.0304 (0.0057-0.0884)	0.063	1.1	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0160 (0.0047-0.0547)	0.063	1.1	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0140 (0.0023-0.0286)	0.063	1.1	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0094 (0.0032-0.0180)	0.063	1.1	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0157 (0.0075-0.0270)	0.063	1.1	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
BIRD (DIET)											
3,198 (712-11,054	scoter and scaup	diet	5.3 (=13.8 egg)	10	0.1498 (0.0280-0.4353)	0.31	5.3	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0818 (0.0233-0.2693)	0.31	5.3	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0700 (0.0115-0.1408)	0.31	5.3	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0460 (0.0157-0.0886)	0.31	5.3	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0774 (0.0371-0.1328)	0.31	5.3	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
3,198 (712-11,054	scoter and scaup	diet	4.4 (=11.4 egg)	05	0.1244 (0.0233-0.3613)	0.26	4.4	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0679 (0.0193-0.2235)	0.26	4.4	April 13-14, 1999 16 day residence	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034

calculated K_d mean and range	food web: particulate> *C. amurensis* >bird	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, residence time, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se (mean and range) µg/g dw	Net Delta Outflow Index (daily average per month cfs)
								wet/high			
6,501 (2,202-26,912)					0.0581 (0.0096-0.1168)	0.26	4.4	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0382 (0.0130-0.0736)	0.26	4.4	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0642 (0.0308-0.1103)	0.26	4.4	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1.58)	9,632
3,198 (712-11,054	scoter and scaup	diet	2.3 (=6.0 egg)	0	0.0650 (0.0122-0.1889)	0.13	2.3	June 16-17, 1998 11 day residence wet/high	0.181 (0.101-0.303)	0.518 (0.150-1.59)	73,732
5,824 (1,151-13,317)					0.0355 (0.0101-0.1169)	0.13	2.3	April 13-14, 1999 16 day residence wet/high	0.116 (0.076-0.165)	0.636 (0.190-1.41)	35,034
6,501 (2,202-26,912)					0.0304 (0.0050-0.0611)	0.13	2.3	October 7-8, 1998 22 day residence wet/low	0.120 (0.077-0.164)	0.713 (0.289-2.21)	12,251
7,614 (3,496-19,785)					0.0200 (0.0068-0.0385)	0.13	2.3	November 4-5, 1999 70 day residence above normal/low	0.102 (0.070-0.137)	0.746 (0.428-1.66)	6,951
4,652 (2,333-8,349)					0.0336 (0.0161-0.0576)	0.13	2.3	November 5-6, 1997 68 day residence wet/low	0.192 (0.101-0.320)	0.842 (0.470-1n58)	9,632

Table 19. Predicted allowed dissolved Se concentrations for landward transects at different effect guidelines and associated levels of protection (USFWS, 2009b) for a suspended particulate material>aquatic insect>juvenile salmon food web. Also shown are 1) observed dissolved Se concentrations, suspended particulate material Se concentrations, and calculated K_ds; and 2) hydrologic conditions including water-year type, flow season, and NDOI. [Assumptions: TTF_{fish} = 1.1; $TTF_{aquatic\ insect}$ = 2.8. Means and K_ds are based on individual data points, not composites.]

calculated K_d mean and range	food web: particulate >insect >fish	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se mean and range (µg/g dw)	Net Delta Outflow Index (daily average per month cfs)
FISH (WHOLE-BODY)											
2,268 (554-3,503)	insect-eating fish	whole-body	5.0 generic		1.05 (0.463-2.93)	1.6	4.5	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.701 (0.254-1.29)	1.6	4.5	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924
2,684 (927-4,351)					0.772 (0.373-1.75)	1.6	4.5	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.382 (0.128-0.997)	1.6	4.5	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350
2,268 (554-3,503)	juvenile salmon	whole-body	1.8	10	0.388 (0.170-1.078)	0.60	1.6	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.258 (0.0934-0.476)	0.60	1.6	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924
2,684 (927-4,351)					0.284 (0.137-0.644)	0.60	1.6	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.140 (0.0472-0.367)	0.60	1.6	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350
2,268 (554-3,503)	juvenile salmon	whole-body	1.5	05	0.316 (0.139-0.897)	0.50	1.4	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.210 (0.0761-0.388)	0.50	1.4	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924
2,684 (927-4,351)					0.232 (0.112-0.525)	0.50	1.4	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.114 (0.0385-0.299)	0.50	1.4	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350
2,268 (554-3,503)	juvenile salmon	whole-body	1.0	0	0.211 (0.0927-0.586)	0.33	0.91	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.140 (0.0507-0.258)	0.33	0.91	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924

calculated K_d mean and range	food web: particulate >insect >fish	tissue	target Se (µg/g dw)	EC	predicted allowed dissolved Se mean and range (µg/L)	predicted allowed particulate Se (µg/g dw)	predicted allowed invertebrate Se (µg/g dw)	hydrologic condition (transect, water year/flow season)	observed dissolved Se mean and range (µg/L)	observed particulate Se mean and range (µg/g dw)	Net Delta Outflow Index (daily average per month cfs)
2,684 (927-4,351)					0.154 (0.0746-0.350)	0.33	0.91	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.076 (0.0257-0.199)	0.33	0.91	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350
FISH (DIET)											
2,268 (554-3,503)	juvenile salmon	diet	2.7 (=3.0 wb)	10	0.632 (0.278-1.758)	0.97	2.7	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.421 (0.152-0.775)	0.97	2.7	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924
2,684 (927-4,351)					0.463 (0.224-1.051)	0.97	2.7	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.229 (0.0770-0.598)	0.97	2.7	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350
2,268 (554-3,503)	juvenile salmon	diet	2.2 (=2.4 wb)	05	0.506 (0.222-1.406)	0.80	2.2	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.337 (0.122-0.620)	0.80	2.2	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924
2,684 (927-4,351)					0.371 (0.179-0.841)	0.80	2.2	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.183 (0.0616-0.479)	0.80	2.2	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350
2,268 (554-3,503)	juvenile salmon	diet	1.5 (=1.65 wb)	0	0.348 0.153-0.967	0.54	1.5	January 22, 2003 above normal/high	0.245 (0.111-0.599)	0.411 (0.27-0.58)	50,847
2,981 (1,256-6,398)					0.231 0.0837-0.426	0.54	1.5	January 15, 2004 below normal/high	0.215 (0.114-0.523)	0.519 (0.23-1.0)	30,924
2,684 (927-4,351)					0.255 0.123-0.578	0.54	1.5	April 22-23, 2003 above normal/high	0.356 (0.115-1.008)	0.614 (0.28-1.31)	21,218
5,855 (1,628-12,650)					0.126 0.0423-0.329	0.54	1.5	October 10, 2003 below normal/low	0.174 (0.068-0.532)	0.751 (0.37-1.53)	4,350

Table 20. Prediction scenarios using Suisun Bay-Carquinez Strait transects for a suspended particulate material>*C. amurensis*>white sturgeon food web.

fish Se target (µg/g wb, dw)	K_d	predicted dissolved Se µg/L	predicted particulate Se µg/g	predicted prey Se µg/g
TTF_{fish} = 1.1; TTF_{clam} = 17				
8	1,180 (June 98, 11 days)	0.363	0.428	7.27
5		0.227	0.267	4.55
1.8		0.082	0.096	1.64
8	2,666 (Apr 99, 16 days)	0.160	0.428	7.27
5		0.100	0.267	4.55
1.8		0.036	0.096	1.64
8	3,435 (Oct 98, 22 days)	0.125	0.428	7.27
5		0.078	0.267	4.55
1.8		0.028	0.096	1.64
8	5,986 (Nov 99, 70 days)	0.071	0.428	7.27
5		0.045	0.267	4.55
1.8		0.016	0.096	1.64
TTF_{fish} = 1.1; $TTF_{clam + amphipod}$ = 8.8[a]				
8	1,180 (June 98, 11 days)	0.700	0.826	7.27
5		0.438	0.517	4.55
1.8		0.158	0.186	1.64
8	2,666 (Apr 99, 16 days)	0.310	0.826	7.27
5		0.194	0.517	4.55
1.8		0.070	0.186	1.64
8	3,435 (Oct 98, 22 days)	0.241	0.826	7.27
5		0.150	0.517	4.55
1.8		0.054	0.186	1.64
8	5,986 (Nov 99, 70 days)	0.138	0.826	7.27
5		0.086	0.517	4.55
1.8		0.031	0.186	1.64
TTF_{fish} = 0.8; TTF_{clam} = 17				
8	1,180 (June 98, 11 days)	0.499	0.588	10
5		0.312	0.368	6.25
1.8		0.112	0.132	2.25
8	2,666 (Apr 99, 16 days)	0.221	0.588	10
5		0.138	0.368	6.25
1.8		0.050	0.132	2.25
8	3,435 (Oct 98, 22 days)	0.171	0.588	10
5		0.107	0.368	6.25
1.8		0.039	0.132	2.25
8	5,986 (Nov 99, 70 days)	0.098	0.588	10
5		0.061	0.368	6.25

fish Se target (µg/g wb, dw)	K_d	predicted dissolved Se µg/L	predicted particulate Se µg/g	predicted prey Se µg/g
1.8		0.022	0.132	2.25
TTF_{fish} = 0.8; $TTF_{clam + amphipod}$ = 8.8 [a]				
8	1,180 (June 98, 11 days)	0.963	1.14	10
5		0.602	0.710	6.25
1.8		0.217	0.256	2.25
8	2,666 (Apr 99, 16 days)	0.426	1.14	10
5		0.266	0.710	6.25
1.8		0.096	0.256	2.25
8	3,435 (Oct 98, 22 days)	0.331	1.14	10
5		0.207	0.710	6.25
1.8		0.074	0.256	2.25
8	5,986 (Nov 99, 70 days)	0.190	1.14	10
5		0.119	0.710	6.25
1.8		0.043	0.256	2.25

[a] TTF = 8.8 is a composite TTF of TTF_{clam} + $TTF_{amphipod}$ where diet is assumed as 50% *C. amurensis* (TTF = 17) and 50% amphipod (TTF = 0.6). Predicted prey concentrations also are a composite that would need to be separated into components to assess the allowable *C.* amurensis Se concentration and the allowable amphipod Se concentration.

Table 21. Prediction scenarios using Suisun Bay-Carquinez Strait transects for a suspended particulate material>*C. amurensis*>clam-eating bird species food web.

bird egg Se target (µg/g wb, dw)	K_d	predicted dissolved Se µg/L	predicted particulate Se µg/g	predicted prey Se µg/g
$TTF_{bird\ egg}$ = 2.6; TTF_{clam} = 17				
12	1,180 (June 98, 11 days)	0.230	0.271	4.62
7.7		0.148	0.174	2.96
5.9		0.113	0.133	2.27
12	2,666 (Apr 99, 16 days)	0.102	0.271	4.62
7.7		0.065	0.174	2.96
5.9		0.050	0.133	2.27
12	3,435 (Oct 98, 22 days)	0.079	0.271	4.62
7.7		0.051	0.174	2.96
5.9		0.039	0.133	2.27
12	5,986 (Nov 99, 70 days)	0.045	0.271	4.62
7.7		0.029	0.174	2.96
5.9		0.022	0.133	2.27
$TTF_{bird\ egg}$ = 2.6; $TTF_{clam + amphipod}$ = 8.8 [a]				
12	1,180 (June 98, 11 days)	0.444	0.524	4.62
7.7		0.285	0.337	2.96
5.9		0.219	0.258	2.27
12	2,666 (Apr 99, 16 days)	0.197	0.524	4.62
7.7		0.126	0.337	2.96
5.9		0.097	0.258	2.27
12	3,435 (Oct 98, 22 days)	0.153	0.524	4.62
7.7		0.098	0.337	2.96
5.9		0.075	0.258	2.27
12	5,986 (Nov 99, 70 days)	0.088	0.524	4.62
7.7		0.056	0.337	2.96
5.9		0.043	0.258	2.27

[a] TTF = 8.8 is a composite TTF of TTF_{clam} + $TTF_{amphipod}$ where diet is assumed as 50% *C. amurensis* (TTF = 17) and 50% amphipod (TTF = 0.6). Predicted prey concentrations also are a composite that would need to be separated into components to assess the allowable *C.* amurensis Se concentration and the allowable amphipod Se concentration.

Table 22. Prediction scenarios using landward-focused transects for suspended particulate material>aquatic insect>juvenile salmon or steelhead.

fish Se target (µg/g wb, dw)	K_d	predicted dissolved Se µg/L	predicted particulate Se µg/g	predicted prey Se µg/g
TTF_{fish} = 1.1; $TTF_{aquatic\ insect}$ = 2.8				
8	2268 (50,847 cfs)	1.145	2.597	7.27
5		0.716	1.623	4.55
1.8		0.258	0.584	1.64
8	2981 (30,924 cfs)	0.871	2.597	7.27
5		0.545	1.623	4.55
1.8		0.196	0.584	1.64
8	2684 (21,218 cfs)	0.968	2.597	7.27
5		0.605	1.623	4.55
1.8		0.218	0.584	1.64
8	5855 (4,350 cfs)	0.444	2.597	7.27
5		0.277	1.623	4.55
1.8		0.100	0.584	1.64

Table 23. Prediction scenarios using landward-focused transects for suspended particulate material>aquatic insect>rail.

fish Se target (µg/g wb, dw)	K_d	predicted dissolved Se µg/L	predicted particulate Se µg/g	predicted prey Se µg/g
$TTF_{bird\ egg}$ = 2.6; $TTF_{aquatic\ insect}$ = 2.8				
12	2268 (50,847 cfs)	0.727	1.648	4.62
7.7		0.466	1.058	2.96
5.9		0.357	0.810	2.27
12	2981 (30,924 cfs)	0.553	1.648	4.62
7.7		0.355	1.058	2.96
5.9		0.272	0.810	2.27
12	2684 (21,218 cfs)	0.614	1.648	4.62
7.7		0.394	1.058	2.96
5.9		0.302	0.810	2.27
12	5855 (4,350 cfs)	0.282	1.648	4.62
7.7		0.181	1.058	2.96
5.9		0.138	0.810	2.27

External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Contract No. EP-C-12-021
Work Assignment 1-43

Submitted to:

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September 25, 2014

Contents

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Dr. Kevin V. Brix

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

I found the overall clarity of the document to be good, although there are several specific areas that require clarification (detailed in comments to specific charge questions). I also found the construction of the criterion statement to be quite clear and logical.

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Yes, primacy of the egg-ovary element is sound and well supported by the scientific literature. EPA has cited all of the key references for support of this approach.

        ii. Is the primacy of the whole-body/fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Yes, in general a tissue-based criterion should have primacy over a water-based criterion for Se due to the complex site-specific nature for Se bioaccumulation. This is well documented in the literature. As discussed by EPA, an egg-ovary based criterion is highly desirable but may not always be achievable due to logistical constraints or the potentially significant impacts on populations of terminal sampling of ovaries for some threatened or endangered species. In such cases, whole body or muscle plugs provide a reasonable surrogate for the egg-ovary element. One item lacking from the WQC is guidance on when use of whole body or muscle elements is acceptable. Some questions that come to mind:

1.) Can WB or muscle elements be used instead of EO even when collection of EO samples is considered logistically and environmentally feasible?

2.) Are there seasonal considerations to use of WB and muscle samples? For example, is it acceptable to use WB or muscle samples collected in the Fall for a species that spawns in the Spring?

iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

There are of course a number of uncertainties in the tiered approach proposed by EPA. I provide specific comments on these uncertainties throughout this review. Overall though, I do not believe there are any currently available data sources, models or alternative approaches that EPA has not considered that would significantly reduce the uncertainty.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

I have provided specific comments on these issues in response to the questions below.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10^{th} centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

It is unclear to me why EPA has selected the EC10 as the measurement endpoint for these studies. EPA argues because it is a tissue-based criterion, the measure of exposure is less variable than might occur for a water-based criterion. I understand the point EPA is making and agree that a tissue-based criterion is more integrative of exposure than a water-based criterion. However, following this logic, EPA is then stating that for a chemical with a water-based criterion in a system where the exposure concentration is consistently above the EC10 (e.g., very stable at a concentration equivalent to the EC15) that it is not sufficiently protective.

It seems to me that the ECx selected should be based on the level of protection EPA intends to provide and that this is independent of variability in exposure. Variability in exposure is more appropriately addressed via averaging periods as EPA has done with the intermittent exposure element of the criterion. In fact, by considering both an intermittent exposure element and using an EC10, EPA is addressing the same issue twice.

Given the above, I do not believe EPA has provided a scientific rationale for use of the EC10 in a tissue-based criterion as providing an equivalent level of protection as an EC20 in a water-based criterion. I recommend EPA evaluate how use of the EC20 would affect the final criteria calculations. I suspect given the sharp dose-response relationships for Se, it will not dramatically change the final criteria calculations. Alternatively, if EPA now believes the EC10 is an appropriate level of protection for WQC, then this should be applied across chemicals.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

    a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

Overall, I found EPA's use of the available data to derive the egg-ovary element to be scientifically sound. However, see caveats in b and c below. I did find EPA's use of the data for *Gambusia* to be questionable. Given the variability in the EO:WB ratio across species and the complete lack of data on this ratio for ovovivaprous fish, the EO-based threshold for this genus is highly questionable. Given this uncertainty and that these are the only data used in the WQC calculation in which EO Se was not directly measured, in my opinion, data for this genus should not be used in the WQC calculation.

    b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

I agree with EPA that currently available data indicates oviparous fish are more sensitive than aquatic invertebrates to Se. However, it is important to note that there is a paucity of data for invertebrates. I agree with EPA's approach to translate available invertebrate data to an EO threshold for purposes of developing a species sensitivity distribution (SSD). However, I strongly disagree with the addition of 2 hypothetical crustaceans to the SSD. This is scientifically indefensible (just making up data) and the WQC calculation should be based only on taxa for which there are actually data available. By this logic, why add only 2 crustacean taxa, why not 3 or 5?

Note, EPA needs to include the data from Conley et al. (2011, 2013, and 2014) in its assessment of Se toxicity and trophic transfer to mayflies.

Overall, given the limited data, I think EPA has overstated the certainty with which we can conclude fish are more sensitive than invertebrates. All we can really say is that based on a relatively small data set, available data suggests the tissue based WQC will be protective of invertebrates.

    c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

I agree with EPA, that generally, the reproductive endpoint is more sensitive than other endpoints such as juvenile growth. However, in the case of salmonids, there is at least some evidence (e.g., Hamilton et al.,

1990) that juvenile growth is comparable in sensitivity to reproduction. It is also worth pointing out that these studies did not include pre-exposure of the parents and subsequent maternal transfer, so it is possible that exposure and subsequent effects on juvenile growth have been underestimated. Further, juvenile salmonids have a much more limited home range and potentially higher intensity of exposure if they rear in Se contaminated areas compared to adult salmonids (particularly migratory species). Given this, it is unclear to me that placing primacy on the egg-ovary element will necessarily be protective of these species. EPA should consider the potential that juvenile whole body Se concentrations for migratory salmonids may need primacy or at least concurrent compliance monitoring to ensure the protection of these important species.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

Yes, it was reasonable to reject these studies for the reasons stated by EPA. In my opinion, there is currently insufficient information to have confidence that injection studies replicate realistic environmental exposures with respect to Se homeostasis. Indeed, the fact that the catfish study resulted in such an unusually low effect level suggests there may be different processes occurring in these types of studies. EPA has adequately documented that catfish do no appear to be uniquely sensitive based on available field abundance data in Se-impacted systems, counter to the lab-based injection study.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

Yes, I found the egg-ovary to muscle and whole body translations to be understandable and scientifically defensible.

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

I appreciate that EPA is dealing with a very difficult issue in terms of translating a tissue-based criteria to water for routine monitoring and screening purposes. I agree with the general conceptual model EPA has developed for making this translation. Having said that, the details of how EPA has implemented this conceptual model I think are very problematic. My concerns center on two major themes – compounding multiple uncertain values in the food chain transfer models and lack of transparency on what level of protection the proposed water elements provide.

I am very concerned that EPA is placing too much value on extrapolated and modeled values. The translation approach involves building food chain models for 69 sites that in many cases have significant data gaps (e.g., dietary composition, extrapolated TTFs, extrapolated CFs, etc.). To address these uncertainties, EPA developed a series of protocols for filling in the data gaps (e.g., using TTFs for species in the same order). While I appreciate the logic and largely agree with these protocols, ultimately, information derived in this manner is not measured data. This approximated information is then used in a very quantitative manner for setting the water-based WQC. Figure 11 in particular I find very misleading. How many of the data points in those two distributions (lotic and lentic) are based on sites where all parameters in the food chain models were actually measured? I did not take the time to calculate this, but EPA must explicitly provide this information. I suspect the percentage will be quite low. What do these distributions in Figure 11 look like if based on only studies where all parameters were directly measured? In my view, use of such data provides a potentially very inaccurate picture of what we actually know about the distribution of waterborne Se concentrations associated with the tissue-based WQC. This seems to be a significant departure from previous WQC criteria derivation processes where if data for a particular study were insufficient, the study was simply excluded and the resulting uncertainty from having relatively few complete data sets was reflected in a lower WQC (e.g., a WQC less than the most sensitive taxa tested if $n<20$).

An important element of previous WQC was transparency in the level of protection being provided (e.g., 95 % of taxa) and the assumptions underlying that protection (e.g., that tested taxa were representative of aquatic communities in the US). It is entirely unclear to me what level of protection is being provided by

the water element of proposed WQC. The proposed water-based WQC is based on the 20th percentile for lotic and lentic sites that were modeled (see concerns about this in the previous paragraph). But even this is not correct, because for some sites, multiple fish species were modeled per site. This raises numerous questions regarding independence of values in the distribution, whether the sites evaluated are biased towards those with known Se issues, etc. EPA has also not made it clear why protection of 80% of sites is a desirable regulatory objective. Why not 70%, 90%, or 95%? I appreciate that EPA has undertaken a ground truthing exercise to evaluate the proposed water element WQC. However, it is unclear exactly how EPA undertook this analysis. Were there truly over 3,000 independent sites that EPA evaluated? If this exercise concluded that <10% of sites would result in false negatives, then what does this say about the representativeness of the 69 sites and what is the real level of protection being provided?

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

In general, EPA has used a scientifically defensible method to derived TTFs. However, I am concerned that the TTFs derived from field data by EPA are biased low and potentially not protective. I note that the data in Figure 16 appear to show a rather significant bias towards underprediction of EO selenium concentrations, consistent with this concern. As recognized by EPA, there is typically an inverse relationship between the exposure concentration and the TTF such that low dietary Se will result in relatively high TTFs for a given predator-prey species pair. Many of the field data sets used by EPA are from sites with high levels of Se contamination (10's to 100's $\mu g\ l^{-1}$ waterborne Se). Conversely, a number of the data sets are from extremely low Se environments (e.g., mayfly). Perhaps, for TTF derivation purposes, EPA should constrain calculation of the median TTF to conditions that approximate the range of WQC (e.g., 0.5-10 $\mu g\ l^{-1}$ in water) that EPA might consider on a site-specific basis, or the range range of concentrations typically associated with the EC10 for sensitive fish species. Otherwise, individual TTFs have the potential to be biased either low or high depending on the site(s) from which they were collected. EPA should carefully review the biokinetic data using similar criteria.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

EPA has used a scientifically defensible method for deriving CFs. I am not aware of any other data EPA should consider. It could be argued that a regression based approach be used instead of the ratio approach EPA has adopted. In some cases, it appears that residuals are structured, suggesting that assumptions of the CF approach may be violated. At least for the 4-5 most sensitive taxa, EPA should conduct a sensitivity analysis of the regression-based approach versus the ratio approach and particularly consider confidence in the CF at concentrations that approximate the EC10.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

Yes, the method for deriving EFs was scientifically defensible and appears to have been applied in a consistent manner. However, similar to my comments regarding TTFs, there is frequently an inverse relationship between water Se and EF. EPA should carefully examine the distribution of EFs as a function of water Se and assess whether their data set is unduly biased by EFs measured in systems with unusually low or high waterborne Se. It would be helpful if Table 12 included the mean or median water Se concentration at the site. Note, in the section on calculation of EFs, there is no reference to where the EFs for the 69 individual sites can be found (i.e., Appendix L).

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

EPA's proposed method for addressing intermittent and time-varying discharges appears reasonable given available data. Ideally, intermittent criteria would be based on a biokinetic modeling approach and EPA's effort to evaluate their proposed approach using biokinetic modeling is encouraging. However, given the limited biokinetic data currently available, it is probably premature to implement such an approach for setting WQC. Further use of such an approach may be unnecessarily complicated if the simpler approach proposed by EPA continues to achieve the same objective as the biokinetic approach. A major uncertainty in the approach and subsequent biokinetic evaluation is the near complete lack of kinetic data for EF. If depuration kinetics are slower than EPA has assumed for primary producers, then this will have significant impacts on the validity of this approach.

The issue of generating pulse loads of Se that may ultimately result in Se accumulation in sensitive downstream systems (e.g., pulse loads in a river that discharges to a wetland) is a legitimate concern. However, in my opinion, this is a site-specific issue and it is not reasonable to establish national WQC that ensure protection of these sites without dramatically increasing the false positive rate for the WQC. However, it would be useful for EPA to provide specific language on the need to consider loading to downstream environments when regulating intermittent discharges or developing site-specific WQC.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

After reviewing the public/stakeholder comments, I highlight the following comments which I would also make above and beyond responses to specific review questions EPA has asked:

1.) Because some states will continue to use an acute WQC for Se, I agree EPA needs to clarify its position on the scientific credibility of the existing acute WQC.

2.) There were a number of comments indicating that use of an instantaneous averaging period and "never to exceed" for the tissue element is inappropriate and inconsistent with the Guidelines. I disagree with these comments and support EPA's decision.

3.) I agree with several commenters that EPA must develop rigorous definitions of lentic and lotic as guidance for regulators.

4.) EPA needs to provide some guidance on how small first order and ephemeral streams that naturally do not support fish populations should be regulated. There are a large number of these streams in the western US that have Se issues. Note, in these types of systems or in small wetland systems without fish, aquatic-dependent birds may be the most sensitive receptor. These leads to the obvious comment that if this WQC is intended to protect all US surface waters, EPA must develop guidance on the protection of aquatic-dependent wildlife.

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Dr. Gregory A. Cutter

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

In general the document is clearly written, but there are numerous typographical errors, missing references (e.g., EPRI, 2006 cited first on p. 16), and incorrect citations (e.g., Table 12 cites Appendix L, but the Appendices only go to K). Some key words are poorly chosen (the freshwater criterion parts are called "elements"), especially considering that this document concerns an aquatic trace ELEMENT, and other elements such as mercury are also discussed; I recommend selecting another key word for this. The use of acronyms and abbreviations are unavoidable in a document like this, and while they provided a table listing them all (which should be numbered Table 1 on page xi), it would make the document more readable to those only looking for some specific details to periodically redefine these in the text, for example the first time it is used extensively in a new section. The criterion statement (largely in Section 3.8) is clearly written and presented, although I have serious scientific problems with parts of it to be elaborated below. While this was not directly requested in our charge, but has direct bearing on the problems in this document, the review section on the aquatic biogeochemistry of selenium (pp. 9-17) has factual errors that may reflect on the authors understanding of the selenium or on some biases. First, in Section 3.2 the statement that "…the effects are integrated across forms of selenium; thus water column values are based on total selenium exposure." is an oversimplification that leads to conceptual errors later. The amount of dissolved selenium that enters the food web through the first trophic level is strongly linked to the speciation of dissolved selenium (e.g., Reidel et al., 1991; Baines and Fisher, 2001; Baines et al., 2001; Baines et al., 2004), which for freshwater and marine/brackish species is: selenite=organic selenide>>selenate. So for a lotic or lentic water body that is dominated by selenate, the incorporation of selenate into the phytoplankton biomass is much lower than that if the selenium was in the +4 oxidation state. In the next section 3.2.1, it starts off with serious errors, in particular "organo-selenide" being selenomethionine. Data on the speciation of dissolved organic selenide show it to be in soluble peptides and proteins, not free amino acids (e.g., Cutter, 1982; Cutter and Cutter, 1995), so phytoplankton uptake studies using free selenomethionine are not using the actual dissolved forms and likely overestimating uptake.

A following sentence says that selenite tends to dominate in "slow moving waters", presumably lentic environments. However, there are no data in the literature to support this statement (e.g., see compilations in Cutter, 1989a); selenite is only dominant when there is a large, fossil fuel-derived input, regardless of water residence time (e.g., Cutter, 1989a, 1989b). In this respect, on p. 14, 2nd complete sentence, they state that geologic AND anthropogenic sources often release mostly selenate, but most anthropogenic sources produce selenite (e.g., Cutter, 1989a, 1989b; Cutter and Church, 1986), only geological sources (weathered or irrigated) yield selenate; the presence of selenite in surface waters can in fact be used as a fossil fuel-combustion source indicator (e.g, Cutter, 1989a, 1989b). Interestingly, the last paragraph on p. 14 is largely correct in stating that the concentration of particulate selenium in the first trophic level (algae) is highly dependent on the dissolved speciation; this begs the question of why the authors later ignore speciation and calculate EF on total (presumably dissolved) selenium in the water column and particles; see later comments.

In the Bioaccumulation section (3.2.2), the major error, and this is significant in terms of bioavailability, is that dissolved selenium uptake results in elemental selenium and organoselenium (2nd to last sentence on p. 15). Elemental selenium is only produced by dissimilatory (heterotrophic) reduction under low oxygen conditions (many works of Oremland, but they correctly cite Oremland et al., 1989); autotrophs perform assimilatory reduction to selenide that is then coupled with acetyl CoA, serine, etc to produce seleno amino acids. Also, the use of the term "absorbed" is poorly chosen in that it implies simple exchange with no chemical reactions; dissolved selenium is assimilated (or incorporated) into autotrophic organic matter, which in the case of selenite uptake/assimilation/incorporation involves a change in oxidation state and chemical form (i.e., selenite is reduced to selenide and bonded with carbon to produce seleno amino acids like selenocysteine).

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Given the known, well documented, and published in the peer-reviewed literature information, choosing the egg-ovary compartment/vector/whatever (not element) is very well justified. The accuracy of then selecting a suitable value for various fish species depends on a critical evaluation of the literature, or new experiments.

        ii. Is the primacy of the whole-body/fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Again, this is well documented and the only proviso would be the choice/selection of the CF value

        iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

While the approach is scientifically justifiable, the propagation of errors that combine to make the total uncertainty is a bit daunting. Indeed, their frequent use of r or r^2 values for log/log plots completely masks the overall uncertainty; what are the correlations for direct concentration comparisons? I suspect they are much less than 0.4 and the p values would make them far less significant. Having said this, the trophic level transfers between higher levels (1 and above) are well described and parameterized in the literature, so the authors really should do a complete error/sensitivity analysis to quantify the overall error/uncertainty.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

I found the time and frequency evaluations of the factors (not elements) well justified, with the exception of the EF, to be explained below.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10^{th} centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

This seems like a statistically-valid approach to setting the threshold, but toxicology is not my field of expertise.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

    a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

In as much as fish are the most vulnerable to Se toxicity, and it is manifested primarily at reproduction, the egg-ovary focus is justified. The availability of data that passed the EPA criteria is somewhat limited, but statistically valid. Having said this, I am not well-versed in fish toxicity literature, so I rely on the other reviewers to point out data sets that may have been overlooked (e.g., I know they missed many water column data).

b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

In the aquatic systems in which I have worked with selenium, we have never encountered Se problems with invertebrates, and the literature seems to bear this out. So it seems to me that setting the criteria for the most at risk population is the best approach.

c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

Again, fish toxicity is not my expertise, so I cannot adequately respond to this question.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

I cannot recommend using any artificial means of introducing selenium to tissues; exposure must be through food and the assimilation pathways it follows for a given species. In this respect, chemical speciation is very important, so the exact form of organic selenide (peptide vs free amino acid, seleno methionine vs seleno cysteine; cytosol vs proteins) is critical to its uptake and eventual assimilation (e.g., Reinfelder and Fisher, 1994; Luoma et al., 1992).

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

The methodology is well described and documented, but as above I would like to see a more thorough error analysis for the resulting CFs.

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The overall approach of considering selenium's pathway from the water column, dissolved state, through trophic levels, and into tissues such as reproductive organs is well justified, particularly the trophic transfer model that is dynamic and rate/kinetically based (uptake rate * assimilation efficiency/elimination rate); the trophic transfer approach largely developed by Nick Fisher and collaborators. However, the water to first trophic level approach is completely unacceptable in that it is not dynamic or rate-based (actually assumes equilibrium) and completely ignores the effects of speciation. The latter is curious in that they seem to be relying on the Chapman et al (2009 and 2010) recommendations from the SETAC Pellston workshop which specifically states, "Understanding Se speciation is critical to understanding its mobility, transformation, partitioning in the environment, and potential risk to aquatic ecosystems." and "The single largest step in the bioaccumulation of Se occurs at the base of food webs, characterized by an "enrichment function"; thermodynamic or equilibrium-based principles are not appropriate for predicting Se bioaccumulation at the base of food webs." The choice of the Presser and Luoma model used in this EPA

document is completely contrary to these recommendations since the water/particle ratio called the Enrichment Factor (EF) is only a renamed equilibrium distribution coefficient (K_d) that was used long ago for metal cations. Dissolved and particulate selenium speciation cannot be modeled with equilibrium approaches, it must consider the kinetics of the transfers/transformations (e.g., Cutter, 1992). Since the transfer of dissolved selenium in any of its chemical forms to the particulate state (largely assimilation by phytoplankton and conversion to organic selenide – seleno amino acids in proteins) changes the chemical forms, how does one calculate a distribution coefficient (EF)? For selenium, dissolved selenite or selenate are not what are in the particulate state (organic selenides), so which dissolved species and which particulate species do you use to calculate EF? And, they are certainly not reversible (selenite uptake followed by regeneration does not return selenite, but rather organic selenide…which may later oxidize back to selenite and selenate; Cutter, 1982; Cutter and Bruland, 1984). In this EPA document, they "solve" this issue by only considering total dissolved selenium, in contradiction to the recommendations at the Pellston workshop.

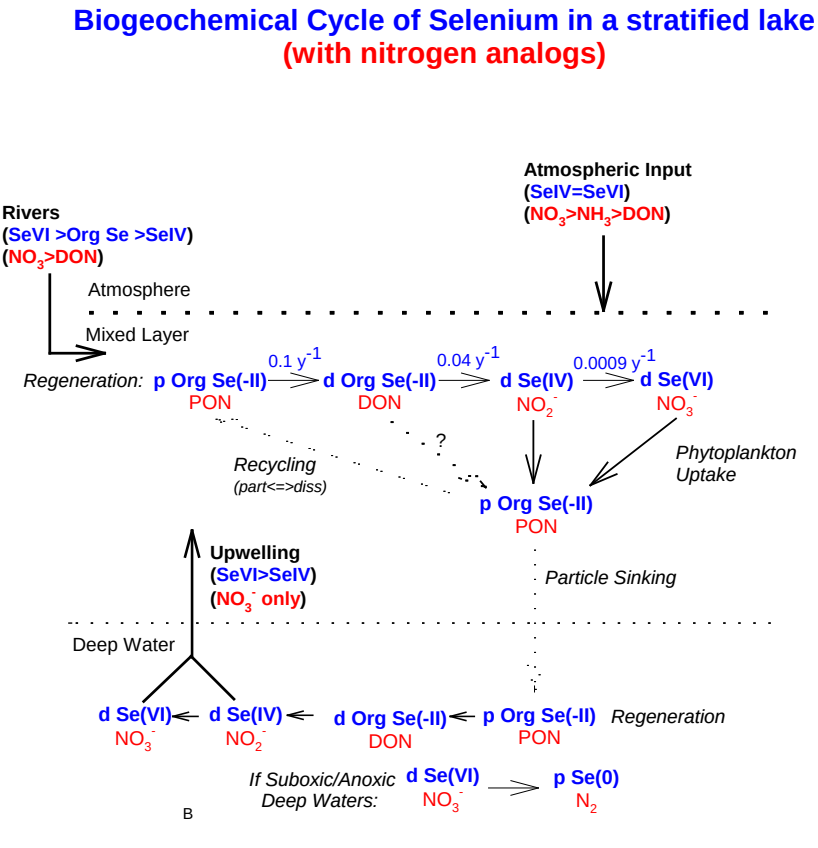
The use of the Presser and Luoma (2006, 2010) model for any aquatic ecosystem to predict dissolved or particulate concentrations is questionable for the simple reason that while it acknowledges the importance of chemical speciation, and the rates of processes (kinetics as opposed to equilibrium thermodynamics), it largely ignores them in application. It is a totally empirical model designed for the San Francisco Bay-Delta system, so its application to other systems may not work. To reiterate the preceding paragraph in detail, the primary problem with this model is the exchange between the dissolved and particulate phases, in this case the first trophic level (autotrophs/primary producers). While there is some adsorption of dissolved selenite and selenate to suspended particles (e.g., Doblin et al., 2006), most particulate selenium in organic matter is organic selenide in the form of seleno-amino acids in proteins (Wrench, 1978). In other words, the uptake of dissolved selenite and selenate from the water column by phytoplankton changes their chemical forms, it is reductively incorporated (Cutter, 1982; Cutter and Bruland, 1984).

Biological uptake of dissolved nutrients such as nitrogen, and metals, is best (most accurately) modeled using Michaelis-Menten kinetics, or at least pseudo-first order rate expressions. The release of this particulate organic selenide back into the water column as dissolved organic selenide is coupled to oxic (or anoxic) respiration (Cutter, 1982; Cutter and Bruland, 1984), which is also modeled using an appropriate rate expression (e.g., first order; see discussion in Meseck and Cutter, 2006). The critical point here is that the speciation of particulate selenium has no relation to that in the water column – reductive incorporation and subsequent regeneration obliterates this relationship and only a rate-based (kinetic) approach can accurately quantify it. However, the Presser and Luoma (2006, 2010) model uses equilibrium distribution coefficients (K_d or in this EPA document EF) to quantify how particulate selenium in the first trophic level reflects the dissolved concentration in the water column. The distribution coefficient approach works well for divalent metal cations where no oxidation state change occurs. For a given K_d value, if the dissolved concentration goes up, more adsorbs to the particles (to maintain equilibrium), and when the dissolved concentration drops, the particulate-bound metal desorbs. But, when there is a redox change between dissolved and particulate conversions, the equilibrium concept is violated. For example, if the concentration of selenite goes up, the rate of uptake increases, and the concentration of particulate organic selenide increases; in a crude fashion, the use of a K_d could mimic this biochemical process. But, when the concentration of dissolved selenite goes down, particulate organic selenide doesn't desorb to balance it; they are different chemical species. Particulate organic selenide is only released through respiration/regeneration, not adsorption/desorption (for which the K_d concept was created). So in this scenario, the Presser and Luoma (2006) cannot accurately predict the response to a change in dissolved concentration, and more importantly cannot predict the speciation of selenium.

Interestingly, Presser and Luoma (2006) note that as more recycling (i.e., the regeneration part of the selenium cycle depicted in Cutter and Bruland, 1984) occurs, organic selenide concentrations increase. Indeed, they do, but their model cannot reproduce this**, a problem if you "reverse" their model to predict water column dissolved concentrations of selenium for a given particulate concentration in**

the food web (e.g., 11.8 ppm Se in fish muscle; this document). This latter (highlighted) point is exactly what Section 4.2 is doing. On a related matter, the Presser and Luoma model suggests that it handles selenium speciation, but only in the dissolved phase, and then rather than using separate K_ds for each species, and presumably summing the contributions from each from to derive the particulate selenium concentration, they simply average the K_ds to one value and omit speciation.

To put this modeling approach into another perspective, it has been observed (Cutter, 2005) that the aquatic selenium and nitrogen cycles are very similar/parallel. Adding N cycling to the Se cycle depicted in Cutter and Bruland (1984) gives:



Thus, I ask those who wrote this document if they would use the Presser and Luoma (2006, 2010) approach to model nitrogen cycling and therefore set N discharge, etc limits? I suspect the answer would be no, and my response then would be, why use it for selenium?

To be constructive, what modeling approach should be used? In Cutter (1992) it was argued that a kinetic/rate approach, and not an equilibrium thermodynamic one (EFs are an equilibrium concept) is the only way to quantify the selenium cycle. There are at least two existing kinetic models for the selenium cycle: for lakes there is the one described in Porcella et al. (1991) and Bowie et al. (1996), and one for estuaries, Meseck and Cutter (2006). The Meseck and Cutter model focuses on the dissolved to first trophic level dynamics and includes the full speciation of selenium in the dissolved and particulate states in an estuary (San Francisco Bay/Delta). The Bowie et al. (1996) model uses a kinetic approach to modeling selenium speciation and dynamics from the dissolved state to all trophic levels in freshwaters, and was designed to assist in mitigation/restoration efforts. The Meseck and Cutter (2006) model also has direct applications to mitigation via scenario modeling (what if…). However, this model includes components to simulate sediment resuspension, mixing and dispersion, and primary production (light-

limited in this case), so it may be too complicated for the application needed here. Indeed, all that is needed is a model that covers dissolved to first trophic level interactions, and from there the existing biodynamic part of the Presser and Luoma (2006; 2010) could be employed. In this case, using Equations 4-6, and 7, in the Meseck and Cutter (2006) paper (and related equations in the Appendices) could suffice. Or, use simple Michealis-Menten equations and values in the literature (e.g., Riedel et al., 1991), and simple first order rate equations (and values) described in the literature (e.g., Cutter, 1982; Cutter and Bruland, 1984; Reinfelder et al., 1993).

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

After the dissolved to first trophic level particulate selenium part of the model that I am criticizing above, the rest of the Presser and Luoma (2006) model (including the derivation of TTFs) is excellent and accurately predicts bioaccumulation through the various parts of the food web (and earlier documented in the Luoma and Rainbow (2005) peer-reviewed paper). The reason here is that once into the first trophic level, the primary speciation of particulate selenium is organic selenide, and the concepts of assimilation efficiency, trophic transfer factors, ingestion and depuration (egestion) work well for selenium (and any other metal or nutrient).

The screening of data followed well-set protocols and are quite defensible. I am not aware of additional data to be included, but I'm sure there must be some in the grey literature.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

The calculation of the CF values was rather straightforward, with my only concern, as noted above, being a thorough quantification of the resulting errors in the CF values. As an overall statement, error propagation seems to have been largely ignored in this document.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

See above comments; I feel the EF values are completely useless and in fact incapable of being calculated given that they really need to include the chemical speciation of dissolved selenium. They did however

miss lots of dissolved and particulate data, many examples including: Cutter, 1989a; Cutter, G. A. 1991., Riedel and Cole, 2001 in their reference list, and river data in Cutter, 1989b and Cutter and San Diego-McGlone that are also in their reference list.

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

If a realistic concentration can be established using a more appropriate modeling approach (as above), then the calculation for intermittent discharges is fine. However, the propagation of errors must be carefully evaluated.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

I examined the public comments AFTER I had reviewed the document and written the above comments, so as to not bias my own evaluation. The comments (by my count, 429) ranged from editorial ones, to simple criticisms, to detailed scientific evaluations and suggestions. Of the later, the most common concerned "implementation" (16% of total), followed by "translation" (to water column criteria; 14%), and site specific criteria (13%). If we combine all the "criteria" comments (site, tiered, tissue, intermittent), these received the most comments (30%). Of these, most dealt with the details of developing the criteria (justifying the calculation methods, literature missed, apparent oversights or conflicts with existing procedures). Thus, the peer-review community (it seems that most of these comments came from consulting companies, municipal and state agency scientists, and some from the academic sector) feels the document needs considerable attention to reformulating the criteria. The next most important topic was then implementing the criteria (16% by itself) and in this respect most comments (actually criticisms) were directed to the water column formulation. Related to this was the "translation" of the tissues (all)-based criteria to the water column (14% of comments), and most of these comments were directed to the inappropriate use of the Presser and Luoma model. Considering my review above and the community

response, it would seem that the EPA needs to reformulate their methodology for setting water column criteria.

References for Cutter evaluation that are not in the existing EPA reference list:

Baines, S.B., N.S. Fisher, M.A. Doblin, and G.A. Cutter. 2001. Uptake of dissolved organic selenides by marine phytoplankton. Limnol. Oceanogr., 46: 1936-1944.

Baines, S. B., N.S. Fisher, M.A. Doblin, G.A. Cutter, L.S. Cutter, and B. Cole. 2004. Light dependence of selenium uptake by phytoplankton and implications for predicting selenium incorporation into food-webs. Limnol. Oceanogr., 49: 566-578..

Cutter, G.A. 1982. Selenium in reducing waters. Science 217: 829-831.

Cutter, G.A. and T.M. Church. 1986. Selenium in Western Atlantic precipitation. Nature 322: 720-722.

Cutter, G.A. 1989a. Selenium in fresh water systems. In: *Occurrence and Distribution of Selenium* (M. Ihnat, ed.). CRC Press, Florida, Chap. 10.

Cutter, G. A. 1991. Selenium biogeochemistry in reservoirs. Volume 1: Time series and mass balance results. Electric Power Research Institute, EPRI EN-7281, 97 pp.

Cutter, G.A. 1992. Kinetic controls on the speciation of metalloids in seawater. Mar. Chem., 40: 65-80.

Cutter, G.A. 2005. Biogeochemistry: now and into the future. Palaeogeogr. Palaeoclimatol. Palaeoecol. 219: 191-198.

Meseck, S.C. and G.A. Cutter. 2006. Evaluating the biogeochemistry of selenium in San Francisco Bay through modeling. Limnol. Oceanogr., 51:2018-2032.

Porcella, D.B., G.L. Bowie, J.G. Sanders, and G.A. Cutter. 1991. Assessing Se cycling and toxicity in aquatic ecosystems. Water Air Soil Pollut., 57-58: 3-11.

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Mr. David DeForest

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

There is a lot of information to digest and it may be difficult for non-technical readers to follow, but I feel that the document was organized in a logical manner and that the approaches were adequately described. Although I have technical comments relative to the criterion statement, I feel that format for presenting the selenium criteria based on multiple elements is clearly presented and easily digestible to the reader.

I have included here a few miscellaneous typos and editorial suggestions that I noted during my review:

p. 59, Table 7a: Correct spelling of "Onchyrhynchus" to " Oncorhynchus "

p. 60, paragraph below Table 7b: Correct spelling of "Leopmis" to "Lepomis"

p. 62, 1st paragraph: Correct spelling of "Oncorhyncus" to " Oncorhynchus "

p. 89, footnote a in Table 12: Appendix L should be Appendix K

p. 114, 1st paragraph, last sentence: Correct spelling of "criteirion" to "criterion"

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Yes, in my opinion the tiered construction of the chronic selenium criterion is logical and scientifically defensible. First, the critical exposure route for fish is dietary organic selenium (Janz et al. 2010), which is the basis for all of the studies in which egg or ovary selenium concentrations are linked to toxicity in offspring. Dietary organic selenium exposures are implicit in those studies in which adult females were exposed in the field and explicit in those studies in which adult females were exposed in the laboratory (primarily through the use of diets enriched with organic selenium, such as selenomethionine). Second, the critical toxicity endpoint for fish exposed to selenium is larval mortality, deformities, and/or edema following exposure to selenium during absorption of the yolk-sac. The selenium concentration in the egg or ovaries is the most relevant exposure metric for this exposure route and toxicity endpoint. Third, and related to the second point, is that fish species partition varying amounts of their total selenium burden to

the ovaries and eggs (deBruyn et al., 2008). Direct measurement of the selenium concentration in the eggs or ovaries addresses this between-species variability in selenium partitioning within tissues. Fourth, fish egg- or ovary-based selenium toxicity values (e.g., EC10s) are not highly variable among fish species, regardless of whether adult females were exposed to dietary organic selenium in the field or in the laboratory or whether species may be considered "warm-water" or "cold-water" species.

Some studies have also shown that juvenile fish survival and growth can be relatively sensitive to dietary organic selenium. For this toxicity endpoint, of course, an egg or ovary selenium criterion would not be applicable (but a whole-body selenium criterion would be). An important question, therefore, is whether compliance with an egg or ovary selenium criterion would be protective of juvenile fish. DeForest (2008) evaluated this question by comparing dietary Se toxicity data for juvenile growth and effects on larvae via maternal transfer. Although data were limited to bluegill sunfish (*Lepomis macrochirus*) for that evaluation, it was concluded that juvenile bluegill are not more sensitive than bluegill larvae exposed to selenium via maternal transfer. This would indicate that an egg or ovary selenium criterion should be protective of effects on juvenile survival and growth (if the observations for bluegill are translatable across fish species).

Although I agree that the primacy of each criterion element is logical, it is not clearly stated whether a water Se criterion could be adopted into a permit limit. For example, if compliance with the lotic or lentic Se criterion is demonstrated, is measurement of fish tissue Se concentrations necessary? If a water body meets a fish tissue-based Se criterion, but not a surface water criterion, would the water body be considered in compliance? I believe the answer to the latter is "yes", but this does not seem to be clearly stated in the draft AWQC document.

Literature cited:

deBruyn A, Hodaly A, Chapman P. 2008. Tissue selection criteria: Selection of tissue types for the development of a meaningful selenium tissue threshold in fish. Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field Prepared for the North American Metals Council - Selenium Working Group, Washington, DC.

DeForest D. 2008. Threshold development endpoints: Review of selenium tissue thresholds for fish: Evaluation of the appropriate endpoint, life stage, and effect level and recommendation for a tissue-based criterion. Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field Prepared for the North American Metals Council - Selenium Working Group, Washington, DC.

Janz DM, DeForest DK, Brooks ML, Chapman PM, Gilron G, Hoff D, Hopkins WA, McIntyre DO, Mebane CA, Palace VP, Skorupa JP, Wayland M. 2010. Selenium toxicity to aquatic organisms. 141-231 in Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, eds. Ecological assessment of selenium in the aquatic environment. SETAC Press, Pensacola, FL, USA.

ii. Is the primacy of the whole-body/ fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Yes, in my opinion the primacy of the whole-body or muscle selenium criterion over the water column criterion is scientifically sound. Selenium bioaccumulation potential from water to fish is highly site-specific (Brix et al., 2005; Presser and Luoma 2010; Stewart et al., 2010), so it is appropriate that a whole-body or muscle selenium criterion is given a priority over a water column selenium criterion.

Consideration of only a water column selenium criterion (or a water column selenium criterion that is given priority over a fish tissue-based selenium criterion) would necessarily have to be very low to ensure protection of the sites with the greatest selenium bioaccumulation potential. However, this would potentially be problematic because it would trigger concerns (i.e., selenium criterion exceedances) at locations where selenium bioaccumulation potential is lower and not of ecological concern.

Literature cited:

Brix KV, Toll JE, Tear LM, DeForest DK, Adams WJ. 2005. Setting site-specific water-quality standards by using tissue residue thresholds and bioaccumulation data. Part 2. Calculating site-specific selenium water-quality standards for protecting fish and birds. Environ Toxicol Chem 24:231-237.

Presser TS, Luoma SN. 2010. A methodology for ecosystem-scale modeling of selenium. Integr Environ Assess Manag 6:685-710.

Stewart R, Grosell M, Buchwalter D, Fisher N, Luoma S, Mathews T, Orr P, Wang W-X. 2010. Bioaccumulation and trophic transfer of selenium. 93-139 in Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, eds. Ecological assessment of selenium in the aquatic environment. SETAC Press, Pensacola, FL, USA.

iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

Overall, I believe that the tiered approach is scientifically appropriate. I do have specific comments on the actual selenium criteria at each tier, which are provided under specific charge questions below.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

The comments below are organized first by magnitude, duration, and frequency, and then by criterion element (i.e., fish egg or ovary, fish whole-body or muscle, and water column) within each of these categories.

Magnitude

Fish Egg/Ovary Se Criterion

<u>Brown Trout</u>

The draft fish egg/ovary selenium criterion is 15.2 mg/kg dw. This draft criterion is driven by brown trout (*Salmo trutta*), which had an EC10 of 15.91 mg/kg dw in the EPA's draft AWQC document. This study, conducted by Formation Environmental (2011a), has received tremendous scrutiny in how to best interpret the results and derive a defensible EC10. In my earlier review of that study on behalf of the Eastern Research Group (ERG) and EPA, I had concluded that the most relevant egg selenium EC10s that could be derived from that study ranged from 20.70-21.60 mg/kg dw. In that same review, however, I concluded that an egg selenium EC10 of 16.76 mg/kg dw was on the lower end of the range of possible EC10s that could be derived from the study. Accordingly, in my opinion, the EC10 of 15.2 mg/kg dw used by the EPA is an overly conservative interpretation of the brown trout Se toxicity study.

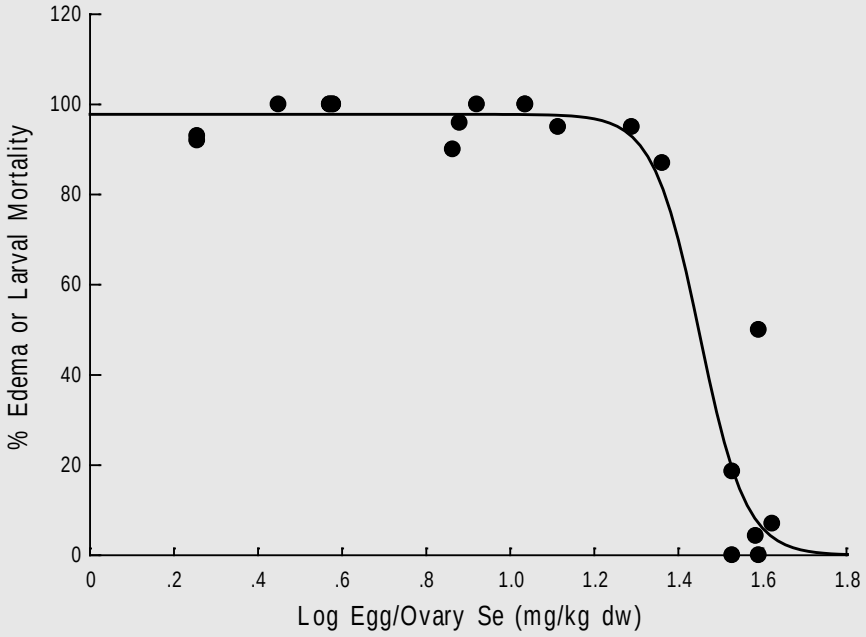
Bluegill

The second lowest species mean chronic value (SMCV) was 18.41 mg/kg dw for bluegill sunfish (*Lepomis macrochirus*). This SMCV was based on the geometric of EC10s from three studies: (1) an EC10 of 20.05 mg/kg dw from Doroshov et al. (1992); (2) an EC10 of 24.55 mg/kg dw from Coyle et al. (1993); (3) an EC10 of 12.68 mg/kg dw from Hermanutz et al. (1992, 1996). The latter EC10 is much less than the other two EC10s for bluegill and less than even a very conservative interpretation of the EC10 for brown trout. I agree with the interpretations of the Doroshov et al. (1992) and Coyle et al. (1993) studies, but disagree with the interpretation of the Hermanutz et al. (1992, 1996) study. The EC10 of 12.68 mg/kg dw from Hermanutz et al. (1992, 1996) is driven by two treatments from Study 1: these were Streams 3 and 8 which had an ovary Se concentration of 17.71 mg/kg dw and 80% edema was observed and Steam 4 which had an ovary Se concentration of 15.46 mg/kg dw and 50.3% edema was observed. At first glance, there are three issues that stand out:

- First, the water Se treatment concentration that resulted in an ovary Se concentration of 17.71 mg/kg dw in Study I was 10 µg/L—in the 10 µg Se/L treatment in Study II the ovary Se concentrations averaged 36.39 mg/kg dw and the average rate of edema was 83%. Thus, the rates of edema were consistent between the 10 µg Se/L treatments in Study I and II, on average, but the ovary Se concentrations were widely different. The mean macroinvertebrate Se concentrations in the 10 µg Se/L treatments in Study I and II were similar (grand means among all invertebrate taxa were 21.6 and 22.8 mg/kg dw for Study I and Study II, respectively [Hermanutz et al., 1996]). The relatively large difference in the bluegill ovary Se concentrations in Study I compared to Study II, therefore, is unexpected.
- Second, in Study I, the ovary Se concentration of 17.71 mg/kg dw in the 10 µg Se/L treatment was greater than the ovary Se concentration of 15.46 mg/kg dw in the 30 µg Se/L treatment. This is also unexpected because the grand mean Se concentration in invertebrate taxa collected from the 10 and 30 µg Se/L streams were 21.6 and 44.7 mg/kg dw, respectively. Thus, a higher ovary Se concentration in the 30 µg Se/L stream would be expected. This basis for this discrepancy is not clear, although the ovary Se concentration measured in the 30 µg Se/L stream was based on a single fish, which may have randomly had a lower ovary Se concentration.
- Third, a potentially more important source of uncertainty is that the ovary Se concentrations in the Hermanutz et al. (1992, 1996) study were reported on a wet weight basis. Dry weight ovary Se concentrations were estimated assuming a moisture content of 76%, which was based on the average from Gillespie and Baumann (1986), 85%, and Nakamoto and Hassler (1992), 67%. If the true moisture content was 85%, the bluegill Se EC10 from Hermanutz et al. (1992, 1996) would be 20.3 mg/kg dw (almost identical to the EC10 derived from Doroshov et al. [1992]). In contrast, if the true moisture content was 67%, the bluegill Se EC10 from Hermanutz et al. (1992, 1996) would be 9.2 mg/kg dw.

In my opinion, the uncertainty in the moisture content of the bluegill ovaries in the Hermanutz et al. (1992, 1996), along with uncertainties in the ovary Se concentrations in Study I, are sufficiently great that this study should not be included in the SMCV for bluegill, as there are two other studies (Doroshov et al. [1992] and Coyle et al. [1993]) for which dry weight ovary Se concentrations were reported and the EC10s from those two studies were very comparable. The SMCV for bluegill based on those two studies would be 22.2 mg/kg dw. Alternatively, if data from Study I of Hermanutz et al. (1992, 1996) are pooled with data from Doroshov et al. (1992) and Coyle et al. (1993), the consistency in the concentration-response data is apparent and an EC10 of 21.4 mg/kg dw can be derived (Fig.1).

Fig. 1. Concentration-response relationship for bluegill based on data pooled from Study I of Hermanutz et al. (1992, 1996), Doroshov et al. (1992), and Coyle et al. (1993). EC10 = 21.4 mg/kg dw based on logistic regression analysis in TRAP.



Other Fish Species in the SSD

The draft fish egg/ovary Se criterion derived following EPA guidelines is based on the four lowest GMCVs and the total number of GMCVs. The two lowest GMCVs in the EPA's draft document are for *Salmo* (represented by brown trout) and *Lepomis* (represented by bluegill), which were both discussed above. The 3rd and 4th lowest GMCVs are for *Micropterus* (represented by largemouth bass) and *Oncorhynchus* (represented by cutthroat trout and rainbow trout). I do not disagree with EPA's interpretation of the studies for those genera.

The *Esox* GMCV of <34 mg/kg dw, represented by northern pike, is an EC24 because the data were not amenable to derivation of an EC10 using TRAP. The EPA compared this EC24 to the EC24 that could be derived for rainbow trout and noted that the two species appear to be similar in sensitivity, with northern pike perhaps slightly less tolerant. In contrast, the original study authors for the northern pike study, Muscatello et al. (2006), reported an EC10 of 20.38 mg/kg dw based on linear regression. The EC10 of 20.38 mg/kg dw would make the *Esox* GMCV the 4th lowest in the EPA's dataset. This change alone, however, would have a negligible influence on the draft fish egg/ovary Se criterion—it would raise it slightly from 15.2 mg/kg dw to 15.6 mg/kg dw (lowering the 4th lowest GMCV steepens the slope of the line through the four lowest GMCVs, which increases the 5th percentile).

Number of GMCVs Assumed in Fish Egg/Ovary Se Criterion Calculation

The logic for setting the number of GMCVs to 14 is flawed in my opinion. This number is based on 9 fish genera, 3 invertebrate genera with tissue-based toxicity data available, and 2 crustacean genera that were waived. In my opinion, a genus sensitivity distribution based on Se toxicity values for fish eggs/ovaries, and for which the resulting criterion will be a Se concentration in fish eggs/ovaries, and for which compliance will be determined by measuring Se concentrations in fish eggs/ovaries, cannot include data for non-fish taxa. It must be remembered that a criterion based on an internal tissue concentration is not the same as a criterion based on an external concentration to which the entire aquatic community may be

exposed. One will not be able to measure Se concentrations in invertebrates in order to determine compliance with the fish tissue-based Se criterion, so they should not be included in the SSD. Further, if I understand correctly, the three whole body Se EC10s for invertebrates (37.84 mg/kg dw for *B. calyciflorus*, >140 mg/kg dw for *L. variegatus*, and 24.2 mg/kg dw for *C. triangulifer*) were multiplied by a (1) diet-to-whole body fish TTF and (2) a whole body-to-egg/ovary conversion factor in order to estimate the Se concentrations in fish eggs/ovaries that may result from the toxicity thresholds for invertebrates. These values were then used as "SMCV & GMCV as estimated EO concentration in an accompanying fish assemblage (mg Se/kg dw EO)" in Table 6b of the draft AWQC document. However, these are simply predicted concentrations in fish eggs/ovary and are not effect concentrations for fish. I believe that n should equal the number of fish genera, which is 9 based on the draft AWQC document.

Additional Genera that Could be Added to the Total N

Although the EPA did not include the egg/ovary Se toxicity data for white suckers (*Catostomus commersonii*; de Rosemond et al. 2005) and razorback suckers (*Xyrauchen texanus*; Hamilton et al. 2005a,b) because reliable toxicity thresholds (EC10s or other) could not be derived, there does appear to be sufficient evidence that they would be among the four most sensitive genera. Thus, the number of GMCVs used in the criterion calculation could be increased from 9 fish genera to 11 fish genera.

Toxicity Data for an Additional Fish Species

Nautilus Environmental in Burnaby, British Columbia has conducted a Se maternal transfer toxicity study with mountain whitefish (*Prosopium williamsoni*). This species does not appear to be especially sensitive (i.e., it would not be among the four lowest GMCVs), but it would added another genus to the sensitivity distribution. I recommend that the EPA investigate whether this study is publically available and, if so, whether it meets the EPA guideline for test acceptability and inclusion in the sensitivity distribution. The Se toxicity study with Yellowstone cutthroat trout (Formation Environmental 2011b) should also be considered.

Influence of Potential Changes to GMCVs and N

As summarized above, in my opinion, the most conservative and reasonable EC10 that can be derived for brown trout is 16.76 mg/kg dw (although the weight-of-evidence suggest to me that the EC10 falls between about 20.7-21.6 mg/kg dw) and that the bluegill SMCV should be 22.2 mg/kg dw. If the four lowest GMCVs were 16.76 mg/kg dw for *Salmo*, 20.35 mg/kg dw for *Micropterus*, 22.2 mg/kg dw for *Lepomis*, and 22.53 mg/kg dw for *Oncorhynchus*, and the total number of fish genera was set equal to 11 (with inclusion of the two sucker genera), the resulting criterion would be 16.0 mg/kg dw. Alternatively, if the *Esox* (northern pike) GMCV was adjusted from <34 mg/kg dw to 20.4 mg/kg dw, the resulting criterion would change slightly to 16.1 mg/kg dw.

Fish Whole-body and Muscle Se Criteria

The draft fish whole-body and muscle selenium criteria are 8.1 and 11.8 mg/kg dw, respectively. In general, I believe that the approach for deriving these draft criteria is reasonable and that the magnitudes of these criteria are consistent with the toxicological literature. My only suggestion is that the EPA consider using empirically measured whole-body Se (or muscle Se) data for those species where it is available, rather than applying CFs to egg/ovary Se data. It would be interesting to see whether that has a significant influence on the draft whole-body or muscle Se criteria. And of course if any modifications are made to the egg/ovary Se GMCVs, this would influence the draft whole-body and muscle Se criteria, as would a change to the number of genera, if my suggestions above are considered.

Surface Water Se Criteria - Monthly Average

The draft water column selenium criteria are 4.8 and 1.3 µg/L for lotic and lentic waters, respectively. In general, I do not agree with the approach used by the EPA in deriving these water column criteria.

Although I do not agree with the approach, I do believe that the draft criterion of 4.8 µg/L for lotic waters is reasonable and consistent with our understanding of the range of Se bioaccumulation potential into fish across a wide range of lotic sites. However, for the draft lentic Se criterion of 1.3 µg/L, the approach used by the EPA results in this criterion being almost exclusively driven by data for two reference locations. This in turn is mostly due to what I perceive as a flaw in the approach, where site-specific Se data in invertebrates and fish are ignored and instead non-site-specific TTFs and CFs are applied that are inconsistent with the site-specific data. This resulted in cases where erroneously high modeled Se concentrations in fish tissue are linked with low water Se concentrations (i.e., reference site concentrations), and then these become the "drivers" for the draft lentic criterion of 1.3 µg/L. Please see my detailed comments on this issue in Part III.

Surface Water Se Criteria - Intermittent Exposure

The draft intermittent exposure Se criteria represent a mathematical manipulation of the monthly average criteria in order to derive values that would still result in 30-day average concentrations of 4.8 and 1.3 µg/L for lotic and lentic waters, even if those were exceeded for *x* number of days. A limitation of this approach is that it does not consider the uptake and elimination kinetics of Se in aquatic food chains and the influence of exposure duration and magnitude on these biokinetic parameters. In my opinion, a biokinetic modeling-based approach would be more appropriate for deriving intermittent, or acute, criteria that are protective against exceeding fish tissue-based Se criteria. More details are provided in my comments in Part III below.

Duration

Fish Egg/Ovary, Whole-body, and Muscle Se Criteria

The draft fish tissue-based selenium criteria (eggs, ovaries, whole-body, muscle) are "instantaneous measurements" as "Fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish at a given site" and "Selenium concentrations in fish tissue are expected to change only gradually over time in response to environmental fluctuations." I agree with the EPA's decision that the duration for fish tissue Se measurements should be an instantaneous measurement since, for most scenarios and fish species, the Se concentrations in fish tissue will be reflective of a longer term exposure.

Surface Water Se Criteria - Monthly Average and Intermittent Exposures

In my opinion, 30 days for an average exposure duration is reasonable, especially since an intermittent criterion is being considered (although, as noted, I believe the intermittent criterion would best be derived using a biokinetic modeling approach). Biokinetic data for algae and several freshwater invertebrates indicate that steady-state Se concentrations in the food chain may be achieved within this time frame.

Frequency

Fish Egg/Ovary, Whole-body, and Muscle Se Criteria

Although the EPA's AWQC, including the draft water Se criteria, are not to be exceeded more than once in three years, the fish tissue-based Se criteria are "never to be exceeded." To my knowledge, the "frequency" component of AWQC is rarely incorporated into permit limitations, so the implications of fish tissue-based Se criteria "never to be exceeded" are not entirely clear to me. The "frequency" component was initially incorporated into AWQC based on the premise that ecosystems will not be harmed if the number of criterion excursions is limited and/or there are compensating periods of time below the criterion over which the ecosystem can recover. As far as I can tell, the draft AWQC document for Se does not explain the basis for the "never to be exceeded" frequency decision for fish tissue. It seems that there should be some level of consistency between the allowable "frequency" for fish tissue-based and water-based Se criteria.

Surface Water Se Criteria - Monthly Average and Intermittent Exposures

The "frequencies" of "not more than once in three years on average" are consistent with the EPA guidelines and AWQC for other chemicals. As noted above, however, I am not aware of the "frequency" component of AWQC being incorporated into most effluent limitation so am unsure of the significance of this component. The fixed monitoring benchmark (FMB) approach, which has initially been developed for copper and biotic ligand model (BLM)-based criteria, represents a method that does explicitly account for exceedance frequency (USEPA 2012). However, this approach is for use under a site-specific context and would not apply to the national (non-site-specific) Se criteria. A reasonable excursion frequency for Se in water should be determined carefully, however, as Se is bioaccumulative and has variable persistence depending on receiving water conditions. For example, more frequent excursion frequencies may not be consequential in lotic systems with low biological productivity and short resident times, while an excursion frequency greater than once every three years may be warranted for lentic systems with high biological productivity and long residence times. In summary, I think the "frequency" decisions should be evaluated and explained in more detail.

Literature cited:

Coyle JJ, Buckler DR, Ingersoll CG, Fairchild JF, May TW. 1993. Effect of dietary selenium on the reproductive success of bluegills (Lepomis macrochirus). Environ Toxicol Chem 12:551-565.

de Rosemond SC, Liber K, Rosaasen A. 2005. Relationship between embryo selenium concentration and early life stage development in a white sucker (Catostomus commersoni) from a northern Canadian lake. Bull Environ Contam Toxicol 74:1134-1142.

Doroshov S, Van Eenennaam J, Alexander C, Hallen E, Bailey H, Kroll K, Restrepo C. 1992. Development of water quality criteria for resident aquatic species of the San Joaquin River. University of California, Davis.

Formation Environmental. 2011a. Brown trout laboratory reproduction studies conducted in support of development of a site-specific selenium criterion. Prepared for J.R. Simplot Company. Pocatello (ID): Smoky Canyon Mine.

Formation Environmental. 2011b. Yellowstone cutthroat trout laboratory reproduction studies conducted in support of development of a site-specific selenium criterion. Prepared for J.R. Simplot Company. Pocatello (ID): Smoky Canyon Mine.

Gillespie RB, Baumann PC. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans Am Fish Soc 115:208-213.

Hamilton SJ, Holley KM, Buhl KJ, Bullard FA. 2005a. Selenium impacts on razorback sucker, Colorado River, Colorado. II. Eggs. Ecotoxicol Environ Saf 61:32-43.

Hamilton SJ, Holley KM, Buhl KJ, Bullard FA. 2005. Selenium impacts on razorback sucker, Colorado River, Colorado. III. Larvae. Ecotoxicol Environ Saf 61:168-189.

Hermanutz RO, Allen KN, Roush TH, Hedtke SF. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ Toxicol Chem 11:217-224.

Hermanutz RO, Allen KN, Detenbeck NE, Stephan CE. 1996. Exposure of bluegills (*Lepomis macrochirus*) to selenium in outdoor experimental streams. U.S. Environmental Protection Agency, Duluth, MN, USA.

Nakamoto RJH, T.J. 1992. Selenium and other trace elements in bluegills from agricultural return flows in the San Joaquin Valley, California. Arch Environ Contam Toxicol 22:88-98.

USEPA. 2012. Calculation of BLM fixed monitoring benchmarks for copper at selected monitoring sites in Colorado. Office of Water, USEPA. 820R12009.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10^{th} centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

The draft AWQC document notes that "an EC10 was determined to be a more appropriate endpoint for tissue-based criteria given the nature of exposure and effects for this bioaccumulative chemical. EC20s have historically been used in the derivation of EPA criteria applicable to the water medium. While water concentrations may vary rapidly over time, tissue concentrations of bioaccumulative chemicals are expected to vary gradually. Thus, where concentrations of selenium in fish tissue approach an effect threshold, there is potential for sustained impacts on aquatic systems, relative to chemicals that are not as bioaccumulative."

I agree with this logic for using the EC10 as the measurement endpoint for tissue-based toxicity values, where this effects statistic can be derived. I also agree with the use of an EC10 rather than a no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), or geometric mean of the two, for the reasons discussed in the draft AWQC document.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

    a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

I agree with the EPA's approach of only considering fish data in the genus sensitivity distribution as fish are the most sensitive aquatic taxa (although the sensitivity of amphibians relative to fish is still uncertain).

There is a fundamental difference in a criterion that is based on an internal organism concentration versus an external environmental concentration (such as a water concentration). If fish are accepted to be the most sensitive taxa, and if selenium criteria are to be based on the selenium concentration in fish tissue (either eggs/ovaries or whole body), then the toxicity data and genus sensitivity distribution need to necessarily be based only on selenium concentrations in fish tissue. Development of a tissue-based genus sensitivity distribution that includes toxicity data for other taxa would not be relevant to the application of any criterion that could be derived using such an approach.

b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

Although it has perhaps not been rigorously evaluated at all levels of food chain structure and function, field data indicates that adverse Se-related effects on fish can occur when there is no evidence of effects to food chain organism communities, including invertebrates. Selenium trophic transfer factors (TTFs) for invertebrates-to-fish typically average about 1 for whole body Se concentrations in fish and ≥2 for egg/ovary Se concentrations in fish (with the latter being more variable). Thus, a whole body Se criterion of 8.1 mg/kg dw and an egg/ovary Se criterion of 15.2 mg/kg dw may, on average, both be associated with an invertebrate Se concentration of about 8 mg/kg dw.

Based on a review of Se toxicity to invertebrate taxa, deBruyn and Chapman (2007) identified two studies in which whole body invertebrate Se concentrations of <8 mg/kg dw were associated with adverse effects. Both of these studies were based on growth effects in larval midges (*Chironomus decorus*). deBruyn and Chapman (2007) reported an EC40 of 1.0 mg/kg dw from Alaimo et al. (1994) and an EC15 and EC46 of 2.6 and 4.1 mg/kg dw, respectively, from Malchow et al. (1995). However, in Alaimo et al. (1994), Se was below the detection limit in the treatment with a 40% reduction in growth relative to the control, which suggests the growth reduction was due to other factors. In Malchow et al. (1995), whole-body Se LOECs of 2.6 and 4.1 mg/kg dw in midges were observed after 96-hr exposures. It is unclear whether growth effects would be related to tissue concentrations under such a short exposure period, but perhaps the water concentrations themselves (10 µg/L of either selenate or selenite) were directly responsible for the reduced growth. More recent data for a mayfly (*C. triangulifer*) suggest that the whole-body Se toxicity threshold for this species is also >8 mg/kg dw (Conley et al. 2009, 2011, 2013).

Overall, in my opinion, the above provides support that a fish tissue-based Se criterion should ensure protection of the aquatic community as a whole, including invertebrates.

Literature cited:

Alaimo J, Ogle RS, Knight AW. 1994. Selenium uptake by larval *Chironomus decorus* from a *Ruppia maritima*-based benthic/detrital substrate. Arch Environ Contam Toxicol 27:441-448.

Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ Sci Technol 43:7952-7957.

Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicology 20:1840-1851.

Conley JM, Funk DH, Hesterberg DH, Hsu L-C, Kan J, Liu Y-T, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the

mayfly *Centroptilum triangulifer*. Environ Sci Technol 47:7965-7973.

deBruyn AMH, Chapman PM. 2007. Selenium toxicity to invertebrates: Will proposed thresholds for toxicity to fish and birds also protect their prey? Environ Sci Technol 41:1766-1770.

Malchow DE, Knight AW, Maier KJ. 1995. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum*. Arch Environ Contam Toxicol 29:104-109.

c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al, 1990)?

Overall, I generally agree with the EPA's interpretation of the non-reproductive effects data and the draft whole-body Se criterion appears to be protective of the toxicity endpoints evaluated in those studies (at least the GMCVs reported in Table 17 of the draft AWQC document certainly are). The one study that could be interpreted somewhat differently is the juvenile Chinook salmon study conducted by Hamilton et al. (1990). The EPA derived whole-body Se EC10s of 7.355 and 11.14 mg/kg dw for juvenile growth based on a seleno-DL-methionine spiked diet and San Luis Drain (SLD)-spiked diet. For comparison, DeForest and Adams (2011) had derived a whole-body Se EC10 of 6.4 mg/kg dw based on the seleno-DL-methionine spiked diet, using a different concentration-response model (they excluded the SLD-spiked diet due to concerns associated with other contaminants). Overall, the model fit by the EPA to the data using TRAP appears to be quite good and the greater EC10 that they derived based on SLD-diet provides support that other contaminants did not adversely affect growth in the juvenile Chinook. Accordingly, I do not disagree with the SMCV (and GMCV) of 9.052 mg/kg dw that the EPA derived from juvenile Chinook salmon. This would also support that the draft whole-body Se criterion of 8.1 mg/kg dw based on reproductive effects would be protective against growth effects in juvenile Chinook.

Literature cited:

DeForest DK, Adams WJ. 2011. Selenium accumulation and toxicity in freshwater fishes. 193-229 in Beyer WN, Meador JP, eds. Environmental contaminants in biota: Interpreting tissue concentrations Second edition. CRC Press, Boca Raton, FL, USA.

Hamilton SJ, Buhl KJ, Faerber NL, Wiedmeyer RH, Bullard FA. 1990. Toxicity of organic selenium in the diet to chinook salmon. Environ Toxicol Chem 9:347-358.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

In my opinion it was reasonable to exclude microinjection studies because there are sufficient questions as the environmental relevance of the exposure. For example, Linville (2006) exposed white sturgeon larvae to selenium using two different approaches: (1) by microinjection of L-selenomethionine into larval yolk sacs immediately after hatching and (2) by exposing parent females to dietary selenium (as selenized yeast) for up to six months before they deposited eggs (i.e., maternal transfer exposure). In larvae that received L-selenomethionine microinjections, mortality was a more sensitive endpoint than developmental-

related effects. In contrast, in the maternal transfer test, larval developmental effects was a more sensitive endpoint than larval mortality. Further the egg Se EC10 for white sturgeon was 15.8 mg/kg dw in the maternal transfer study versus 6.77 mg/kg dw in the microinjection study (as derived by Beckon [2012]). The microinjection methodology has not been validated in other studies and the results from Linville (2006) suggest that it is not an appropriate substitute for maternal transfer. Further, to my knowledge, studies on injection of Se into muscle tissues and subsequent maternal transfer of Se to the ovaries and eggs, and comparison to maternal transfer data following dietary Se exposures, have not been conducted.

(Although the data from Linville [2006] are sufficient to make some comparisons between maternal transfer and microinjection studies, the concentration-response data are too limited to derive an EC10 that would be considered reliable in a sensitivity distribution for criteria development. Further, the egg Se EC10 from the maternal transfer test was estimated from the larval Se EC10 using a regression relationship between egg and larval Se concentrations from a microinjection test.)

Literature cited:

Beckon WN. 2012. Evaluation of the toxicity of selenium to white and green sturgeon. U.S. Fish and Wildlife Service, Sacramento, CA.

Linville RG. 2006. Effects of excess selenium on the health and reproduction of white sturgeon (*Acipenser transmontanus*): Implications for San Francisco Bay-delta. Ph.D. Thesis, University of California, Davis. 232 pp.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

In general, I am hesitant about considering tissue-to-tissue Se relationships in order to estimate toxicity thresholds for one tissue based on measured concentrations in another tissue. However, the "EO/WB" ratios shown in Table 7a appear bracket the ratios typically observed, while still reflecting the variability observed between different species and families. The resulting draft whole-body Se criterion of 8.1 mg/kg dw is not inconsistent with other whole-body fish Se guidelines that have been recommended based on direct whole-body Se measurements. DeForest and Adams (2011), for example, recommended a whole-body fish Se guideline of 8.1 mg/kg dw following a different approach. However, per my above comment, I believe that the number of GMCVs should be 11 rather than 14 (or 12 if a recently conducted study for mountain whitefish were added to the sensitivity distribution.

In addition, for those species with measured Se concentrations in whole-body tissue or muscle, why not use the empirical measurements? For example, for Dolly Varden, McDonald et al. (2010) reported a whole body Se EC10 of 44 mg/kg dw based on the site-specific relationship between egg and WB Se in their study (this would not influence the draft whole-body Se criterion because *Salvelinus* is not among the four most sensitive genera, but it would be more accurate). Likewise, Coyle et al. (1993) and Hermanutz et al. (1992, 1996) report whole body Se concentrations in bluegills. This could be checked for other species as well.

Finally, perhaps it should be noted that, if possible or desired, site- and species-specific relationships between egg/ovary Se and whole-body or muscle Se could be derived and used in place of the draft criteria of 8.1 and 11.8 mg/kg dw.

Literature cited:

Coyle JJ, Buckler DR, Ingersoll CG, Fairchild JF, May TW. 1993. Effect of dietary selenium on the

reproductive success of bluegills (*Lepomis macrochirus*). Environ Toxicol Chem 12:551-565.

DeForest DK, Adams WJ. 2011. Selenium accumulation and toxicity in freshwater fishes. 193-229 in Beyer WN, Meador JP, eds Environmental contaminants in biota: Interpreting tissue concentrations Second edition. CRC Press, Boca Raton, FL, USA.

Hermanutz RO, Allen KN, Roush TH, Hedtke SF. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ Toxicol Chem 11:217-224.

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McDonald BG, deBruyn AMH, Elphick JRF, Davies M, Bustard D, Chapman PM. 2010. Developmental toxicity of selenium to Dolly Varden char (*Salvelinus malma*). Environ Toxicol Chem 29:2800-2805.

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

I believe that the EPA's translation method is not unreasonable, but I have three primary concerns: (1) TTFs and CFs derived for taxa from other studies are applied to sites regardless of whether those TTFs and CFs are reflective of site-specific trophic transfer data; (2) the EFs and TTFs are treated as constants regardless of exposure concentrations; and (3) the level of protection associated with the draft criteria is unclear. These are discussed further below (in response to questions 1 and 2).

Model for translating fish egg/ovary Se criterion to lentic and lotic water Se criteria is not always consistent with site-specific information:

The EPA identified sites where Se EFs could be calculated based on reported co-located Se concentrations in surface water and particulates (algae, detritus, sediment). Information on the fish species present at those sites was then used to develop food web models, which determined the CFs and TTFs that were then applied in translating from the draft fish egg/ovary Se criterion back to corresponding water Se concentrations. Site-specific food web information was used where reported, but the EPA mostly relied on the NatureServe database (http://www.natureserve.org) for information on the typical diet and/or eating habits of the fish at each site.

A limitation of this modeling approach is that it ignored site-specific information on Se bioaccumulation in fish and their diets. The EFs used were site-specific, but Se modeling up the rest of the food chain and into fish was based on assumed model parameters. This becomes particularly important when considering the data "drivers" for the draft lentic Se criterion of 1.3 µg/L. This value is driven almost exclusively by data for two reference lakes (Badin Lake and High Rock Lake, NC, USA). Badin Lake was reported to have a water Se concentration of 0.32 µg/L and High Rock Lake a water Se concentration of 0.67 µg/L (Lemly 1985). For comparison, the mean water Se concentrations translated from a fish egg/ovary Se criterion of 15.2 mg/kg dw were 0.54 µg/L for Badin Lake and 1.2 µg/L for High Rock Lake. The former falls between the water Se concentrations reported for these two reference lakes and the latter almost equals the draft lentic criterion of 1.3 µg/L. Since six fish species were assumed to represent each of these two sites, these two reference sites are the drivers for the draft lentic Se criterion of 1.3 µg/L.

In addition to two reference sites being the drivers for the draft lentic Se criterion of 1.3 µg/L, the model for translating a fish egg/ovary Se criterion of 15.2 µg/L to a water Se concentration does not appear to be correct for these two sites. Although fish egg/ovary Se concentrations were not reported for Badin Lake and High Rock Lake, muscle Se concentrations were. Those muscle Se concentrations were reported on a wet weight basis and converted to a dry weight basis by assuming a moisture content of 75%. The muscle-to-egg CFs reported in Table 12 of the draft AWQC document were then used to estimate fish egg Se concentrations. These estimated fish egg Se concentrations for the two reference sites were, on average, less than one-half of the draft fish egg/ovary Se criterion of 15.2 mg/kg dw. Further, the muscle Se concentrations at the references sites ranged from 2.3 to 5.8 mg/kg dw, which are well below the draft muscle Se criterion of 11.8 mg/kg dw. The above demonstrates that the food web model for these two reference sites does not accurately reflect Se bioaccumulation potential at these two sites and in fact greatly overestimates Se bioaccumulation potential.

Overall opinion on method for translating from a fish tissue criterion to water Se criteria:

In my opinion, the approach should rely more on empirical data in order to eliminate cases where the food web models do not reflect the site-specific data. One alternative approach is that described in DeForest et al. (2014). That approach was also based on multi-step Se partitioning, but rather than using EFs and TTFs, the empirical relationships between (1) water and particulate Se; (2) particulate and invertebrate Se; and (3) invertebrate and fish egg/ovary Se were used. Quantile regression was used to work backward from an egg/ovary Se threshold to conservative Se concentrations in lentic and lotic water bodies. This regression-based approach accounts for the breadth of data on Se enrichment and trophic transfer potential, which can essentially represent the bounds of Se bioaccumulation potential from water to fish eggs/ovaries. The regression-based approach also accounts for the slopes of the relationships between water and particulate Se, particulate and invertebrate Se, and invertebrate and fish Se. This would be one example of an alternative model that could be considered.

Level of protection associated with draft water selenium criteria unclear:

The draft lentic and lotic criteria are based on the 20th percentiles of the data points plotted in Fig. 11 of the draft AWQC document. Those data points in Fig. 11 are for individual fish species at a given site. For example, 18 of the 51 data points for lentic systems (35%) are for just three water bodies (six fish species per water body). It is unclear what the 20th percentiles of those lentic and lotic distributions are protective of, as they do not represent 20% protection of sites or 20% protection of fish species. The latter was presumably not the intent, as those levels of protection would not be acceptable for national AWQC recommendations.

Literature cited:

DeForest DK, Brix KV, Gilron G, Hughes SA, Tear LM, Elphick JR, Rickwood CJ, DeBruyn AMH, Adams WJ. 2014. Selenium partitioning between water and fish tissue in freshwater systems: Development of water-based selenium screening guidelines. http://www.namc.org/docs/Selenium%20Integrated%20Report%20-%20Final%20(2014-05-20).pdf

Lemly AD. 1985. Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and safety. Ecotoxicol Environ Saf 10:314-338.

2. Regarding the trophic transfer factor (TTF) values, did EPA use a scientifically defensible method to derive the TTF values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in TTF values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

Overall, I generally agree with how the EPA derived TTFs from either physiological coefficients or from field data. Following are specific comments.

TTFs from empirical measurements in laboratory studies:

Laboratory-based TTFs were calculated from physiological coefficients (AE, IR, ke), but it does not appear that TTFs were calculated from laboratory data in which Se concentrations were empirically measured in invertebrates or fish and their diets. This approach is analogous to the field-based TTFs calculated by the EPA, but there is less uncertainty in the dietary Se concentration because the dietary Se

concentration is known in laboratory studies. Is there a reason why these studies were not considered?

TTFs are not constants across exposure concentrations:

As previously noted, one potential limitation of the modeling approach is that TTFs tend to be inversely related to exposure concentration (i.e., TTFs are inversely related to the corresponding dietary Se concentration). However, the TTFs in the model used by the EPA are constants that are specific to the exposure concentration in the test from which they were derived. The EPA did note, on p. 74, that the "distribution of ratios could be biased high toward larger values if the data are obtained from aquatic systems with low selenium concentrations" and on p. 75 a regression-based approach was considered. EPA ultimately used what was described as a hybrid approach, in which ordinary least squares (OLS) linear regression was used to confirm that a significant ($p \leq 0.05$) and positive relationship was observed, and then the median of individual ratios was used to estimate central tendency and avoid bias from systems with very low or very high selenium concentrations. This helps to partially address the issue, but a regression-based approach may still be more appropriate (see previous comment).

TTFs for insect larvae:

The draft AWQC document includes Se TTFs of 1.97 for a dragonfly (Anisoptera), 2.88 for a damselfly (Coenagrionidae), 1.28 for a mayfly (*Centroptilum triangulifer*), 1.90 for a midge (Chironomidae), and 1.48 for a corixid (Corixidae).

- **Dragonflies and damselflies:** The dragonfly and damselfly TTFs do not always appear to be calculated as described. On p. B-63 it is noted that the Se concentration in dragonfly and damselfly food is the median selenium concentration in all invertebrate tissues that co-occur with an Odonate species. For Site 29 in Birkner (1978), however, only corixids are considered in the damselfly diet, even though data for chironomids are available. The damselfly Se concentration at this site was 55.0 mg/kg dw and the corixid Se concentration was 29.4 mg/kg dw, which resulted in a TTF of 1.87. However, if chironomids were also considered part of the diet, which had a Se concentration of 58.2 mg/kg dw, the median Se concentration in the damselfly diet would be 43.8 mg/kg dw and the TTF would be 1.26. I recommend that the EPA double-check the dietary data used to calculated the TTFs for these taxa.
- **Mayfly (*C. triangulifer*):** The Se TTF of 1.28 for this species may be too low. This value was based on biokinetic data from Riedel and Cole (2001). However, empirical laboratory data from Conley et al. (2009, 2011, 2013) indicate that the Se TTF may range from about 1-3, with a mean of about 2 depending on exposure and test conditions. I recommend that the EPA consider these studies, which may result in a higher Se TTF for *C. triangulifer*.
- **Midges (Chironomidae):** The Se TTF of 1.90 for this taxa may be high when considering laboratory-based TTFs, for which the dietary Se concentration is known. Based data for chironomids from Malchow et al. (1995) and Rickwood and Jatar (2013), mean and maximum Se TTFs are 0.3 and 1.4. The chironomid Se TTFs derived from field data by the EPA include dietary Se assumptions that may underestimate the dietary Se concentration and result in relatively high Se TTFs. For example, the TTFs from Saiki et al. (1993) average 1.0 when a detritus-based food chain is assumed, as suggested by the study authors. I recommend that the EPA consider the dietary assumptions in the field studies in light of the laboratory data.
- **Corixids (Corixidae):** Additional Se TTF data for corixids are available from a laboratory study with *Trichorixa reticulata* (water boatman). In this study, the TTF was very high (32.6) in the control with a low dietary Se concentration of <0.1 mg/kg dw, but then TTFs were <1 at dietary Se concentrations of about 6 to 86 mg/kg dw. It is recommended that this laboratory study be included in deriving the corixid and be used to check the dietary assumptions in the field studies.

Additional potentially relevant TTF data sources:

Laboratory data:

- Conley et al. (2009, 2011, 2013) - *Centroptilum triangulifer* (mayfly)
- Malchow et al. (1995) - *Chironomus decorus* (chironomid)
- Rickwood and Jatar (2013) - *Chironomus dilutus* (chironomid)
- Besser et al. (1989) - *Daphnia magna* (cladoceran)
- Besser et al. (1993) - *Daphnia magna* (cladoceran)
- Guan and Wang (2004) - *Daphnia magna* (cladoceran)
- Thomas et al. (1999) - *Trichorixa reticulata* (water boatman)

Literature cited:

Besser JM, Huckins JN, Little EE, La Point TW. 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. Environ Pollut 62:1-12.

Besser JM, Canfield TJ, La Point TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ Toxicol Chem 12:57-72.

Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ Sci Technol 43:7952-7957.

Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicology 20:1840-1851.

Conley JM, Funk DH, Hesterberg DH, Hsu L-C, Kan J, Liu Y-T, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. Environ Sci Technol 47:7965-7973.

Guan R, Wang W-X. 2004. Dietary assimilation and elimination of Cd, Se, and Zn by *Daphnia magna* at different metal concentrations. Environ Toxicol Chem 23:2689-2698.

Malchow DE, Knight AW, Maier KJ. 1995. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum*. Arch Environ Contam Toxicol 29:104-109.

Rickwood CJ, Jatar M. 2013. Investigation into the fate and effects of selenium on the life-cycle of a benthic invertebrate (*Chironomus dilutus*). CanmetMINING, Project: 603994. Natural Resources Canada (NRCan), Ottawa, Canada.

Riedel GF, Cole L. 2001. Selenium cycling and impact in aquatic ecosystems: Defining trophic transfer and water-borne exposure pathways. Chapter 3 in EPRI Report 2001. EPRI, Palo Alto, CA.

Thomas BV, Knight AW, Maier KJ. 1999. Selenium bioaccumulation by the water boatman *Trichocorixa reticulata* (Guerin-Meneville). Arch Environ Contam Toxicol 36:295-300.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

I think the EPA used a reasonable approach for deriving CFs. As a partial confirmation of those values, fish species for which diet-to-egg TTFs can be derived could be compared to the combined CFs and TTFs values.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

Overall, I believe that the EPA used a reasonable approach in calculating EF values. However, I do not necessarily agree that Se concentrations should be available for at least two particulate types in order to derive an EF. Periphyton, for example, may be the dominant particulate in certain lotic systems and in my opinion such data should be included. I do agree that Se concentrations in sediment alone is insufficient for deriving EF values. I have greater reservations in how the EFs (and CFs and TTFs) were ultimately used to translate from the draft fish egg/ovary Se criterion to water Se criteria.

Potential sources of additional EF data may include:

Bowie GL, Sanders JG, Riedel GF, Gilmour CC, Breitburg DL, Cutter GA, Porcella DB. 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. Water Air Soil Pollut 90:93-104.

Casey R. 2005. Results of aquatic studies in the McLeod and Upper Smoky River systems. Alberta Environment. 64 pp.

Fan TW-M, Swee JT, Hinton DE, Higashi RM. 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquat Toxicol 57:65-84.

Greater Yellowstone Coalition. 2005. Technical Reports on selenium concentrations in water, macrophytes, macroinvertebrates, and fish.

Hamilton SJ, Buhl KJ. 2003a. Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern Idaho near phosphate mining operations: September 2000. US Geological Survey. 64 pp.

Hamilton SJ, Buhl KJ. 2003b. Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern Idaho near phosphate mining operations: May 2001. US Geological Survey. 61 pp.

Hamilton SJ, Buhl KJ, Lamothe PJ. 2002. Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern Idaho near phosphate mining operations: June 2000. USGS, Yankton, SD and Denver, CO. 72 pp.

McDonald LE, Strosher MM. 1998. Selenium mobilization from surface coal mining in the Elk River basin, British Columbia: A survey of water, sediment and biota. Ministry of Environment, Land and Parks, Cranbrook, BC. 46 pp. + appendices.

Orr PL, Guiguer KP, Russel CK. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicol Environ Saf 63:175-188.

Orr PL, Wiramanaden CIE, Paine MD, Franklin W, Fraser C. 2012. Food chain model based on field data to predict westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) ovary selenium concentrations from water selenium concentrations in the Elk Valley, British Columbia. Environ Toxicol Chem 31:672-680.

Presser TS, Luoma SN. 2009. Modeling of selenium for the San Diego Creek watershed and Newport Bay, California. US Geological Survey, Open-File Report 2009-1114. 48 pp.

Zhang Y, Moore JN. 1996. Selenium fractionation and speciation in a wetland system. Environ Sci Technol 30:2613-2619.

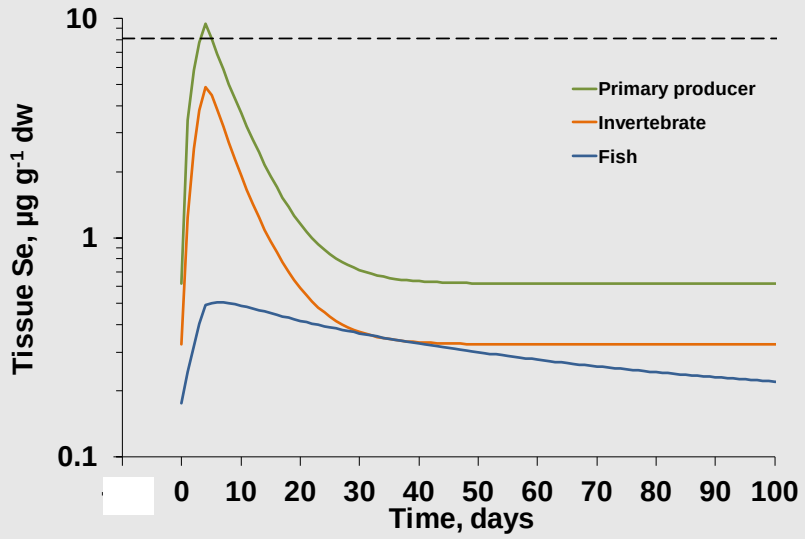
5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

I am not sure that the criterion equation for intermittent dischargers is meaningful, as it is basically a mathematical manipulation and does not in any way account for selenium uptake and elimination kinetics. An alternative approach that the EPA may want to consider is based on biokinetic modeling, such as that described in Brix and DeForest (2008). The method they described was based on modeling of a food chain comprised of periphyton, an invertebrate (mayfly), and a fish (fathead minnow). Inputs to the model include the background water Se concentration, the magnitude of an intermittent Se pulse, and the duration of the Se pulse. This provides a tool for evaluating whether a Se pulse of a given magnitude and duration could result in exceedance of a whole-body fish Se criterion, or short-term Se criteria could be derived for given short-term durations.

For a comparison of the biokinetic-based approach to the intermittent criterion equation in the draft AWQC document, I assumed that the background water Se concentration is 1 µg/L, the lotic criterion is 4.8 µg/L, and the number of days elevated is 4. The intermittent criterion would be 29.5 µg/L. Just as an example, if a lotic food chain consisting of periphyton→mayflies→fathead minnows were assumed, a 4-d pulse of 29.5 µg Se/L would not be nearly sufficient to reach a whole body Se concentration of 8.1 mg/kg dw (Fig. 2). There is a rapid increase in predicted Se concentrations in periphyton and mayflies and then a rapid elimination, but uptake is slower in fathead minnows.

In my opinion, a biokinetic-based modeling approach would be more appropriate for deriving acute or intermittent water Se criteria.

Fig. 2. Example of modeled selenium uptake and elimination in a model food chain exposed to a 4-d pulse of 29.5 µg Se/L.



Literature cited:

Brix KV, DeForest DK. 2008. Selenium. Pages 123-172 in Gensemer RW, Meyerhoff RD, Ramage KJ, Curely EF, eds. Relevance of ambient water quality criteria in ephemeral and effluent-dependent watercourses of the arid western US. SETAC Press, Pensacola, FL, USA.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

A substantial number of comments from stakeholders and the public were provided. These comments covered a large variety of topics and were often conflicting. I did not identify any comments that would lead me to think that the scientific direction of the criterion should be changed. The comments relative to interpretation of toxicity studies and derivation of EC10 values should all be carefully reviewed by the EPA, as some suggested that certain EC10 values should be lowered and other suggested they should be raised (although I personally believe that the GMCVs values derived by the EPA were generally conservative, especially for *Salmo* and *Lepomis*). Aside from the technical comments and disagreements that are related to magnitudes of the various Se criterion elements, it appears that there is a desire (or need) for the EPA to more clearly define how the draft Se criteria should be implemented by the states. Perhaps

case studies could be provided as examples? It is also apparent that the basis of the intermittent criterion, and its relationship to an acute criterion (if there is a relationship), needs to be more clearly explained. Although some comments seem to agree that an acute Se criteria is not necessary any longer, there does still appear to be a need for acute Se criteria from the perspectives of certain states. Finally, again related to implementation, is the question of whether the lotic and lentic water Se criteria can be replaced by a different metric, such as residence time. In my opinion, the latter would be worthy of further consideration by the EPA, although I wonder whether more reliable categories could be developed based on existing datasets.

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Dr. Nicholas S. Fisher

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

Reasonably clear, although some phrases and terms need further clarification.

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

The tiered construction makes sense for most natural conditions, but not when acutely high Se levels are present (e.g., Kesterson reservoir). But for most sublethal concentrations this approach makes sense as a general approach for the EPA to adopt.

        ii. Is the primacy of the whole-body/fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

This approach is wholly justifiable because Se is accumulated by animals almost exclusively through diet rather than directly from the dissolved phase in ambient water. In fact, Se and perhaps methylmercury would be extreme examples in which this approach is appropriate.

        iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The EPA can provide further levels of uncertainty with regard to toxicity associated with fish egg/ovary contamination. How many studies is this approach ultimately reliant upon? The report is based on a limited number of studies, but more studies are warranted before we can be assured that this approach is rock-solid.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

I do not see obvious errors in their approach.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10^{th} centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

Strikes me as rather arbitrary.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

    a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

I have no particular insight on this issue.

    b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

Until we find more Se-sensitive groups of freshwater animals than fish, the fish tissue-burden approach seems warranted.

c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

I'm not sure.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

It is hard to argue on behalf of egg injection studies in favor of dietary uptake (the obviously more natural process) studies. This is particularly the case if the Se contents of the tissues and eggs are measured during the dietary exposure.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

It seemed reasonably clear to me.

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The EPA is justified in simplifying the bioaccumulation equations by eliminating the growth rate constant (g) because it is negligible compared to the loss rate constant of Se from aquatic animals. This is generally the case for most metals and metalloids, with some notable exceptions where the loss rate constants are very low (e.g., methylmercury). Their equations 2 and 3 (pages 64-65) have already been published, and the reference for this should be cited. (Reinfelder, J.R., N.S. Fisher, S.N. Luoma, J.W. Nichols, and W.-X. Wang. 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Science of the Total Environment 219: 117-135.) The authors should note that the loss rate constant of some contaminants can differ following uptake from the aqueous phase and uptake from diet---this is because the contaminant may deposit in different tissues from these two uptake routes. As such, the term ke should be converted to kef and kew (following uptake from food and uptake from water). For Se, fortunately, this correction is unlikely to be an important one because uptake from the aqueous phase (water) is negligible compared to dietary uptake. But strictly speaking, the mathematical expression (Eq. 2) should reflect two different loss rate constants.

By using tissue concentrations of Se in fish to calculate dissolved Se concentrations in ambient water, one must ultimately calculate the Se concentration in organisms at the base of the food chain, namely phytoplankton. This is because none of the animals in the food chain appreciably take up Se from the aqueous phase. The problem of inferring Se concentrations in water from phytoplankton Se concentrations is that the enrichment factors (or bioconcentration factors) of Se in phytoplankton can vary by up 2 or 3 orders of magnitude, depending on the type of phytoplankton that happen to be dominant in the water. Chlorophyceae (green algae), for example, bioconcentrate Se far less than diatoms, and so the variability in these calculations would depend heavily on which types of phytoplankton happen to be dominating the community, and this can change temporally and geographically.

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

I am more familiar with the marine literature and am not well-versed in the freshwater literature regarding Se TTF values.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

See my response to question 2.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

See my response to question 2.

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

See my response to question 1.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

Some of the comments made about acute toxicity are valid, but are unlikely to be relevant to most real-

world situations. Note that acute toxicity can affect other than reproduction, but such effects are rarely seen (I think).

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Dr. David M. Janz

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

The document is generally well-written and is based on a comprehensive evaluation of the extensive body of freshwater Se literature. This said, I found many typographical and other errors throughout the document, which I will address in a marked-up copy (Adobe would not let me use the edit text functions so I simply highlighted the text in yellow and provided a comment if necessary). There were also several areas that I believe require significant clarification, which I will address in my subsequent review comments found below.

I agree with the concept of the tiered criterion approach, particularly that tissue (i.e., ovary, egg, muscle, or whole-body)-based Se concentrations ([Se]) are key to accurately assess the toxicological risk posed to fishes, and that egg/ovary [Se] overrides/supersedes whole-body or muscle [Se]. However, I do not fully agree with the approach, in the absence of tissue [Se] data, that a water-column criterion will be protective of aquatic species. There are many examples of aquatic systems, due to their specific biogeochemistry, ecology, and physiology, where very low dissolved [Se] (i.e., less than the proposed criteria for lentic or lotic systems) results in toxicologically significant bioaccumulation in fishes and their prey, and elevated frequencies of larval abnormalities. I suggest that dissolved [Se] be used as a "trigger" to initiate further monitoring (i.e., collection of fishes to determine tissue [Se]). I also do not agree with the intermittent exposure criterion; it is unclear why it was developed, how it could be implemented consistently and reliably, and in general I think it just adds too much complexity to an already complex (indeed perhaps the most complex) water quality criterion.

These are my general comments, and more specific details can be found in my subsequent review comments.

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Yes, it has been clearly shown in the scientific literature that egg/ovary [Se] provides the greatest certainty in predicting the toxicological risk associated with Se exposure in fishes. This is because (a) embryo-larval

abnormalities are the most sensitive toxicological response, and (b) maternal transfer of Se to the eggs by adult female fishes provides the ultimate dose received by their offspring (i.e., during yolk resorption prior to swim-up). In addition, the frequency and severity of early life stage abnormalities caused by Se has clear ramifications for population dynamics; impaired recruitment of individuals into fish populations can alter demographics and ultimately result in extirpation. This is Ecotoxicology 101. Indeed, documented Se poisoning events (e.g., Belews Lake) provide some of the most convincing evidence of a cause-effect relationship between exposure to a toxic substance and resulting negative impacts on fish populations and communities. This is the goal of aquatic ecotoxicology: to protect populations and communities of organisms, not individuals.

ii. Is the primacy of the whole-body/fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Yes, in the absence of egg/ovary [Se], the next best thing is whole-body or muscle [Se]. Practically, whole-body or muscle samples are more reliably collected throughout the year since most adult female fishes do not have appreciable ovarian tissue mass during non-reproductive periods. This is especially true in small-bodied fishes. In addition, muscle tissue can be collected non-lethally in larger fishes, which may be particularly relevant to threatened species.

It is important to note that [Se] in ovarian tissue containing only primary oocytes or pre-vitellogenic ovarian follicles (i.e., during the non-reproductive period spanning most of the year in many fishes) will likely provide similar information on Se risk as whole-body or muscle [Se]. This is because the ultimate Se dose is maternally delivered to eggs during the period of vitellogenesis in fishes. Eggs will not be present in the ovary of most fish species for much of the year. During vitellogenesis (the period of egg "growth"), adult females synthesize the yolk precursor protein, vitellogenin, in their liver, where it is transported via the bloodstream to the ovary and taken up by growing (vitellogenic) ovarian follicles (eggs). Thus, the [Se] in the <u>liver</u> of adult female fishes may provide a better predictor of Se risk than whole-body or muscle [Se]. To be even more scientifically correct, it is the concentration of the seleno-amino acid, selenomethionine, in the liver of adult female fishes that is incorporated into vitellogenin in a non-specific, dose-dependent manner (replacing the amino acid methionine) that defines the ultimate dose of Se received by their offspring. For more details see the following paper, which was not cited in the EPA document:

Janz, D.M. 2012. Selenium. Pp. 327-374 In: C.W. Wood, A.P. Farrell and C.J. Brauner (Eds.) *Fish Physiology Vol 31A, Homeostasis and Toxicology of Essential Metals*. Elsevier, San Diego, CA.

Thus, I do not agree with the statement on page 27 (line 4) that "concentrations of Se in ovaries are considered equivalent to concentrations of Se in eggs…" because fish ovarian tissue during the non-reproductive phase contains somatic cells responsible for ovarian maturation processes (i.e., steroidogenic cells), and gametes (primary oocytes and pre-vitellogenic follicles), and the [Se] in these cells do not necessarily reflect the dose of Se that will be received by the eggs (i.e., in the yolk) during vitellogenesis. Further studies are needed to examine the relationship between [Se] in ovarian tissue vs. eggs. It is strongly suggested that the EPA inspect the ovary and egg data carefully and attempt to derive the potential relationship between [Se] in ovarian tissue vs. eggs.

iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

One major source of uncertainty is the translation of whole-body or muscle [Se] to egg/ovary [Se]. This relationship has been documented for 10 fish taxa in the document (Tables 7a and 8a). These ratios vary about two-fold among taxa (1.21-2.44 for EO:WB and 0.95-1.92 for EO:M). Not all fish taxa have been studied, and more work is needed in this area. Importantly, in a given fish species these ratios may vary considerably among aquatic ecosystems due to differences in the food web, biogeochemistry of Se, and other factors. These ratios may also vary across seasons. Nonetheless, the data sources, models and approaches used by the EPA to derive these ratios are valid; we simply need more data to more accurately define these conversion factors.

The major source of uncertainty in the tiered approach is the conversion of tissue (egg, ovary, muscle or whole-body) [Se] to water column [Se]. The approach used by the EPA is appropriate and uses, for the most part, the recent biodynamic modeling approach to derive water column [Se] from tissue [Se]. However, to use water column [Se] as a criterion in of itself in the absence of tissue [Se] data is a recipe for inappropriate conclusions, which may penalize industry (i.e., false positives) or cause harm to certain fish populations (i.e., false negatives). I strongly believe that water column [Se] should be used more as a "trigger" to initiate further monitoring that includes collection of fish for tissue [Se] determinations. I also think that a safety factor should be applied to the proposed 1.3 ug/L and 4.8 ug/L criteria for lentic and lotic systems, respectively, which would reduce these values as triggers for further ecosystem monitoring. There are many examples of lentic systems with < 1 ug/L dissolved [Se] where negative effects of Se on early life stage development of fishes have been demonstrated.

This is an appropriate place to discuss the problems with a crude classification of systems as lentic vs lotic. Many rivers in the USA are impounded, essentially creating lentic systems for a significant portion of their river-miles, although they would still be classified as lotic. I think the EPA needs to more clearly define these terms. One suggestion is to use water residence time and/or mean annual flow velocity as more quantitative descriptors. Many of the studies that have shown lower Se bioaccumulation in lotic systems have been conducted in fast-flowing mountain streams, creeks and rivers. To classify a river in the southern USA that has numerous dams as a lotic system does not make sense.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

The egg/ovary criterion of 15.2 mg/kg relies strongly on the reassessment of brown trout data, in particular the Formation study. It seems that much of the issue is related to the lab accident where larval trout were removed from an aquarium due to a faulty standpipe. The EPA has chosen to assume the worst-case, that 100% of the fish that escaped were dead and/or deformed, resulting in an EC10 of 15.91 mg/kg egg. However it is plausible that certain of these fish were not dead or deformed, as discussed in certain public comment documents. The EPA has reanalyzed these data to account for different scenarios, and shown that the EC10 varies from 15.91 to 21.16 mg/kg egg. It seems to me that the 15.91 mg/kg EC10 may be overly conservative. Due to the lack of knowledge regarding the status of these escaped fish (dead, deformed, or healthy), perhaps the assumption could be made that 50% of the escaped fish were dead/deformed, and 50% were normal. This would only slightly increase the EC10 value from which the 15.2 mg/kg egg/ovary criterion is being largely driven. This is only a suggestion of a reasonable compromise given the diverse opinions on this lab occurrence.

For the egg/ovary criterion, the timing of fish sampling is absolutely critical, and the EPA provides no

guidance on sampling design for determining egg/ovary [Se] in the document. As discussed above in 2a(ii), it is the [Se] in eggs that drives early life stage toxicity, so adult female fish absolutely must be collected during the late vitellogenic or preovulatory periods of oogenesis for this criterion to be scientifically and toxicologically meaningful. Measuring [Se] in ovarian tissue during other periods of oogenesis will be much less informative (i.e., about as informative as muscle or whole-body [Se]). The EPA must provide guidance for specific times of the year to collect adult female fish for egg [Se] determinations. For synchronous spawning species (e.g., salmonids, esocids, catostomids, ictalurids), this will be a defined period of 1-2 months on average (usually spring). For asynchronous (batch) spawning species (e.g., cyprinids), this period will be less defined and will usually be 3-6 months (usually spring to late summer or early fall).

For the whole-body and muscle criteria, the EPA has used best available knowledge and approaches to derive these values, and they are of appropriate magnitude, duration and frequency. Collecting fish at any time of the year and determining whole-body or muscle [Se] will provide sufficient information on Se bioaccumulation. Although there will likely be some variation across seasons, due to prey availability, temperature and other factors, this approach should work.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10^{th} centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

The EC10 is absolutely the appropriate endpoint for early life stage toxicity in fish to be used to derive the egg/ovary criterion. This is due to the very steep dose-response relationships observed for larval abnormalities/mortality as a function of egg [Se]. Thus, EC10 provides a toxicologically relevant threshold for appearance of such toxicities, that is, only a marginal increase in egg [Se] will result in a much greater frequency of toxicity. In addition, the main alternative endpoint (EC20) will not differ greatly from EC10 for a given species due to this steep dose-response relationship.

Something the EPA should consider when developing the genus sensitivity distribution is the nature of the experiment for each taxa (lab- vs. field-based). In lab studies, adult female fish are most commonly exposed to selenomethionine (SeMet), which is valid because it is the dominant Se species (60-80% of total Se) found in organisms throughout food webs, particularly at higher trophic levels. In field studies, fish are exposed to SeMet and several other selenium species that likely vary in their toxicity, and in fact are likely less toxic than SeMet. Thus, lab exposures using pure SeMet may overestimate toxicity (i.e., generate lower EC10 values) compared to real-world exposures.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused

primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

This certainly makes the regulator's job easier due to the exquisite sensitivity of oviparous fish species to Se, and the well-established, characteristic and diagnostic response pattern in fishes (larval deformities and edema) that have clear links to population-level impacts. So yes, the egg/ovary tissue element is appropriate. However, it is important to note that we have limited data for all species, whether vertebrate or invertebrate. Recent work in David Buchwalter's lab at NC State U has observed a certain invertebrate taxon (Ephemeroptera I think) to be very sensitive to Se, and should be considered by EPA in the future criterion document. Nevertheless, in my opinion protecting fish based of an egg/ovary criterion will be protective of aquatic ecosystem sustainability.

To my knowledge, the EPA has used a scientifically sound procedure to use available data on 9 fish species to derive the egg/ovary criterion.

b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

See previous comment regarding aquatic insects. In my opinion the tissue-based criteria in fish will protect freshwater aquatic communities.

c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

Since the non-reproductive effects occur at tissue [Se] equal to or more commonly greater than reproductive effects, and since reproductive effects have clearer links to population-level impacts than non-reproductive effects such as reduced growth or altered behavior, the EPA has appropriately chosen not to use non-reproductive effects in their derivation of tissue-based criteria.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

I think the EPA should use studies that use maternal injection of Se as the route of exposure (e.g., the Doroshov et al. (1992) study in catfish). Whether Se is absorbed from the gut or injected into adult female fish, it will reach the systemic circulation and become part of the Se pool, some of which will be

incorporated into vitellogenin in the liver and transported/deposited into eggs. Including the Doroshov et al. (1992) study is thus scientifically sound, and will add an additional fish taxon (ictalurids) into the species sensitivity distribution.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

The EPA used an appropriate approach to translate the egg/ovary element to whole-body and muscle elements. Unfortunately, data are limited to few fish species. As discussed above in 2a(iii), conversion ratios vary by about two-fold for both EO:WB and EO:M. In addition, within-species ratios may vary throughout the year. These aspects all create uncertainty, but these are the data we have and this is the best approach. It is suggested that as more studies measure [Se] in egg/ovary, whole-body and muscle, that these data be used to update criteria through time.

One thing that was not clear. In certain cases it appears that [Se] in egg/ovary and whole-body were determined in the same fish. If eggs were removed for [Se] determination prior to determination of whole-body [Se], then how did the removal of eggs influence the whole-body [Se]? Was the absolute quantity of Se removed by subsampling eggs added back into the whole-body quantity, and was the mass of eggs removed added back to the whole-body?

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The EPA has used the modern and scientifically valid biodynamic model approach to derive water quality elements from tissue-based elements. I am not aware of other data sources, models or approaches that would reduce the inherent uncertainty. However, based on comments provided above (in 1 and especially 2a(iii)), relying on water column dissolved [Se] has a high likelihood of generating both false positive and false negative results with respect to regulatory action. I think the proposed water column criteria (a) should be used as triggers to initiate further monitoring of fish tissue [Se], (b) should be made more conservative (reduced) by application of a safety factor to avoid false negatives, and (c) that the simple classification of a water body as lentic or lotic should be modified to include more quantitative measures of flow such as water residence time and/or mean annual water velocity. Given that many impounded riverine systems in the USA are essentially lentic systems for much of their river-miles, perhaps a water column trigger [Se] could be set at 1 ug/L (same as the current Canadian [CCME] water quality guideline for Se). If exceeded, this trigger value would result in further action in terms of fish collections for tissue [Se].

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

The method used to derive TTF values is scientifically sound by using the widely accepted biodynamic modeling approach, which is particularly appropriate for Se. The EPA also demonstrated that temporal changes in TTF are for the most part not a factor that may cause large data discrepancies. Since the EPA used a large dataset to derive TTF values for insects, any differences between the EPA-derived values and values reported from individual studies are not of concern to this reviewer. I am not aware of any other data, other than the recent work by Buchwalter mentioned in II2a above. It is suggested the EPA include an updated literature search for this and other supporting data prior to the next revision of the document.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

EO:WB conversion factors ranged from 1.38 to 7.39 with a median value of 1.27. As mentioned in II3 above, it was unclear how determination of [Se] in both whole-body and egg were determined in the same fish, and this should be clarified in the document. Similarly when muscle and whole body were determined in the same fish.

Overall, this is a simple method and I am not aware of any alternative methods nor data sources for these analyses.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

EF values were derived from all available data that I am aware of and used scientifically valid approaches, including inclusion/exclusion criteria. See comments above regarding the simple distinction used for lentic vs. lotic systems.

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

As mentioned above, I am not in favor of the intermittent water column criterion. If the EPA decides to go ahead with it, then (a) the rationale for such a criterion should be clarified in the document, and (b) clear guidance on the practical use of the criterion should be provided. In my opinion, the intermittent criterion makes the complex issue of Se aquatic life criteria unnecessarily more complicated, and may be manipulated to either underestimate or overestimate the actual risk posed by Se to fish and other aquatic life.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

I have read through the entire package of views from public and stakeholders, not just the summarized Excel file but the actual documents, some of which are >100 pages. The EPA should pay close attention to these documents, since some excellent scientific issues are raised in many of them. It is good to see that there presently exists such good knowledge of the aquatic ecotoxicology of Se among stakeholders; 10 years ago this would not be true.

The public/stakeholder views represent the classic range, from industry-based opinions that the proposed criteria are too conservative, to conservation group-based opinions that the proposed criteria will not protect all aquatic life. Both sides of the argument present many good points that should be considered carefully by the EPA. I will provide my views on each category of public/stakeholder comments at the end of this section.

The bottom line is that industry would prefer the egg/ovary criterion to be about 20 mg/kg egg (or greater), whereas conservation groups would prefer it to be about 10 mg/kg egg (or lower). Perhaps the 15.2 mg/kg criterion represents a workable compromise between these two extremes? I believe the EPA document for the most part has used current, scientifically sound approaches without significant bias in either direction (but see my comments regarding the Formation brown trout study). Since the proposed EPA criteria would still allow some aspect of site-specific assessment at the State level, then there could be modifications based on site specific issues such as relatively high background [Se] in certain areas, fish species not included in derivation of the egg/ovary criterion, lack of fish species ("fishless" waters), high aqueous sulfate, the presence of listed/threatened/endangered fish species, the presence of critical aquatic-dependent wildlife such as birds, or other biological/chemical/physical factors.

Specific comments on public/stakeholder documents:

An acute criterion is not needed and is not relevant. If you are releasing Se into the aquatic environment at levels that cause acute toxicity to fish, then you have a big problem!

Lentic and lotic systems must be clearly defined and perhaps a more quantitative approach should be used as I have discussed above.

The EPA should read the public/stakeholder input carefully and use these suggestions to come to a final decision on the Formation brown trout study. This is of critical importance since brown trout was found to be the most sensitive fish species and the egg/ovary criteria is driven largely by the brown trout EC10.

Elevated sulfate ion in aquatic systems may reduce Se bioaccumulation in food webs by competing with selenate for uptake by primary producers, particularly algae. However, if regulatory limits are based on fish tissue [Se] then any modification of Se uptake by primary producers will be reflected in fish tissue

[Se]. In my opinion sulfate is not really a regulatory issue when fish tissue [Se] is used.

Ideally freshwater criteria for Se should include aquatic-dependent wildlife such as birds. However this makes the Se criteria more complicated than perhaps it needs to be. The issue of birds could be considered on a site-specific basis in certain ecosystems inhabited by ecologically significant avian populations and migrating water birds.

The EPA must provide guidance on several aspects related to implementation of the tiered criteria approach, at the very least including (a) when to sample fish so that females are in vitellogenic or pre-ovulatory stages of oogenesis, (b) what sample size of fish to collect for tissue [Se] determinations (I suggest a minimum of n=10 female fish per site), (c) recommended analytical procedures for quantification of Se, (d) guidelines for implementation of the 30-day average water column criterion element (how, when, where), and (e) guidelines for implementation of the intermittent water column criterion, if the EPA chooses to keep it in the tiered criterion.

An interesting comment made in one of the public/stakeholder documents (US Fish and Wildlife Service, document 354-A2)) regards the use of recently published studies in zebrafish, a non-native cyprinid, in the species sensitivity distribution for larval deformities as a function of egg [Se]. They present a compelling argument to consider these data in the criterion development.

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Dr. Gregory Möller

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

On an overall basis, the 2014 Selenium Criterion is well-organized and well-written. The major sections of the document serve to critically analyze the scientific and regulatory background of the issue, and to develop and rigorously justify a tiered criterion. Overall, the writing is clear and communicative, with key details, data and background information appropriately appended to the main document. The included tables and figures act to support the analysis of cause for a substantially different approach to risk management and furthermore this information serves to validate this criterion approach by critically evaluating decades worth of peer-reviewed laboratory and field observations in a fair and scientifically valid manner. The concordance observed in many tables exploring and ground-truthing modeled approaches, available data, and a broad array of published study results yields exceptional weight and justification for this new approach developed for the protection of aquatic life.

Importantly, the criterion statement on p. 96 does indicate dry weight basis for tissue analyses, and this is discussed in the text, however Table 15 and the tabular Summary on p. 4 do not carry the dry weight basis notation and this should be included. With the advantage of subsequent key published selenium research targeting trophic transfer and reproductive endpoints in fish, as well as the expert panel contributions published in Chapman et al., 2009, this current document is a significant improvement over the 2004 AWQC draft. In its presentation and treatment of a broad and diverse study and data set, the draft criterion document can be characterized as exhaustive in its attempt to quantitatively and qualitatively address the myriad issues related to this task under the CWA. Furthermore the draft criterion document addresses that task in a manner that synthesizes a new tiered criterion approach well-grounded in our current understanding of selenium risks in aquatic ecosystem and best available peer-reviewed knowledge. The draft approach balances knowns and unknowns, data and data gaps, simplicity and complexity in an overall sound attempt to address the time-value requirement of regulatory science. Although additional implementation guidance for this new tiered approach may be necessary, and observing that the discussion of background science, data and methods used in the intermittent exposure tier of the present criterion needs significant improvement, the draft document is overall remarkable for its clarity and completeness, in a scientifically driven and defendable analysis of a complex risk management challenge.

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

The primacy of the egg-ovary element over other elements of the selenium chronic criterion is logical and broadly scientifically defensible. As identified in the document, numerous published studies outline the major aquatic ecosystem impact of selenium, beyond its nutritional requirement, as a reproductive toxicant. While the specific bio-molecular mechanisms of reproductive toxicity and teratogenesis still require further work, it is well-established from controlled laboratory studies and field studies that the best indicator of the potential for reproductive end effects from selenium is in tissue concentrations, and specifically in egg-ovary concentrations. While the relationships of tissue and water concentrations can be studied, quantified, modeled, and tasked to risk assessment, the now well-established relationship of egg-ovary Se levels to toxicity endpoints fully justifies this primacy of this indicator.

        ii. Is the primacy of the whole-body/ fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

With regards to many chemical exposures in aquatic ecosystems, tissue levels in resident or migratory aquatic animals often help to assess toxic risk by integrating the exposure and revealing the storage, distribution, metabolism, and excretion of the toxicant, regardless of the geography, hydrograph or acute-to-chronic exposure dynamic of the chemical. The bimodal nutrient-toxicant behavior of selenium adds to the complexity of evaluating approaches to risk management. Metabolic and environmental conditions can also add complexity and uncertainty to a full understanding of risk in selenium impacts aquatic ecosystems. It is clear from many studies that the physiological homeostasis (uptake/efflux) of Se is not well controlled and the biochemical metabolic co-relationship of Se and S pathways *in vivo* allows for chronic Se exposure to advance to toxic endpoints. A recurring issue in aquatic ecosystem Se management has been co-exposure to high levels of sulfur, typically as sulfate. While high sulfate co-exposure may impact Se toxicosis, tissue Se levels yield a high quality, aquatic Se toxic impact potential metric regardless of sulfate co-exposure or other co-factors in Se reproductive toxicity (e.g., synergists or antagonists), known or unknown. This Se fish tissue approach, including eggs, ovary, muscle or whole body, is robust with respect to the findings of several decades of peer-reviewed studies. Selenium levels in fish tissue are broadly accepted in the scientific community as a high quality indicator suitable for risk management of aquatic life. The document supports the tissue approach and key toxicological endpoints with a critical review of the peer-reviewed literature and inclusion or rejection of specific studies and the data or findings therein, in an overall transparent, logical and defendable manner.

The tier placement of whole-body/fish muscle is appropriate since egg/ovary assessment may have practical challenges with some ecosystems, with some species, the size of the target fish, and with some aspects of the life-cycle of the target fish. The inclusion and tier level of fish tissue selenium gives

flexibility in aquatic ecosystem risk assessment.

Inclusion of water column Se levels in the tiered criteria will no doubt help screen for potential Se impacts in aquatic ecosystems that have not had a history or occurrence of selenium contamination, and in the prevention of discharges or other anthropogenic activities that present an unacceptable risk to water quality.

iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The tiered approach presented in the criterion embodies the best available scientific knowledge of selenium in aquatic ecosystems actively studied by a broad range of investigators, disciplines and institutions, across a diverse range of water environments and potentially impacted organisms, over more than three decades of focused effort. While all science has uncertainty, the magnitude and diversity of the research effort in the environmental toxicology and regulatory science community to understand the complex risk dynamic of selenium in aquatic ecosystems is unprecedented in the history of U.S. environmental law. The 2014 Aquatic Life Ambient Water Quality Criterion for Selenium balances the available data, models, and approaches to risk management. The document and the tiered criteria within represent a balanced approach where assumptions and data uncertainties are clearly laid out and discussed. Published data or results that were not included in criterion determination were adequately and satisfyingly discussed and defended for exclusion. The data and peer-reviewed studies used in the quantitative and qualitative development of the criterion are sufficiently robust, sufficiently concordant in their conclusions, and sufficiently broad in their scope and number to result in a criterion that can protect aquatic organisms under the requirements of the Clean Water Act.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

The recommended magnitude, duration, and frequency for each criterion element are scientifically sound and appropriate. The derivation of the tissue based criteria are well-supported by including the major published works in the related fields and by rejecting with transparent cause and inclusion/rejection standards those studies that do not attain the stated benchmark for quality and reproducibility (e.g., NOECs). The criterion development satisfyingly addresses a diverse range of major fish types indicative of aquatic ecosystem health in geographically diverse lentic and lotic systems. With chronic exposure, fish egg-ovaries are now recognized as the best indicator of toxic selenium risk, however practical monitoring may require whole body-muscle tissue analysis. Water column selenium values fill the need for screening and analysis of potential for risk, abatement of new contamination pathways, and managing discharge, as well as other activities that may impact water quality.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10th centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

The EC10 is an appropriate endpoint to use in the development of the egg-ovary element of the tiered criterion. Egg-ovary Se concentration is well recognized in the peer-reviewed scientific literature as a high quality indicator of reproductive toxic risk in fish. Because selenium is a reproductive toxicant, special considerations in risk management are warranted. For precedent, the Food Quality Protection Act of 1996 which manages risk of chemical exposure in the human food system, uses an extra ten-fold safety factor for chemicals used in food production that have reproductive toxicology or neurotoxic endpoints. This extra safety factor results from our common understanding in toxicology that those chemicals with repro- or neuro-toxic activity represent an exceptional risk and thus require exceptional safeguards. Reproductive toxicity is a significant threat to the population of the impacted aquatic organisms and thus to the aquatic food-web. There are valid questions whether the EC10 is sufficiently protective of endangered aquatic species and the criterion document should address these concerns more thoroughly. Overall the EC10 egg-ovary endpoint is scientifically consistent and defendable with the intent and required actions of the CWA.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

    a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

The use of fish data to drive the tiered criteria, and specifically the egg-ovary tissue element is fully justified and well-supported in the criterion document and the relevant scientific literature. While the sources, pathways, receptors and controls of chemicals impacting water quality have inherent diversity, selenium demonstrates significant trophic transfer potential and potential for fish reproductive effects in aquatic ecosystems. The reproductive endpoints observed in peer-reviewed, published controlled and field studies strongly suggest the potential for accumulation, magnification, and trophic transfer, and thus population level effects in a higher tropic level organism such as fish. The concomitant food-web impacts and observed impacts to aquatic birds support the criterion approach. The guidelines of Stephan et al., 1985 pre-date much of the knowledge base of Se in aquatic ecosystems, and the somewhat unique behavior and impact potential of this toxicant across trophic levels did not come into a more complete

understanding for nearly two decades since that work. Hence, deviation from prior risk assessment approaches that pre-date our current knowledge base and the evolution of understanding of Se behavior in aquatic ecosystems is broadly justified in the risk management of selenium. Stephan et al., 1985 pre-date much of the knowledge base of Se in aquatic ecosystems, and the somewhat unique behavior and impact potential of this toxicant across trophic levels did not come into a more complete understanding for nearly two decades since that work. Hence, deviation from prior risk assessment approaches that pre-date our current knowledge base and the evolution of understanding of Se behavior in aquatic ecosystems is broadly justified in the risk management of selenium.

b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

The fish tissue-based criterion affords protection to the aquatic community as a whole and is appropriately placed in the tiered criterion. Since tissue Se integrates chronic and intermittent acute aquatic Se exposure, it provides a good quality indicator of impacts and potential impacts to the broader aquatic community. The complex interactions of predator-prey relationships in these environments rely on nominal stability in each tropic level and the food-web as a whole. In field practice and in published controlled studies, fish tissue Se has been shown to provide a valuable assessment and management tool for Se impacted aquatic ecosystems. Except where fish populations are absent, very low, endangered or otherwise insufficient, tissue monitoring is a high quality indicator of water quality with regards to selenium.

c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

The non-reproductive fish data, limited in scope and diversity, were adequately explored and treated in the development of the tiered criterion. The increased concerns over reproductive effects from a risk management perspective, study diversity (e.g., species, geography, lentic/lotic), in addition to the quality and quantity of reproductive toxicity endpoint data and studies reproductive toxic risk the superior driver of selenium risk management in aquatic ecosystems. The summary statement that the non-reproductive data were less reproducible (p. 57) suggests that including them would have added uncertainty to the final criterion values. It is reasonable, acceptable, and scientifically defensible to have reproductive toxicity as the driving endpoint for criterion development, as these criteria appear to afford protection from non-reproductive toxic effects.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

The rejection of injection exposure route studies is reasonable. Injection based toxicology studies have their place in understanding the interface of chemistry and biology. They are of significant value when metabolism of the toxicant is of interest or when digestive and absorption processes (i.e., bioavailability) confound or complicate study goals. Since controlled feed/water laboratory exposure trials, and field observation data and published studies are available in overall sufficient quantity, diversity, and quality for

establishment of the criterion, the rejection of injection-based trials results yield a data set more amenable to generalization of aquatic ecosystem exposure and dose, as well as the subsequent analysis of trophic transfer and potential for toxic end effects. Although injection route studies have scientific value, they are not necessary or required for a qualitative and quantitative understanding of Se aquatic ecosystem risk potential given the other peer-reviewed resources presently available.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

The approach and method of translating the fish egg-ovary criterion into muscle/whole body is transparent and broadly scientifically defensible, and there appears to be sufficient data to make the translation. Although there is some variability in the calculated results of whole body and muscle calculations, the relative consistency across taxon gives significant support to the modeling approach and in the data used to derive the values. The Figure 5 references to Table 10 and 11 should be introduced and explained in the body text prior to using them in a Figure caption since the reader has not seen that data. Some editing in this regard would improve clarity and help the reader understand and follow the approach. The body text of paragraph 1 of page 59 needs to be rewritten for clarity; statements of "it can be seen" assume much and explain little. Because the paragraph references a subsequent Section 4.2, editing page 59 to introduce and summarize the detail of 4.2 would be an improvement in clarity for the reader. Table 7a and 8a would be improved with units (mg Se/kg DW) for tissue concentrations. Footnotes on these important tables cross-referencing the specific source, table or appendix where the data originated would be helpful and aid in reader understanding and transparency.

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The translation equation approach used to convert toxicologically relevant fish tissue concentrations to water-column concentrations is broadly scientifically sound and defensible, and represents our best available understanding of these relationships across trophic levels in an aquatic ecosystem food web. This may be especially true because the approach is based on a straightforward model, and alternative approaches that introduce complexity can also introduce uncertainty from the requirements of additional data beyond that currently available. Risk estimation rarely has perfection due to situational variability and uncertainty involving the integration of exposure, uptake, and biokinetics. The draft criterion approach uses qualified data and reasonable analysis to reduce complexity and increase the transparency of criterion. Modeling dynamic relationships in complex multi-level systems with innate variability is a significant environmental management challenge, however the effort can yield a valuable management tool. Figure 8 (p. 73) graphically demonstrates "hysteresis" with regards to aquatic food chain selenium levels and potential for toxic impact as well as the temporal relationship to periodic sampling. Any challenges in application of this approach across diverse aquatic ecosystem types with variable water chemistries and annual variability (e.g., flow and flux), are equally met by the challenges of sufficiently devising specific criteria to address every subset of variables with less or equal uncertainty in the protection of aquatic life. The duration and frequency requirements of the water column selenium criterion address the potential for system variability (e.g., year to year weather/hydrograph changes) and propagation of system uncertainty (e.g., non-selenium related chemical or biological changes) in this risk management.

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

The trophic transfer factor (TTF) values were developed as an application of a peer-reviewed, published approach that represents our best available scientific information. The method and data used are adequately described, and the approach is satisfyingly direct. The confounding dynamic to this approach could be the bi-modal essential-toxic behavior of selenium where low-level exposure has different metabolic and storage behavior that non-essential metals and therefore different toxicodynamics across a broad range of exposures. This dynamic is adequately discussed (p. 74). The screening criteria for data used in TTF calculations appear defensible and reasonable, and complete with regard to major published works.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

There is inherent uncertainty and variability in deriving conversion factors given the diversity of fish types, lifecycle stage, and environmental conditions. The single 1.27 conversion factor approach appears to be a straightforward and reasonable approach given the limitations of data and species data sets. This is especially true in practice where a criterion will be applied to fish types including those not subjected to controlled studies. While species specific CFs are desirable, this would require considerably more data that currently available especially in regards to life cycle of the target fish analyzed. The conversion factor (CF) method and input data appear to be a reasonable and defendable approach to addressing data limitations and practical application of the criterion. Other numerical approaches can also rise to developing CFs however it is unclear if the absence of data would bias those results or create similar uncertainties as well. The calculation approach in the current draft is straightforward and robust. Appendix B appears to have most freshwater fish data used in the CF analyses addressed in multiple published scientific papers or agency reports. Because of the critical nature of this calculation to criterion development, updating literature searches for new research data is important.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

The enrichment factor (EF) approach and method is scientifically defensible and represents our best understanding of selenium dynamics in aquatic ecosystems. While all modeling approaches have uncertainties and limits in application, the approach is reasonable, transparent, appropriately applied and representative of the present selenium knowledge base. The criterion document uses available data in a consistent manner, and extending the water system terminology used by study authors for data used in EF value determinations is a best practice. The evaluation of categories of aquatic systems is well treated in the analysis. The grouping of streams, drains, washes and creeks into a common category is reasonable. The results of Figure 9 and 10, and furthermore in Figure 11, help to validate the EF approach of the criterion document when measured against our cumulative knowledge base of selenium behavior in different aquatic systems. The use of a 20^{th} percentile approach for water column values accommodates system variability and system uncertainty that is inherent in all modeling approaches. Whereas tissue levels of Se can more reliably predict toxic risk, a 20^{th} percentile affords adequate protection in many risk management situations such as water quality-based effluent limits, especially in light of the primacy of the tissue based components of the criterion.

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

While the need for a criterion tier that addresses intermittent discharges is clear, this part of the document is not well documented for scientific support as evidenced in the main document by no citations in this section beyond that of the general Chapman et al. 2009 reference. Appendix G Part 3.0 documents the modeling approach, however a list of references is missing. Since this is original work, further description of methods, key data inputs, and model run output may be useful for potential replication of the results by others. A citation on page G-6 (EPA 1986; should be USEPA 1986) may be important to sourcing this modeling approach, but it is unclear in the writing whether this is so; without references to Appendix G, validation of scientific defensibility of the intermittent water-column criterion is not possible. Infrequently, some of the writing in Appendix G is informal or tech-speak and should be edited for clarity. Figure captions should contain a short description of all relevant model inputs to increase communication value and transparency. The modeling approach and the results of Appendix G appear to be a reasonable and defendable approach to developing a criterion for intermittent water column selenium values, although the polished execution of this important part of the tiered criterion is lacking in comparison to the other criterion elements. Thus, there appears to be sufficient support for the criterion approach in Appendix G and this information should be summarized and referenced in the main document body. This part of the tiered criterion is the most difficult to study in the field, although our practical and experiential knowledge of Se bioaccumulation in aquatic ecosystems suggests it has high importance in protecting aquatic life. The practical implementation of this tier of the criterion will require enhanced guidance and regulatory sensitivity to the cost of monitoring.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

Acute criterion: The comments largely support or request guidance concerning abandonment of an acute criterion. The intermittent water column tier of the draft selenium criterion does much to address potential ecosystem impact potential from discharge concentrations historically regarded as having "acute" toxic

potential.

Alternative more sensitive endpoint: Comment lacks clarity and method/approach publication or peer-review to fully consider the point being made.

Aquatic dependent wildlife: Sound points are made concerning the potential for impact to aquatic birds. The author overstates that the criterion set a *de facto* limit for invertebrates. While the comments are broadly valid and demonstrate the complexity of the Se aquatic impact issue, equal concerns should be weighed on the relative balance of over- or under- protection of the draft criteria if deployed. The rigor of this present document to address aquatic life ambient water quality is significant, broadly inclusive and broadly defendable. The tier approach may be expected to have significant impact in overall water quality and aquatic dependent wildlife because of the integrative exposure nature of the tissue criterion.

Averaging period: Comment reasonably addresses the need for clearer implementation guidance of the intermittent water column criterion.

Bioaccumulation factors: The context of this question is addressed in the document, however additional clarification may be useful.

Biphasic modeling: The comment author expresses an opinion regarding modeling approach. The available peer-reviewed published studies supporting this approach for selenium in fish/aquatic ecosystems is limited and thus of less value in setting the criterion. The author may have a good point however the availability of published work limits its practical consideration. The Atlantic salmon graph referenced appears to be a Wikipedia selenium entry without attribution.

Bluegill Hermanutz: The conclusion that the Hermanutz data are outliers is not supported in the comment by any numerical/statistical analysis and thus must be treated as opinion, unless otherwise verified. Data variability in biological systems can be tested to determine outliers however it is unlikely the data count would support exclusion, thus inclusion is more defendable.

Brown trout study: The presentation and role of the brown trout study, related serial reviews, and re-reviews in the draft criterion document and supporting resources raises questions in the public comments. While some of the questions addressed in public comments are broadly addressed in the draft document, additional effort should be made by EPA to specifically address concerns outlined in these comments. The use of the study data is confounded by unfortunate experimental system failure encountered during the study.

Clarification: The comment authors state reasonable requests for clarification that can be addressed in the main text body.

Conversion factors: Several of the public comments regarding conversion factors represent valid concerns. Some of the issues are addressed in the draft document and thus additional explanation could be useful. The suggested approach of using species specific CFs and determining a 80 or 90^{th} percentile cut is a solid suggested for an alternative approach.

Correction: These should be validated and corrected.

Criteria are over-protective: these are speculative comments.

Criteria are under-protective: There are valid concerns expressed, especially in the apparent disconnection between agencies working towards similar goals. Concerns over the water column tier of the criteria are adequately addressed by the primacy of the tissue tiers. The risk differentiation argued between 4-6 mg/kg and 8.1 whole body/muscle tissue selenium, in light of the egg-ovary tissue primacy in the draft criterion, is moot.

Data analysis: This comment should be explored for its validity.

Data paucity affecting criterion: This comment appears to somewhat understate the available data. An additional literature search may yield new studies that increase egg-ovary data counts.

Define terms in document: Solid points are made to enhance clarity.

Dietary requirements of Se in fish: The identified citations are of value.

Document process: No comment.

EC10 clarification: Editing error identified; requires correction.

Endangered species protection: This process observation should be considered.

Exclude invertebrates: Risk assessment using extrapolations from animal models is a keystone of toxicology. The approach in the document is a modeling effort based on a similar extrapolation of available data. While not perfect, the data have value.

General comments: Many opinions expressed. Sulfate impacts can be argued to be adequately incorporated into the primacy of the egg-ovary criterion.

GMCV alternative: There are several useful comments, including apparently revised data that should be addressed.

Human health: This comment contains information useful in addressing human health implications of the draft criterion.

Implementation: The public comments express thoughtful concerns and practical implementation questions that can serve as prompts to draft additional guidance.

Importance of Se speciation: The comment expresses academically valid concerns however the practicality and data quality issues of speciated Se analyses for routine sampling and monitoring discount this concern. There are additional confounding issues of analytical sensitivity and result uncertainty at the criterion levels. Total dissolved Se sampling will filter out selenite that is readily adsorbed to suspended sediment particles.

Intermittent criterion: Several good points are raised in the public comments. Suggestions to abandon one model for another do not provide adequate support for the suggestion. Practical implementation concerns are valid and should be addressed.

Lentic lotic clarification: The public comments express thoughtful concerns and practical questions that can serve as prompts to draft additional guidance and supporting information.

Mayfly toxicity: This study should be reviewed for inclusion.

Mercury interaction: This observation is not unequivocal in the scientific literature and thus does not require significant consideration in criterion development.

Misunderstanding of MDRs: Some points are valid, however the practice of extrapolating and translating data is commonplace in toxicology.

Mode of action: The authors correctly identify an oversimplification of the wording in the draft criterion document.

Natural background: The public comments correctly identify concerns of naturally occurring selenium contamination of waters and impacted aquatic life. The draft criterion should explicitly address these concerns in regards to implementation of the draft criterion.

New information: Some of the submitted information has value and should be considered for inclusion. Sulfate modification to selenium impacts are addressed in the primacy of the egg-ovary criterion which

reasonably characterizes endpoint risk regardless of modified uptake.

Number of GMCVs in data set: Draft text should be modified to address clarification.

Other comments: Most labs report 2 significant figures for water Se analysis at these levels.

Rainbow trout study clarification: Clarifying language should be added to the draft text.

Recommend other studies: These studies should be reviewed for inclusion in the data set.

Recommended modifications: This is a summary state of previous suggestions in the list. Data updates once validated are reasonable requests.

Recommended muscle criterion: The approach should be critically reviewed.

Recommended research: While interesting, the method is not used in all studies. Citable references are absent from the comment.

Recommended whole body criterion: The approach should be critically reviewed.

Recommends alternative analysis of Hardy cutthroat trout: The commenter's calculation lacks peer review and detail.

Recommends alternative statistical analysis for Hermanutz bluegill: The commenter's calculation lacks peer review and detail.

Recommends alternatives to Guidelines SSD: Several practical comments are contained in this collection that can assist in drafting clarifying language and guidance.

Recommends including catfish study: The comments are well developed but not necessarily compelling for inclusion, especially in light of previous comments directed at lowering the outcome of the criterion development.

Recommends including zebrafish in data set: A sound argument is forwarded to include this new dataset.

Requests clarification of GEI fathead minnow analysis and its exclusion: This request can be reasonably addressed in the draft document.

Salinity freshwater distinction: Guidance should be included to address these concerns.

Se speciation: The comments addressing plant Se speciation are correct in that the draft text is overly simplified and dated in its discussion of plant Se. Mesocosm studies will also adopt a test water that will influence Se speciation and thus similar Se species exposure concerns will be present as will transferability or differential sediment/particulate/container reactivity of Se species in the test system.

Site-specific criteria: There are numerous public comments that should be addressed in guidance for implementation.

Tiered criteria: There are numerous public comments that should be addressed in guidance for implementation.

Tissue criterion: There are numerous public comments that should be addressed in guidance for implementation.

Translation: There are numerous public comments that should be addressed in the draft document.

Update data set: If practical and possible, this is always a consideration.

Water column values: The concerns should be addressed in the draft document text.

Wildlife criterion: It is apparent from FWS comments that there is significant concern with the draft

criteria potential for protection of aquatic dependent wildlife and fish as well. The pathway for further consideration and development of protection proposed in the draft document appear reasonable to move CWA requirements forward.

Winter stress: Comments opine on winter stress exclusion.

Ww to dw conversions: The comment should be addressed in the draft criterion text as best as possible. It is unlikely that the variability of WW-DW can be uniformly captured in a standardized approach.

PEER REVIEW COMMENTS FROM

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External Peer Review of the Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014

Responses to Charge Questions by Dr. Vince Palace

PART I: OVERARCHING QUESTIONS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

The *Draft Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater* is generally clearly written and logically organized. While there are some issues which require clarification in the document, these generally arise as technical issues (identified in subsequent sections of this review) rather than writing clarity within the document. In contrast to some of the public comments, this reviewer believes that the document clearly states the order of preference for criterion (e.g., egg/ovary over muscle and whole body over water column concentrations) and the ultimate primacy of the egg/ovary criterion. The lone issue of clarity in the document concerns the water column values of selenium. Table 15 (page 97) specifies that water column selenium concentrations are based on "dissolved total selenium in water" however, elsewhere in the document the criterion is described as including "all oxidation states (e.g., selenite, selenate, organic selenium, and any other form)". While clarity regarding the species and analytical methods for assessing water column selenium are provided in Appendix J (Analytical Methods for measuring Selenium), a more precise definition of water column Se is warranted within the body of the document.

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available

    a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

        i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

The tiered construction of the chronic criterion is logical and scientifically defensible, and the primacy of the egg/ovary element over all other elements is also defensible. In fact, the primacy of the egg/ovary criterion was also recognized by a multidisciplinary and international group of selenium experts convened at a workshop in 2009. Proceedings from that workshop were published (Chapman et al. 2009) and in the executive summary it was noted "Selenium concentrations in eggs are the best predictors of effects in sensitive egg-laying vertebrates". Additional sections of that volume further supports the USEPA's Draft Document approach by recommending that measurement endpoints for risk assessment should be as closely associated with reproductive endpoints in egg laying vertebrates as possible and that measurements in eggs or ovaries, or in the absence of these measures, selenium concentrations in muscle or whole body are required. The scientific evidence supporting these conclusions has not changed substantively since the

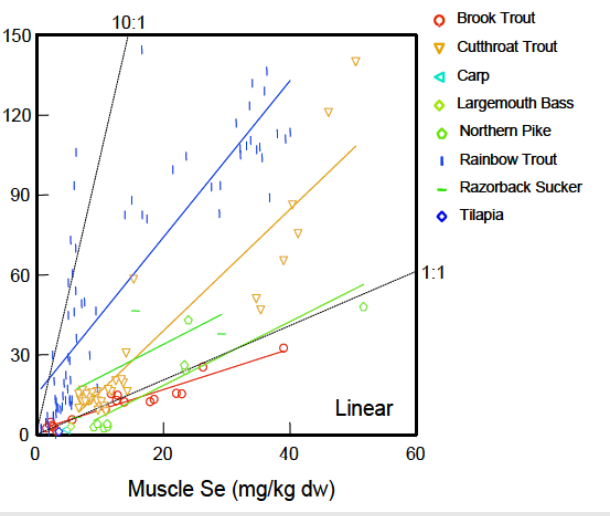
time of that volume's publication and the approach remains the most valid scientifically. In fact, this general approach was also recently adopted by the British Columbia Ministry of the Environment (BC MoE 2014) after an extensive, and peer reviewed, analysis of the literature relevant to the ecotoxicology of selenium.

It is unclear however, how the USEPA will interpret the "never to be exceeded" criteria. Biological variability, coupled with uncertainty regarding the residence of mobile fish species, will make it likely that some fish in a given collection may exceed the guidelines. It is unclear if a result from one fish (i.e. a single exceedance) will render a given management area in non-compliance, or if some average value is intended as the trigger.

ii. Is the primacy of the whole-body/ fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

Affording primacy to the measurement of selenium in tissues over measurements in the water column is scientifically sound. While egg/ovary are recognized as the best predictors of potential impacts of selenium in oviparous vertebrates, there may be situations where these tissues are not available or where technical expertise is not sufficient to allow collection. In this instance, muscle or whole body measures are the next best alternative to egg/ovary as a risk assessment tool. The use of water column concentrations of selenium as environmental assessment tools or as triggers for additional assessment is fraught with uncertainty from several sources, which are discussed in subsequent sections of this review.

However, it is important to recognize that the use of these tissues for monitoring purposes introduces a layer of uncertainty with regard to potential reproductive toxicity assessments. This uncertainty arises because selenium partitions between egg/ovary and muscle/whole body differently in different species. For example, regression plots of selenium concentrations in eggs versus those in muscle of 8 fish species revealed vastly different slopes and strengths of regression between species (see figure below reproduced directly from North America Metal Council ([NAMC] 2008), y axis scale is Egg Se (mg/kg dry weight (dw)). Due to this divergence, in order for muscle to be used as an effective surrogate for concentrations of selenium in egg/ovary, the specific regression for the fish species in question will have to be documented.



With regard to whole body as a criterion, it is unclear whether the USEPA intended to include visceral tissues (e.g., liver, kidney, gonads, gastrointestinal tract) with the carcass for whole body measurements of

selenium. Because these tissues can account for a significant amount of the whole body pool of selenium, when Se concentrations in liver and gonads are elevated (especially during oogenesis [i.e. egg formation]), this requires clarification.

iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

Uncertainty associated with the US-EPA's tiered approach arises from the species specific disposition of selenium into egg/ovary versus muscle and whole body (noted above) and in the sampling methods used to obtain these tissues. In terms of sampling methods, timing may contribute to variability. A recent study showed that some fish may partition selenium to the eggs/ovaries more immediately from the diet than from their tissue stores (Conley 2014). In these species of fish, muscle/whole body might be less reflective of egg/ovary selenium concentrations than concentrations in the diet. However, the authors noted that spawning strategy may play a role in determining the importance of tissue reserves versus dietary sources accounting for selenium partitioned to egg/ovary. Specifically, for species with longer periods of oogenesis and which spawn only once annually, tissue stores may be better predictors of egg/ovary selenium concentrations than dietary sources. However, for multiple spawners, the diet may be a more important determinant. This has relevance to the both the egg/ovary and muscle/whole body criteria recommended by the US-EPA and the variability inherent in each. If muscle/whole body were used as a measure of compliance the timing of sampling within the fishes' reproductive cycle could have an influence on the concentration of selenium in the tissue, especially among single spawners with extended oogenesis periods. Therefore, if muscle/whole body were sampled immediately following the spawning period lower concentrations of selenium might be expected than if the tissues were sampled prior to oogenesis.

Another source of variability concerning the application of muscle and whole body as a criterion concerns a precedent that USEPA has established with regard to conversion of concentrations in one of these tissue types to another. While the Draft Document acknowledges that matched pairs of muscle and whole body concentrations of selenium were assessed for each species, only a few fish species provided data for assessing the conversion (Page 78). As a result, USEPA used the median ratio for all species (i.e., 1.27) to convert muscle selenium to whole body concentrations. In the absence of additional species specific conversion ratios, continued use of this generic ratio would be expected to introduce additional variability. For example, and with reference to derivation of the egg/ovary criterion for the Draft Document, variability would be expected to have arisen from the fact that almost half (i.e., 7 of 16) of the Conversion Factor (CF) values for egg/ovary to whole body were derived using the generic muscle to whole body conversion ratio.

iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

As noted in our response above, there is some confusion regarding how "never to be exceeded" concentrations of selenium in the tissue based criterion will be applied (i.e., is this applied to analysis of single fish or to arithmetic or geometric means from sampled populations?). Clarification on this question is required before the scientific defensibility of the duration and frequency can be assessed for the two tissue based criterion.

With regard to the magnitude of the tissue based elements, it would appear that at least two issues may challenge the scientific defensibility of these criteria. First, it is our understanding that the egg/ovary

criterion was developed from EC10 values derived from the literature. Where multiple results of acceptable quality for a given species were available, a geometric mean was calculated. In the case of the EC10 for bluegill (*Lepomis macrochirus*), the mean EC10 resulted from 4 studies, published by three authors: Hermanutz et al., 1992 and 1996, Doroshov et al. (1992) and Coyle et al. (1993). However, the EC10 value calculated from the Hermanutz et al. studies (=12.7mg/kg) is quite different from the values rom the other two studies (20 and 24.6 mg/kg respectively), indicating cause for investigation of the reasons for the difference, especially in light of their importance for determining the egg/ovary tissue based criterion. One of the supplemental comments provided as additional information with this package (Docket ID EPA-HQ-OW-2004-0019-0331) indicates that the TRAP model plot of the Hermanutz et al. data provide a poor fit. While we were not afforded access to figure 1, which cited in that docket submission, if the data are indeed poorly fit, it is appropriate to consider them questionable and eliminate them from the geometric mean calculation for this species.

A second, and potentially more serious issue with regard to the magnitude of the egg/ovary tissue based criterion, is the reliance on the reanalysis of data from the brown trout (*Salmo trutta)* study (Formation 2011). Uncertainty in this study arises because some fry escaped from their respective incubation chambers and could not be assigned to a given treatment. As a result, several scenarios were calculated based on whether the escaped fry had similar deformity rates relative to the retained fry, were all deformed, or were all normal. While this cannot be resolved, the criterion was calculated based upon the most conservative approach: that all fry were dead or deformed. This conservative approach to calculating an EC10 value for brown trout result in it being the most sensitive species, thereby affecting the overall egg/ovary criterion. Subsequently, because other criterion (i.e., muscle/whole body and the water based criterion) are back calculated based on the egg/ovary value, conservatism is compounded in the values for these criterion as well.

For the water column based criterion, two separate elements are prescribed in the Draft Document: a monthly average and a separate element for intermittent (discontinuous) exposures. Each of these is further delineated to apply to either lentic or lotic systems. Presumably the definitions for lotic and lentic systems would be based on residence time of water or some related criteria, but the Draft Document does not contain an explicit definition of either type of system. Back calculating from egg/ovary to muscle/whole body and then down through trophic levels to derive allowable water column criterion for each of these types of aquatic systems is not scientifically valid, because of the use of generic conversion factors and broadly based trophic transfer factors. These generic terms do not incorporate site specific information, including concentration dependent uptake kinetics and consideration for important influencing factors (e.g., sulfate). The water based criterion is therefore, conservative and variable. As evidence for this, the monthly average exposure value for lentic systems is 1.3 μg/L. This value is at the upper end of background values for freshwater and may be exceeded even in the absence of industrial inputs in areas receiving runoff from seleniferous soils. The value is also lower than recently recommended lentic values based on similar analysis (2 and 2.1 μg/L respectively (Deforest et al., 2104, BC MoE 2014).

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10th centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

The slope of the response curve for selenium rates of deformities plotted against selenium concentrations in eggs/ovaries rises rapidly above the EC10 value. Therefore, use of the 10th percentile as the measurement endpoint is scientifically defensible, appropriate and consistent with USEPA's assessment of toxicity of other compounds as well as the assessment of reproductive toxicity in other jurisdictions (BC MoE 2014).

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

    a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

The use of reproductive effects in fish to derive the sensitivity distribution is appropriate because non-reproductive effects may arise from mechanisms that are not central to the primary ecological effects of selenium; reproductive toxicity in oviparous vertebrates manifested by maternal transfer of selenium to eggs. Additionally, as noted in the Draft Document, non-reproductive effects thresholds are highly variable and provide less confidence for deriving threshold values for selenium. The use of data from fish as the most sensitive organisms is appropriate and likely to be protective of invertebrates. However, it should be noted that sensitivity among invertebrates is highly variable and that some invertebrate taxa do exhibit sensitivity at low μg/L concentrations (see BC MoE 2014 for a review of this data).

While we agree that the Draft Document predominantly uses data from fish generally sensitivity, the approach in the Draft Document is not a complete departure from the principles surrounding the use of eight phylogenetically distinct taxa. The US-EPA has attempted to increase taxonomic coverage of the sensitivity distribution by converting results from studies of three invertebrate taxa into fish reproductive endpoints. Specifically, threshold concentrations of selenium in the invertebrates were converted to predicted fish concentrations of selenium in egg/ovary based on consumption of the invertebrates by fish. These values were then included in the fish distribution (Figure 5, page 58). The variability inherent in this calculation is large because a generic trophic transfer factor of 1.27 was applied to convert invertebrate

concentrations to fish whole body concentrations and then a generic conversion factor of 1.71 was applied to convert whole-body concentrations to egg/ovary. The result is a highly variable, and scientifically questionable, series of three additional data points that were added to the distribution of reproductive effects for fish.

b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

As noted above, the use of data from oviparous fish as the most sensitive aquatic organisms to derive criterion is appropriate and likely to be protective of invertebrates. However, the USEPA may wish to consider sensitivity data for some invertebrate taxa that do exhibit sensitivity at low μg/L concentrations (see BC MoE 2014 for a review of this data).

c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

Because non reproductive tests do not evaluate the most sensitive measure of selenium ecotoxicology, their use as regulatory criteria are questionable. However, the USEPA has provided summaries of non-reproductive tests and compared the results from these studies with the criterion derived using reproductive data. In most cases, the studies have evaluated growth or survival of fish. The species mean chronic values (SMCV) and genus mean chronic values (GMCV) from the non-reproductive tests are generally greater than the egg/ovary criterion and, therefore, it is expected that the criteria derived from the reproductive studies (e.g., Egg/ovary) will be protective of non-reproductive endpoints as well.

d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

The US-EPA rejected the Doroshov et al. (1992) study in which female catfish were injected intramuscularly with seleno-methionine and effects were determined in their offspring. The chemical form of selenium was appropriate for injection into these fish, but it could be argued that injection circumvents dietary uptake, tissue partitioning and timing of muscular uptake with respect to reproductive cycle of the fish. Some may therefore consider this injection study to be invalid. However, relating selenium concentrations in egg/ovary to reproductive effects was the primary focus of the USEPA's assessment. While several compromises have been established to allow data to be included in the development of the criterion (see discussion of the bluegill and brown trout data from earlier comments), the exclusion of the data from the Doroshov et al. (1992) study appears arbitrary. Moreover, citing abundance of Ictalurids in the Hyco Reservoir (Crutchfield (2000) and at Belews Lake (Young et al. 2010) at selenium concentrations that may have affected abundance of other fish species is not sufficient evidence to dismiss the data from the Doroshov et al. (1992) study. A reexamination of the data and consideration to include them in the egg/ovary criterion is warranted.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

The methods used to translate egg/ovary to muscle and whole body criteria are understandable and transparent, but as we noted in our earlier comments, there are scientific issues with some of the transformations. The USEPA attempts to use matched pairs of muscle and whole body concentrations of selenium for each species, but only a few fish species provided data for directly assessing the conversion (Page 78). As a result, US-EPA used the median conversion value for all species (i.e., 1.27) to convert muscle selenium to whole body concentrations where species specific data were not available. Continued use of this generic ratio would be expected to introduce additional variability and uncertainty, particularly for the conversion from egg/ovary to whole body because in many cases this requires a two step conversion (i.e., from egg/ovary to muscle and then from muscle to whole body). More specifically, almost half (i.e., 7 of 16) of the Conversion Factor (CF) values for egg/ovary to whole body were derived by including the generic muscle to whole body conversion ratio. The issue is less important for conversion of egg/ovary to the muscle criteria because for most species (other than desert pupfish) there were data available to calculate the conversion directly.

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).

- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).

- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).

- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).

- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

The scientific method for translating concentrations of selenium in fish tissues to allowable concentrations in the water column is clearly written and understandable. However, while we understand the regulatory need for triggers to initiate site investigation where selenium is suspected of being an issue, the derivation of allowable water column concentrations from eggs or ovaries is oversimplified and likely to need site specific inputs for refinement. Back calculating from egg/ovary to muscle/whole body and then down through trophic levels to derive allowable water column criterion for each of these types of aquatic systems is not scientifically valid, because of the use of generic CF, assumptions regarding proportions of prey items consumed by resident fish and broadly applied trophic transfer factors. These generic terms do not incorporate site specific information, including concentration dependent uptake kinetics and consideration for important influencing factors (e.g., sulfate, organic carbon, temperature,etc.). The water based criterion developed in the Draft Document are therefore, necessarily conservative. As evidence for this, the monthly average exposure value for lentic systems is 1.3 μg/L. This value is at the upper end of background values for freshwater and may be exceeded even in the absence of industrial inputs in areas receiving runoff from seleniferous soils. The value is also lower than recently recommended lentic values based on similar analysis (Deforest et al. 2104, BC MoE 2014).

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

The derivation of Trophic Transfer Factors (TTF) by the US-EPA in the Draft Document is clearly outlined and presented. However there are several issues which, again, result in the introduction of error and therefore an element of conservatism in the data that was derived. For example, the USEPA matched selenium concentrations in consumers and their likely prey items from a thorough investigation of the available data. However, where matched data from more than one prey item was identified from a site, the median of lower trophic organisms was used to calculate a TTF. While we understand the rationale for this practice from a data handling perspective, by not acknowledging that prey items may comprise different proportions of the diet ultimately introduces variability in the calculated TTF, with the potential for an influence in either direction. Additionally, while the US-EPA presents a statistical argument for the validity of matching pairs of samples taken from an aquatic site over a year, it is also acknowledged that some sites may present selenium loads or bioaccumulation kinetics that require different collection time criteria. Recognizing that the Draft Document will largely be applied to impacted receiving environments that are influenced by industrial activity and which present dynamic ranges in selenium loading, it appears likely that establishing a precedent to allow matching concentrations of selenium in aquatic compartments collected a year apart will, in most cases, not be appropriate. Finally, the USEPA designated single TTF

based on the median value of only those regressions that were significant (Page 75). While this is a conservative approach, it does not fully incorporate consideration for differential uptake among lower trophic organisms at varying concentrations of selenium exposure.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

As noted in our response to Charge Question #2, almost half (i.e., 7 of 16) of the Conversion Factor (CF) values for egg/ovary to whole body were derived using a generic (i.e., not species specific) muscle to whole body conversion ratio that was calculated as the median value of the available data for all fish species. This practice will have likely contributed to the variability in the dataset.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

Derivation of Enrichment Factors (EF) based on paired concentrations of selenium determined in water and particulate would have been influenced by the practice of allowing data to be paired if they were collected up to a year apart. In terms of application of EF to categories for lentic and lotic systems it is difficult to judge because of the lack of specific criteria to distinguish between the two types of systems in the Draft Document. While the US-EPA acknowledges the importance of residence time for defining aquatic systems as either lentic or lotic, the criterion for their initial assignment to each category is not apparent (Page 82). Despite statistical comparisons that support their aggregation, it is very likely that lakes, reservoirs, ponds and marshes will have vastly different selenium kinetics, and yet they are all designated as lentic systems. Likewise, selenium uptake into aquatic food-webs of creeks, drains, washes, rivers and streams may differ markedly. The wide range of variability in the aggregated categories (Figure 10, page 84) is compelling evidence in support of this point. Additional specific guidance is required to distinguish between the two types of aquatic systems and the applicability of EFs for each.

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

It is not clear how the intermittent criterion outlined in the Draft Document will be applied. The mathematical expression of the criteria on page 93 is clear but the terms surrounding the application of the

criterion are not. For example, the criterion is not intended to apply to "ordinary smoothly varying concentrations" (Page 94). However, what specifically will constitute a discharge curve that is not "smooth" has not been defined. It is also not clear what magnitude of selenium concentration spikes would designate a discharge as having to be regulated as an intermittent discharge. Finally, designation of an intermittent criterion appears to contradict the data in Appendix G and the statement on page 94 that "kinetics of selenium accumulation and depuration are sufficiently slow that attainment of the water criterion concentration element by ambient 30 day averages will protect sensitive aquatic life species even where concentrations exhibit a high degree of variability. While outside the area of our expertise it is noted that several comments in the public registry suggest that a biokinetic model may be more appropriate than the application of an expansion of the 30-day average calculation for determining intermittent criterion.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

Relevant comments from the public or stakeholders have been acknowledged where they are relevant to the other charge questions above. No further specific issues arising from our review of the public comments are noted.

References

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OW-2004-0019

United States Department of the Interior

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U.S. Environmental Protection Agency
Water Docket
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Washington, DC 20460
Attention Docket ID No. OW-2004-0019

May 19, 2005

Dear Mr. Johnson,

The U.S. Fish and Wildlife Service (USFWS) respectfully submits the following public comment package in response to the U.S. Environmental Protection Agency's (EPA) request for scientific information, data, and views pertaining to the "*Draft Aquatic Life Criteria Document for Selenium*" (Federal Register 69(242):75541-75546; December 17, 2004). Selenium is a particularly potent environmental stressor for fish and wildlife, and USFWS scientists (often in collaboration with the U.S. Geological Survey's Biological Resources Division (BRD), EPA's Office of Research and Development (ORD), and university researchers), have produced a substantive portion of the scientific record documenting the ecotoxicology of selenium through a combination of field and laboratory research. For example, publications by current and former FWS scientists comprise thirty-five percent (81 of 228) of the literature cited in a recent review of the ecotoxicology of selenium published in the "*Handbook of Ecotoxicology*" (Hoffman et al. 2003).

The USFWS examined EPA's Draft Criteria Document, associated documents posted on EPA's selenium web site (http://www.epa.gov/waterscience/criteria/selenium), and documents found in EPA's e-docket for the selenium proposal, Docket ID No. OW-2004-0019 (http://www.epa.gov/edocket). The USFWS has identified technical concerns regarding the Draft Criteria Document (EPA 2004). We are aware of the exceptional complexity of selenium chemistry, its environmental dynamics and partitioning, and its biological effects, and the USFWS realizes the difficulty in deriving Ambient Water Quality Criteria for selenium. The USFWS appreciates EPA's substantial allocation of expertise and other resources in producing the Draft Criteria Document (EPA 2004) and pulling together an enormous and diverse base of scientific information into a single document for review by the wider scientific community. Although our comment package focuses on what the USFWS has identified as technical concerns in the Draft Criteria

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Document (EPA 2004) there was much in the document that the USFWS viewed as appropriate, such as the preference for a tissue-based chronic criterion and the recognition that Lemly's (1993) winter-stress study is environmentally relevant in addition to those conducted under thermoneutral laboratory conditions.

General Comments and Recommendations:

Documentation and background for our comments and recommendations are found in the attached appendices. All tissue values for selenium cited in this letter are on a dry weight basis unless noted otherwise.

The USFWS agrees with the conceptual basis and scientific soundness of tissue-based criteria for bioaccumulative pollutants. The basic components for scientific tissue-based criteria are: (1) the tissue-based numerical value must be scientifically defensible; (2) detailed sampling protocol for measuring the tissue number, and (3) detailed guidance for translating measured tissue numbers back to water-based numerical values since several Clean Water Act (CWA) programs are designed for implementation of water-based numerical values.

The guidelines employed to draft the proposed acute criteria for selenium (Stephan et al. 1985) are recognized both within EPA and throughout the scientific community as not being most relevant for application to highly bioaccumulative pollutants (e.g., Reiley et al. 2003). For proposed acute criteria of a bioaccumulative pollutant, one needs to know toxic risks for fish and wildlife based on their dietary exposures and the risk posed by exposure to the proposed water concentration. Although an acute excursion may be very short-lived in the water column, for bioaccumulative pollutants, the food web effects last much longer (e.g., Maier et al. 1998). The USFWS recommends the USEPA consider bioaccumulation as part of a multipathway exposure in the acute criterion. The USFWS realizes it may be necessary to collect data to evaluate the toxic risks to fish and wildlife based on their dietary exposures.

The USFWS recommends the USEPA employ an effects target level for the chronic criterion which is consistent with that of acute criteria. The USFWS has concluded the proposed selenium chronic criterion of 7.91 ug/g in whole body fish tissue exceeds an LC-20 effects target level. In the study cited by EPA as the basis for the 7.91 ug/g proposal (i.e., Lemly 1993), the lowest observed adverse effects (tissue) concentration (LOAEL) was 5.85 ug/g. The USFWS recommends EPA replace the chronic value of <7.91 ug/g for the winter-stress study (Lemly 1993) with a chronic value of <5.85 ug/g. Furthermore, the USFWS notes because 5.85 ug/g appears to be an LC-40 concentration, a tissue-based chronic criterion in the 4-5 ug/g range may be scientifically warranted and would also be consistent with wildlife toxicity data.

The USFWS recommends EPA give consideration to a new strategy on both water column and tissue based approaches. A national generic safety-net water criterion of 2 ug/L, as has been recommended (DuBowy 1989; Peterson and Nebeker 1992; Sweet 2002) and could be combined with a fish tissue-based criterion for site-specific

implementation. The monitoring of water concentrations in discharges could continue without increased expense of biotic sampling and translation of those sample results back to a water basis. Dischargers would be required to do biotic sampling intermittently (not a routine monitoring burden) on fish tissue relative to the fish tissue criterion. Only when the water column criterion and the fish tissue criterion are both exceeded, or the fish tissue criterion alone, would a full site-specific analysis including development of inter-media translation factors be necessary. Exceedance of the water criterion alone would not require any action. Hamilton (2002) reported a mixed strategy was being employed for mercury criteria in Australia and Canada. Because mercury, like selenium, is a bioaccumulative pollutant, valuable information may be garnered from the Australian and Canadian experiences.

The USFWS is confident that with modifications a revised version of the Draft Criteria Document (EPA 2004) will serve as a scientifically sound basis for updating the national selenium criteria. The USFWS appreciates the opportunity to review and provide comments on this document and looks forward to a continued close working relationship on selenium criteria to achieve our respective Agency's mutual goals and responsibilities for scientifically sound environmental protection and stewardship.

Sincerely,

Everett F. Wilson
Chief Division of Environmental Quality

Attachments:

Technical Comments

Documentation and background for our comments and recommendations are included below. All tissue values for selenium cited in this and following sections are cited on a dry weight basis unless noted otherwise.

1. ***Acute Criteria*****:** The standard guidelines (Stephan et al. 1985) were not developed with highly bioaccumulative pollutants in mind. This is illustrated by referring to EPA's Figure 1 (EPA 1989:8), where bioaccumulation is not considered in the acute criterion flow paths, Reiley et al. (2003) viewed the standard guidelines for deriving water quality criteria as problematic as they give minimal consideration to such concerns. Both the mode of action and critical body residues are affected by the bioaccumulation of a pollutant. Similarly, the expert panel noted: "Little consideration is given to multipathway exposure, leaving criteria to reflect uptake from the water only." The USFWS believes that for highly bioaccumulative compounds in order to develop meaningful acute criteria the potential for residual food chain effects, from even brief acute excursions, must be considered. The USFWS looks forward to working with EPA to develop a model to accomplish this difficult task.

2. Fish Tissue-based Chronic Criterion: The USFWS agrees with the conceptual basis and scientific soundness of tissue-based criteria for bioaccumulative pollutants. The basic components for scientific tissue-based criteria are: (1) the tissue-based numerical value must be scientifically defensible; (2) it needs to be accompanied by a detailed sampling protocol for measuring the tissue number, and (3) it must be accompanied by detailed guidance for translating measured tissue numbers back to water-based numerical values since several Clean Water Act (CWA; 33 U.S.C. 1251 *et seq.*) programs are designed for implementation of water-based numerical values.

The USFWS concluded the proposed tissue value of 7.91 ug/g selenium (parts per million; EPA 2004) is not protective of fish or aquatic-dependent wildlife. In the study cited in the Draft Criteria Document (EPA 2004) as the basis for the 7.91 ug/g proposal (i.e., Lemly 1993), the lowest observed adverse effects (tissue) concentration (LOAEL) was <5.85 ug/g, and this value appears to be an LC-40 (see Attachments 1 and 2). Based on linear extrapolation, an underestimate of effects levels as these curves are exponential, the USFWS has concluded the 7.91 ug/g was greater than an LC-50 for the Lemly (1993) experiment because response curves for selenium are typically very steep (i.e., Lemly 2002; Holm et al. 2003). EPA's standard practice for deriving acute water quality criteria is to divide Final Acute LC-50 Values by a factor of 2 to approximate an LC-01 level of protection (e.g., EPA Water Quality Standards Academy Participant's Manual 1999; Reiley et al. 2003; Keating 2003). The USFWS agrees that a 1-5% effect level is an appropriate target level for setting adequately protective water quality criteria. The USFWS concluded that the Lemly (1993) study demonstrates an EC-20 tissue value for bluegill that is less than 5.85 ug/g. Based on this data and other data presented later in this review the USFWS believes that a tissue concentration less than 5 ug/g would provide an appropriate level of protection, not only for aquatic organisms but also for wildlife. As noted in other sections of this analysis other data also suggests a lower concentration. The USFWS would like to work with USEPA to assist them in developing a protective concentration.

2(a). ***Sampling Guidance:*** EPA states (Federal Register 69(242):75541-75546): "*Because EPA has not yet made decisions on the form or value of its final water quality criteria for selenium, EPA has not yet developed implementation procedures.*" The USFWS believes EPA should promulgate a final tissue-based chronic criterion with developed implementation procedures. The USFWS further notes that in conjunction with this criterion an EPA-approved analytical method for whole-body fish tissue may require promulgation.

2(b). ***Implementation Guidance:*** EPA's proposed tissue-based criterion is founded on the whole-body selenium concentration in juvenile bluegill associated with over-wintering mortality. However, when dealing with a mortality endpoint, and the sampling of surviving fish, it is difficult to get a true measure of tissue selenium due to "survivor-bias" (see Seiler et al. 2003). EPA suggested adult fish tissues should be monitored as they will not be affected by the criterion value and thereby survivor-bias would be avoided (Federal Register 69(242):75541-75546). However, the criterion value would be expected to kill at least twenty percent of juvenile fish; thereby biasing the pool of surviving fish available for tissue monitoring (i.e., introducing survivor bias). The dietary habits, and therefore exposure to selenium, are very different for many species of juvenile and adult fish. This is compounded by the additional summer/fall screening value of whole-body selenium. EPA proposes the monitoring of adult fish as a check on whether exposure at those seasons may exceed the proposed criterion value due to winter-stress syndrome. However, these effects would be expected in juvenile fish (Lemly 1993), but not in adult fish.

EPA's outside formal peer reviewers brought up the issue of implementation guidance and how technically complex many of the implementation issues were likely to be (see peer review comments from reviewers Canton, Lemly, Moller, and Reash; Selenium Docket Document No.s OW-2004-0019-0019 thru OW-2004-0019-0023). In response EPA states (in part): "*We agree that implementation guidance is essential, and needs to address a range of issues, from tissue sampling to BAF calculations. Implementation of selenium criteria will be addressed in a separate publication.*" Elsewhere in response to peer reviewers, EPA states: "*...we recognize that in practice* [i.e., site-specific modifications] *would not be easy to implement in the absence of an EPA protocol.*" In reference to a recommended tissue-based criterion for methylmercury, EPA states: "This the first time EPA has issued a water quality criterion expressed as a fish and shellfish tissue value rather than as a water column value. EPA recognizes this approach differs from traditional water column criteria, and will pose implementation challenges" (EPA 2002:5). The methylmercury precedent serves to reinforce the conclusion a scientifically sound implementation protocol should precede or coincide with promulgation of tissue-based water quality criteria.

The USFWS understands EPA will likely undertake an effort to develop implementation guidance in the near future, as EPA repeatedly noted in response to outside peer reviewers (see EPA responses to peer reviewers Canton, Lemly, Moller, and Reash; Selenium Docket Document No.s OW-2004-0019-0019 thru OW-2004-0019-0023). It is difficult to assess the proposed chronic criterion without the implementation guidance, as the success of the criterion is dependent on an accurate, representative sampling of the target populations in the receiving water. It is possible some states and/or dischargers will prefer to develop site-specific water-based standards. This will require development of bioaccumulation factors (BAFs).

EPA has begun to define the implementation procedures (e.g. whole body sampling vs. tissues, adult vs. juvenile), but other aspects of how the criterion will be used are not well described. Technical implementation issues needing to be addressed include; species selection, age of the fish, development of site-specific bioaccumulation factors, survivor bias, fishless waters, sample locations, and appropriate tissue.

Species Selection: When selecting a species to monitor for regulation of selenium discharges, it is important to consider not only the chemical sensitivity, but also to consider the candidate species life history aspects, which contribute to their vulnerability. Species with long life cycles and low reproductive rates are often more vulnerable to increases in mortality than species with short life cycles and high reproductive rates. These characteristics are important when assessing the potential adverse effects of selenium to threatened and endangered aquatic species. Information on selenium sources, speciation, exposures, site-specific characteristics, lag effects, and integration of ecological effects, must be taken into consideration.

Fishless Waters: Implementing a fish tissue-based chronic criterion is problematic for fishless waters. EPA suggests the possibility of applying the criterion to invertebrate tissue where invertebrate samples are obtained in place of fish samples (Federal Register 69(242):75541-75546). However, in fishless waters, invertebrates are not eaten by fish, but rather become food for aquatic-dependent wildlife. As EPA notes, their proposed criterion was not derived with intent to protect wildlife (Federal Register 69(242):75541-75546).

Bioaccumulation Factors (BAF's): The proposed tissue-based chronic criterion will be problematic for the development of an NPDES permit limit for new discharges. EPA notes "where translation from the tissue benchmark to a water concentration is needed, a bioaccumulation factor (BAF), which may vary substantially from site to site, would need to be established" (Federal Register 69(242):75541-75546). There are difficult technical obstacles to determining representative BAF's required for site-specific standards. The BAF is not a fixed number that can be applied universally. This value is usually dependent upon the concentration of selenium in the water column (cf., McGeer et al., 2003), and thus will vary with temporal and spatial factors affecting water column concentrations. These problems may not be insurmountable (Toll et al., 2005), but considerable time and effort will likely be needed to develop site-specific BAF's.

Alternative Approaches: The USFWS recognizes a tissue-based chronic criterion may be difficult to implement. The USFWS recommends EPA give consideration to a strategy based on both water column and tissue based approaches. A national generic safety-net water criterion of 2 ug/L, as has been recommended (DuBowy 1989; Peterson and Nebeker 1992; Swift 2002) and could be combined with a fish tissue-based criterion for site-specific implementation. For the majority of waters nationwide, permitting and other CWA activities could continue without increased expense of biotic sampling and translation of those sample results back to a water basis. Dischargers could be required to do biotic sampling intermittently (not a routine monitoring burden) on fish tissue relative to the fish tissue criterion. Only when the water column criterion and the fish tissue criterion are both exceeded, or the fish tissue criterion alone, would a full site-specific analysis including development of inter-media translation factors be necessary. Exceedance of the water criterion alone would not require any action. The tissue-based criterion would also be used in the 303(d) listing process. The USFWS notes other advantages of a transitional mixed strategy are to allow collection

of data, which may alleviate uncertainties, both with tissue criteria values and difficulties implementing the criteria. A mixed strategy would have to be developed more fully, but the USFWS believes the concept has merit and recommends EPA give further consideration to this. Hamilton (2002) reported a mixed strategy was being employed for mercury criteria in Australia and Canada. Because mercury, like selenium, is a highly bioaccumulative pollutant, valuable information may be garnered from the Australian and Canadian experiences.

3. ***Analysis of the Protection of Reproductive Endpoints:*** The proposed chronic criterion value of 7.91 ug/g selenium on a whole-body fish tissue basis was developed from EPA's interpretation of an over-wintering survival endpoint (Lemly 1993). Reproductive endpoints are normally considered the most sensitive fish and wildlife biological effects endpoints for selenium (e.g., EPA 2004). Also, winter stress, may not be pertinent to water bodies in climatologically mild regions, nor to coldwater species of fish (Moller 2002; but see Mebane 2005). Therefore, it is necessary to evaluate what the proposed criterion would imply for gravid ovaries/eggs of fish. Also, EPA suggests tissue monitoring would be based on sampling adult tissue (Federal Register 69(242):75541-75546). A regression to relate selenium in bluegill ovaries to selenium in bluegill whole-body tissue was developed and presented in the Draft Criteria Document (EPA 2004:Appendix H), but is employed only to translate fish exposure data from studies for fish ovaries to a whole-body tissue basis so all species chronic values can be reported as whole-body tissue equivalents.

The question of whether a whole-body tissue concentration of 7.91 ug/g selenium would be protective of reproductive endpoints in fish is not addressed in the Draft Criteria Document (EPA 2004). Alternative interpretations of the relevant literature have produced guidelines for reproductive toxicity thresholds ranging from 10-17 ug/g for fish ovaries/eggs (Lemly 1996; DeForest et al. 1999). Using the equation developed by EPA (2004), at 7.91 ug/g whole-body selenium, ovaries would be expected to contain 17 ug/g selenium. However, one set of data (17 of the 23 data pairs; Hermanutz et al. (1996)) used to develop the regression were converted from wet weight to dry weight values without having the percent moistures for the samples, producing inaccurate dry weight values. Doroshov et al.(1992) reported for bluegill that the percent moisture in ovaries varies widely (59.6 – 80.2%) depending on the annual cycle of gonadal development (Gonadal Somatic Index, GSI %). The corrected conversion factor for the Hermanutz et al. (1996) data may be from 2.48 to 5.00 times the wet weight; an uncertainty which can not be resolved. This leaves the six data pairs from Coyle et al. (1993) for valid dry weight basis comparison of whole-body selenium versus ovary selenium in bluegill. One of Coyle et al.'s (1993) treatments resulted in a whole-body selenium concentration of 7.2 ug/g selenium in adult tissue. They found 7.2 ug/g whole-body selenium translated to 25 ug/g ovary selenium in reproductively active female bluegill. Because this exceeds the reproductive toxicity threshold range of 10-17 ug/g, it is reasonable to conclude a whole-body chronic criterion of 7.91 ug/g selenium would not be protective of reproductive endpoints. Doroshov et al. (1992) reported on a reproductive tissue (eggs) toxicity study that yields a chronic value of 12.7 ug/g for bluegill. They did not measure whole-body selenium, however data from Coyle et al (1993), associated 12.5 ug/g in ovary with 4.9 ug/g whole-body; (see also EPA 2004:Appendix H), suggesting a whole-body criterion of 5 ug/g or less would be required to protect bluegill reproductive endpoints.

Although the data for bluegill are limited, relatively few data exist for other species to assess reproductive protectiveness of the proposed chronic criterion. The Draft Criterion Document (EPA

2004:Table 4) reports the reproductive chronic value for rainbow trout was estimated at less than 6 ug/g whole-body selenium. USFWS data for bullhead and catfish from the Colorado River system (USFWS, Grand Junction, CO; written communication) reveal these species commonly exhibit ovaries with selenium concentrations ten-fold the whole-body concentration (e.g., 5.46 ug/g whole-body and 54.2 ug/g ovary). Available data for bluegill and rainbow trout indicate a whole-body tissue-based chronic criterion for selenium would have to be lower than 6 ug/g to be protective of reproductive endpoints for these fish.

4. ***Aquatic-Dependent Wildlife:*** The present chronic criterion for selenium is 5 ug/L (EPA 1987; 2002). Scientists assessing aquatic-dependent wildlife have concluded a range of 1-3 ug/L is required to be protective of wildlife (e.g., DuBowy 1989; Peterson and Nebeker 1992; Sample et al. 1996; Van Derveer and Canton 1997; Skorupa 1998). An analysis of the National Irrigation Water Quality Program (NIWQP) database conducted by Dr. William Beckon (SWG) suggests the proposed tissue criterion may be comparable, to a 7 ug/L water criterion (Figure 1), thus increasing the risk for wildlife.

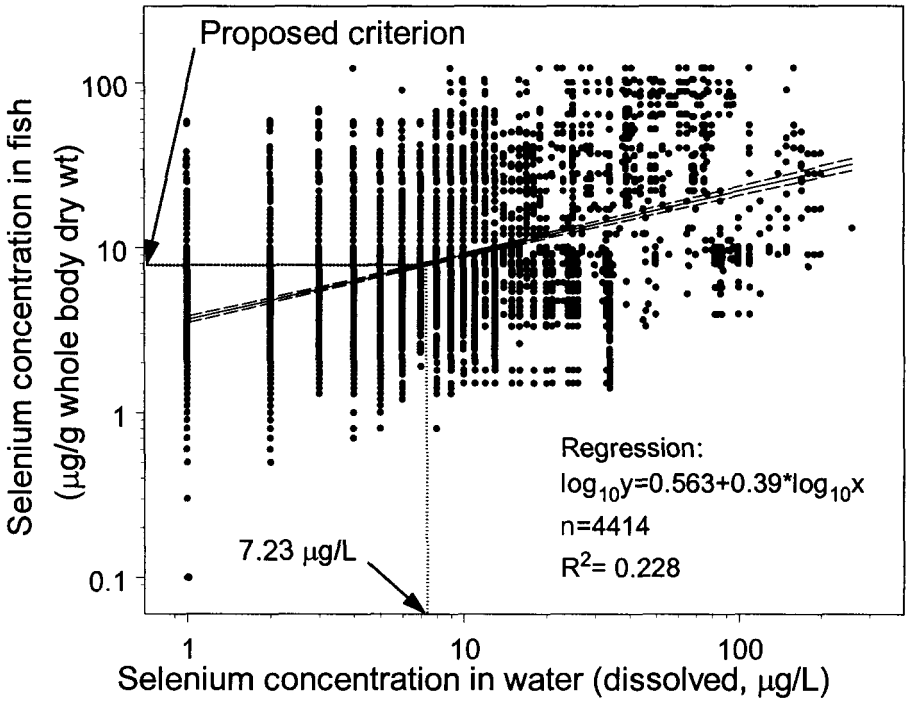

Figure 1.

Selenium in temporally and spatially matched samples of water and fish collected by the National Irrigation Water Quality Program from 26 areas of 14 states in the western United States (Seiler and Skorupa 2001).

National Contaminants Biomonitoring Program (NCBP) data show that the 85th percentile value for fish whole-body tissues has varied between 2.5-3.0 ug/g selenium nationwide with less than five percent of individual sampling sites rising to the level of even 4 ug/g (Walsh et al. 1977; May and McKinney 1981; Lowe et al. 1985; Schmitt and Brumbaugh 1990). The level of selenium loading in fish tissues will be mirrored closely by co-occurring aquatic invertebrates (e.g., May et al. 2001; Swift 2002) important to the diets of aquatic-dependent wildlife such as breeding waterfowl. The USFWS queried the NIWQP biota database (Seiler and Skorupa 2001; Seiler et al. 2003) and summarized the spatial and temporal matched samples of fish and aquatic invertebrates from sampling sites where whole-body fish tissue averaged between 5 and 10 ug/g selenium. The implied invertebrate-to-fish concentration factors from this dataset ranged from 0.67 to 1.36 (Attachment 3). These results suggest the selenium content of aquatic invertebrates would fall in the range of 5.8-11.8 ug/g. This dietary exposure range

for mallards would correspond with an EC20 to EC85 range of effects based on reproductive toxicity (e.g., Ohlendorf 2003). The query results also suggested a central tendency for the implied concentration factors of 1.1 (Attachment 3). Thus, 7.91 ug/g in whole-body fish tissue translates to 7.2 ug/g in aquatic invertebrates. This estimate exceeds the upper 95% statistical confidence boundary (6.64 ug/g) of the dietary EC20 for mallards and equals the EC40 (Ohlendorf 2003). Fish whole-body tissue containing 7.91 ug/g selenium would allow levels of aquatic food chain contamination to exceed the dietary EC20 for reproductive toxicity in mallards (>95%), with an estimate of an EC40.

5. *Threatened and Endangered Species:* There are about one hundred species of aquatic-dependent wildlife in the United States listed as threatened and endangered pursuant to the Endangered Species Act (ESA; 16 U.S.C. 1531 *et seq.*) which is roughly equal to the number of listed species of fish. There are no promulgated national "Wildlife Criteria" for selenium. The California Toxics Rule wildlife criterion process cited by EPA (Federal Register 69(242):75541-75546; December 17, 2004), has been initiated and is projected to require a minimum of five more years to produce a wildlife criterion recommendation (EPA, written comm.). Promulgation of USFWS recommended tissue-based chronic criterion using present practices for acute criteria (i.e., LC-01) would be consistent with the purposes and goals of the CWA/ESA Memorandum of Understanding (MOA) between EPA, FWS, and NOAA-Fisheries (formerly National Marine Fisheries USFWS; see Federal Register 66(36):11202-11217; February 22, 2001).

6. Data Screening and Analyses: Appropriate studies should be included for analyses such as the Hamilton et al. studies; the Beyers and Sodergren studies of razorback sucker (though Beyers and Sodergren studied a less sensitive life stage; see Hamilton, In Press); the Hamilton and Palace (2001) critical review of the Kennedy et al. (2000) study; and including the Hamilton et al. (1990) 90-day results based on performance of controls.

The USFWS is concerned about bias in the Draft Criteria document due to wet to dry weight conversion factors. Conversions of data from wet weight to dry weight basis and vice-versa were done using inaccurate percent moistures which leads to a 25% overestimation of chronic values for whole-body analyses. In one case, calculation of wet weight data from an unpublished manuscript (Hermanutz et al. 1996) was done using a percent moisture value, derived from other studies, whereas a published paper from the same study (Swift 2002) employs the value from the cited study.

The USFWS is concerned about the potential bias in the Draft Criteria Document by not accounting for the hormetic status of selenium. EPA should use a hormetic model rather than the sigmoid logistic model for regressions in deriving a chronic criterion for selenium (EPA 2004:59), especially for data sets that span optimum and deficiency side of selenium exposures. (e.g. Hilton et al. 1980, cited at EPA 2004:I-13). Hormetic models are available (e.g., Brain and Cousens 1989; Van Ewijk and Hoekstra 1993; Svendsgaard 1993; Bailer and Oris 1997; Devidas et al. 1993) and widely used (Schabenberger et al. 1999; Stephenson et al. 2000; Chèvre et al. 2002). However, a non-hormetic statistical model is used to estimate LC and EC 20's.

Specific examples are presented below for Chinook salmon and rainbow trout.

1. Laboratory exposure of juvenile Chinook salmon to dietary selenium: (Hamilton et al. 1990, cited in EPA (2004: Appendix I-5)

This experiment indicates juvenile Chinook salmon with the proposed chronic criterion tissue concentration of 7.91 ug/g whole-body selenium would experience 59 percent mortality after 90 days of exposure.

The EPA analysis included 60-day results but excluded the 90-day results of this study of fall run Chinook salmon (*Oncorhynchus tshawytscha*) juveniles because "control survival declined significantly" during the final 30 days of experiment. However, the survival of all treatments declined substantially during the period, exhibiting a clear concentration-response relationship, with about 30 percent baseline mortality not attributable to selenium (Figure 2a). This general decline may have been caused by some unknown health problem, but it also may have been due in part to the physiological stress Chinook salmon of this strain experience during this developmental period as they undergo the genetically programmed osmoregulatory changes associated with the normal pattern of migration from freshwater breeding areas to the ocean (Scott Foott, Project Leader, California-Nevada Fish Health Center; James Smith, Project Leader, Red Bluff Fish and Wildlife Office; personal communication). The diet of the control group included a low concentration of selenium (1.0 µg/g) intended to represent background exposure. Thus, the control group effectively constituted the low end of a spectrum of exposures rather than a distinctive zero-exposure treatment. The model suggests the "controls" may have been slightly deficient in selenium (Figure 2a). The control survival rate of 66.7 percent accorded well with hormetic concentration-response models (Brain and Cousens 1989). Additionally, mortality due to selenium might be expected to increase during this period if the effects of dietary exposure involve some lag time associated with assimilation and incorporation of selenium into enzymes or tissues (Beckon In Prep.).

The results of this study indicate the proposed criterion of 7.91 µg/g (whole body dry weight) would result in 59 percent mortality of young Chinook salmon attributable to selenium (Figure 2a). The fish tissue criterion would need to be lowered to 2.5 µg/g to reduce mortality to 20%. These projections are based on a standard hormetic model while a sigmoid logistic model used by in the Draft Criteria Document (EPA 2004) does not account for hormesis. Sigmoid models indicate the proposed criterion would cause 64.5 percent mortality in young Chinook salmon (Figure 2b), and a criterion of 1.0 µg/g (whole body dry weight) would be needed to limit selenium-caused mortality to 20 percent.

The experiment included two parallel series of dietary selenium treatments. One set was spiked with seleno-DL-methionine (SeMet), the other set, was mosquitofish collected from the San Luis Drain (SLD), which carried seleniferous agricultural drainwater from a subsurface tile drainage system in the Westlands Water District in the San Joaquin Valley of California. The Draft Criteria Document (EPA 2004) suggests the SLD diets may have included other contaminants, such as pesticides, which may have contributed to the adverse chronic effects measured in this experiment. The data indicate, once selenium is incorporated into fish tissue, there is no difference in the tissue concentration-response relationship due to the different selenium (SLD or SeMet) sources. The experiment indicates the other contaminants effects were not detected. Therefore, all data from both diet series were used in the analysis presented here (Figure 3). The experimental data demonstrate assimilation into tissue of salmon larvae was more efficient with the SLD diet. This however, may be due to the

racemic mix of SeMet isomers used in the spiked diet rather than interaction with other contaminants.

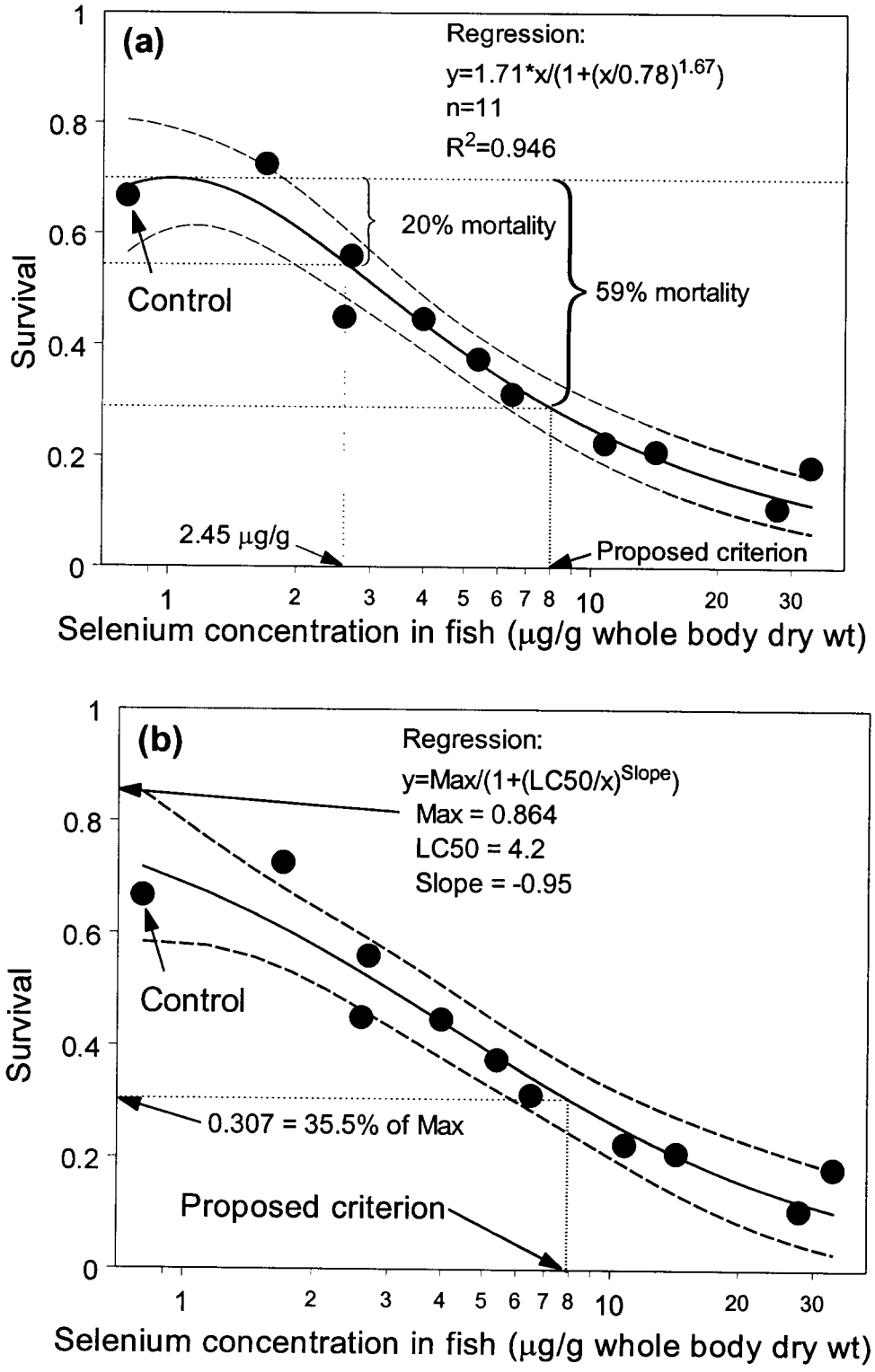

Figure 2. Survival as a function of selenium concentration in tissue of juvenile Chinook salmon after 90 days of exposure to dietary selenium. Hormetic model (a) and logistic model (b) fitted by least squares regression. Dashed lines indicate 95% confidence bands around the regressions in this and following figures.

Guidelines currently used in the U. S. do not address "controls" for hormetic substances. Furthermore, these guidelines explicitly apply only to waterborne, not dietary, exposure (ASTM 2004).

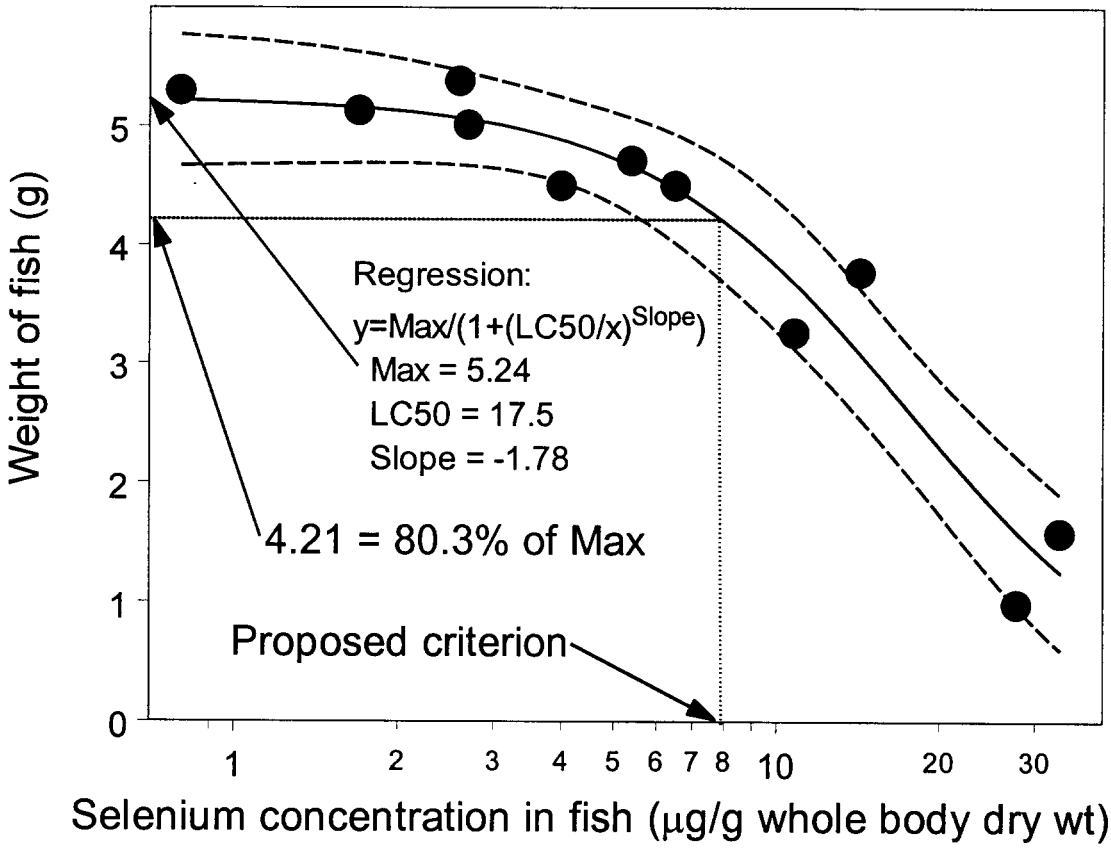

Figure 3. Juvenile fall run Chinook salmon weight 90 days after swim up, in fresh water with dietary exposure to selenium.

The surviving juvenile Chinook salmon at 90 days after swimup exhibit 20 percent weight loss due to selenium (Figure 3). Other studies performed on salmonds mirror those results and confirm the sensitivity of salmonids to selenium.

EPA failed to consider another major component of the Hamilton et al. (1990) study. A separate experiment of Hamilton et al. (1990) reared Chinook salmon fingerlings in reconstituted brackish water with dietary exposure to selenium for 120 days. These fingerlings were then challenged by 10 days of emersion in reconstituted seawater. The results indicate proposed chronic criterion concentration of selenium in salmon rearing in brackish water will result in 2.3 percent reduction in growth within 120 days (Figure 4), and upon entering the ocean will experience an additional 15 percent mortality within 10 days, due to selenium (Figure 5).

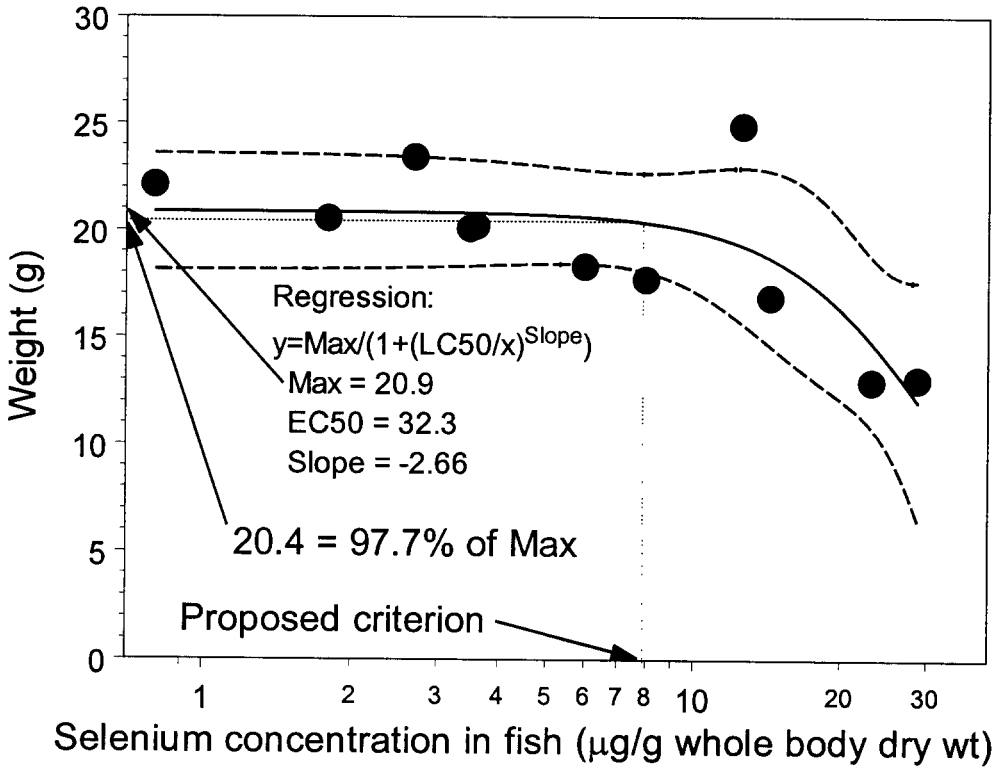

Figure 4. Juvenile fall run Chinook salmon weight after 120 days of rearing in brackish water with dietary exposure to selenium.

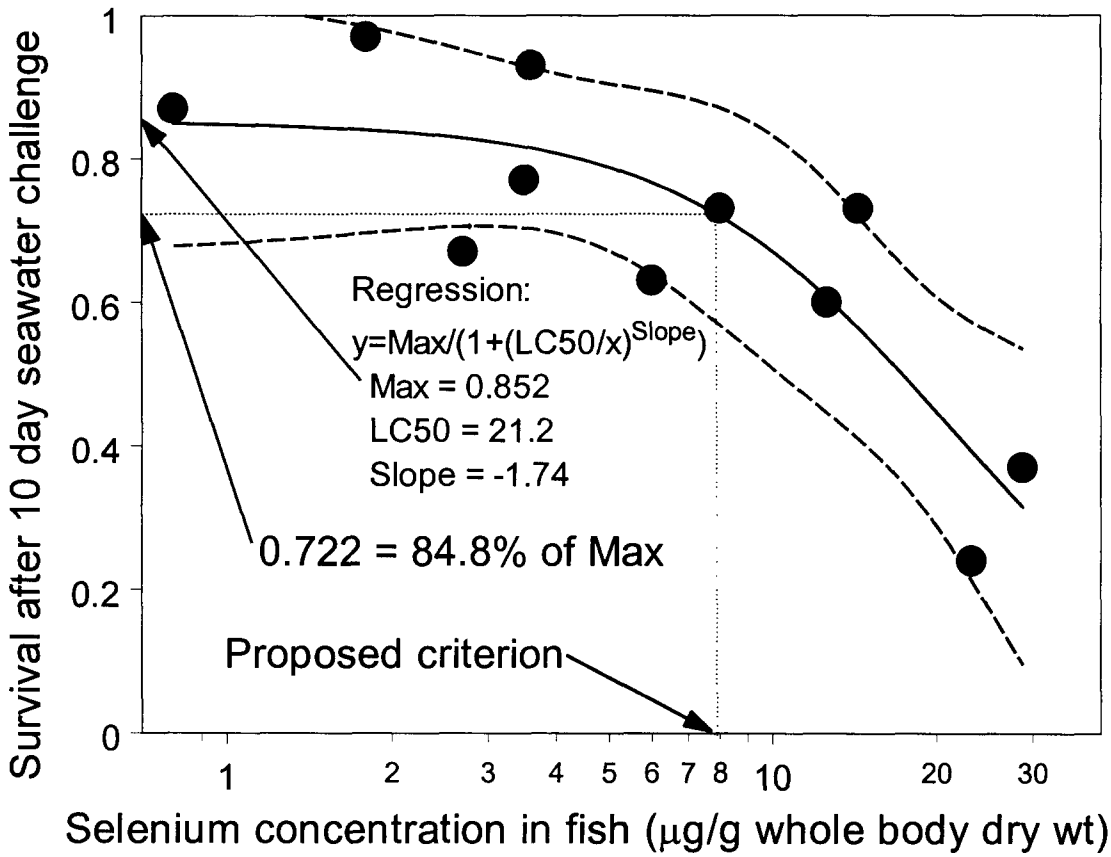

Figure 5. Survival of juvenile fall run chinook salmon after 10 day seawater challenge following rearing for 120 days in brackish water with dietary exposure to selenium.

2. Effects of selenium on fry of rainbow and brook trout exposed in streams in Alberta, Canada: (Holm 2002 and Holm et al. 2003, cited in the Draft Criterion Document (EPA 2004: Appendix I-15).

This study indicates female rainbow trout in the wild with the proposed criterion concentration of selenium in their (whole-body) tissue would produce eggs and swimup stage fry with 44.2 percent mortality. Among the 55.8% swimup survivors, 96 percent would suffer edema and 42 percent would have craniofacial deformities. To protect rainbow trout at an EC_{20} level, this study calls for a criterion of 3.51 µg/g whole-body dry weight.

Data for regressing egg selenium concentration against adult muscle selenium concentration in rainbow trout are displayed on a linear-scaled graph rather than on a log-log scaled graph (EPA 2004:I-23). This led to using an incorrect regression method to minimize the influence of a single datum (1.9 µg/g muscle wet weight). However, plotting the data on a log-log scale reveals this datum is not an outlier. The lowest egg selenium concentration datum (0.01 µg/g), is an extreme outlier (Figure 6). This datum is questionable as it is below typical analytical detection limits. Pending confirmation of this datum, it should be omitted from the regression (Figure 7).

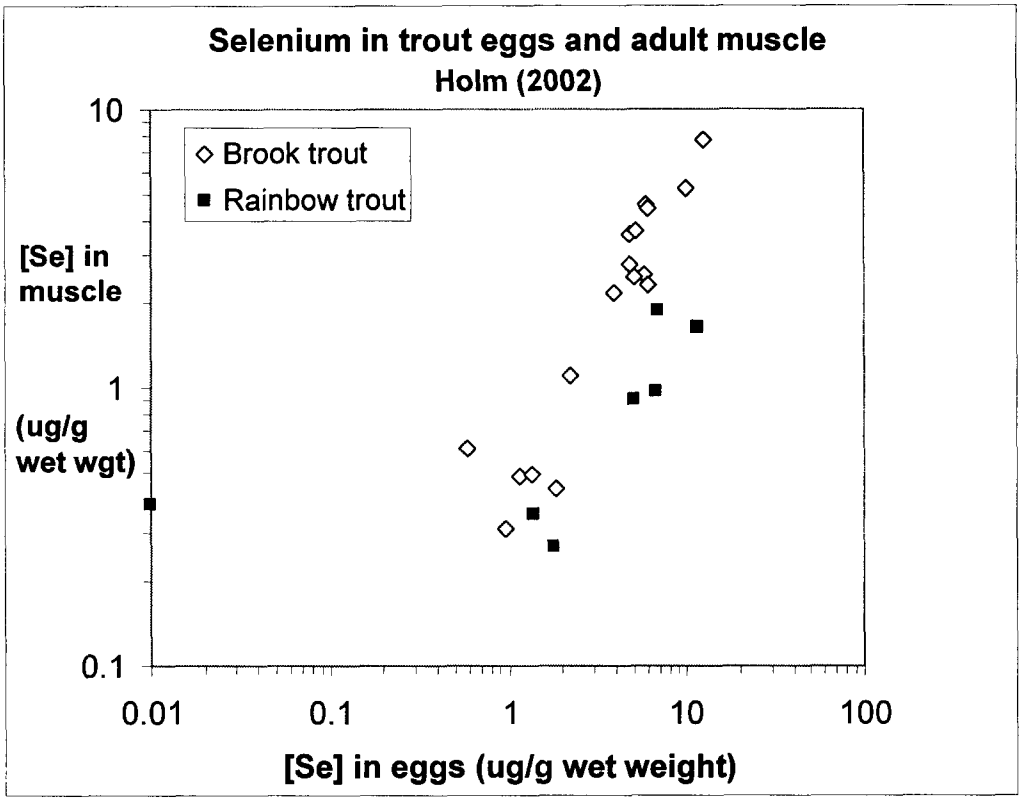

Figure 6. Relationship between maternal muscle and egg selenium concentrations in rainbow and brook trout from streams in northeastern Alberta (Holm 2002 and pers com).

Because contaminant concentrations are log normally distributed (Ott 1990) least squares regression should be performed on log-transformed concentrations (Figure 7).

The Draft Criterion Document (EPA 2004) used Holm's (2002) rainbow trout data to project selenium concentration in brook trout muscle from brook trout eggs even though better data for brook trout egg-muscle selenium relationship were available (Figure 6, Holm 2002, Holm et al. 2003).

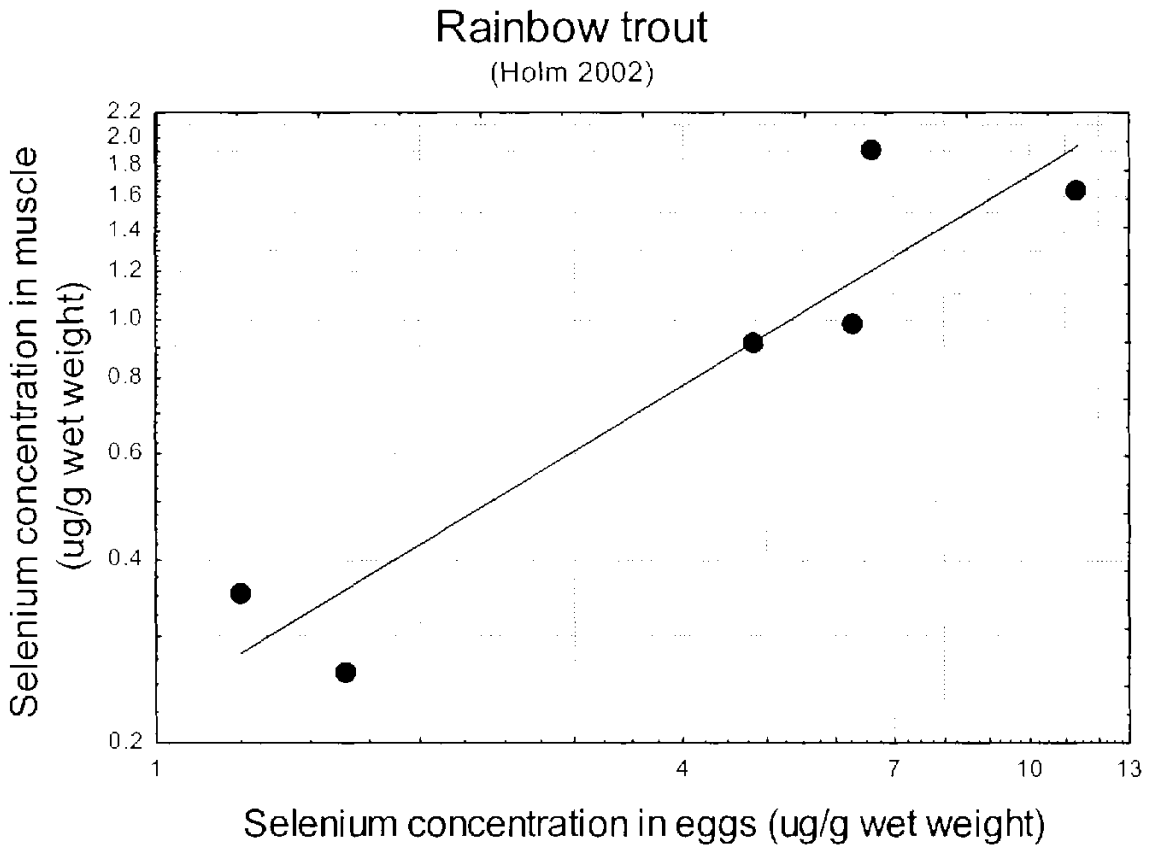

Figure 7. Regression performed on log-transformed Holm (2002) data excluding questionable outlier.

Using the regression in Figure 7, the percent moisture used by the EPA converting dry weight to wet weight (EPA 2004:I-23), and the muscle-whole body regression Equation I (EPA 2004:58), adult female rainbow trout would produce eggs with a selenium concentration of 12.47 µg/g wet weight at the criterion tissue concentration of 7.91 µg/g.

The Draft Criterion Document (EPA 2004:I-16) states a logistic curve could not be fitted to the 2001 rainbow trout edema data shown in "Holm Figure 3." However, a standard logistic curve can be fitted to these data, and shows the proposed tissue criterion will result in more than 80 percent edema in rainbow swimup fry (Figure 8).

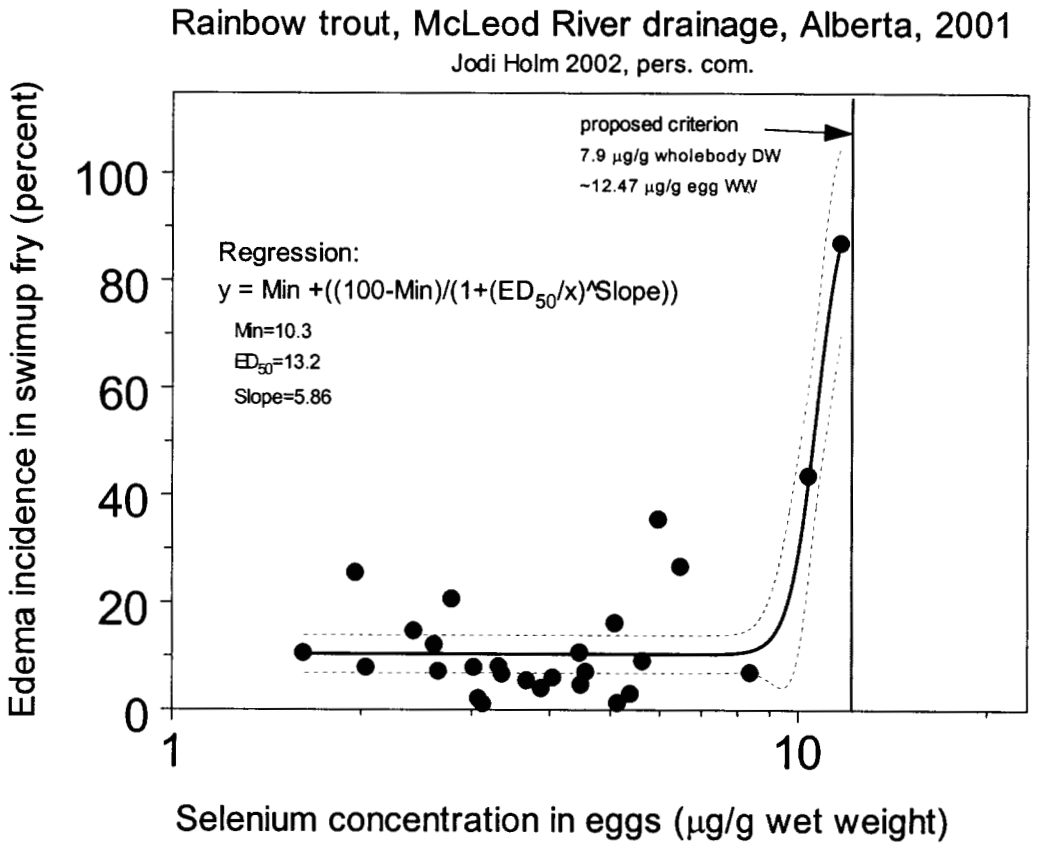

Figure 8. Relationship between selenium in rainbow trout eggs and edema in surviving swimup fry, data from the year 2001 only.

Inclusion of data from years 2000 and 2002 of the same study (Holm pers. com.) extends the regression, projecting 96 percent of the surviving swimup fry will suffer edema, 86.5 percent attributable to selenium in addition to a baseline of 9.5 percent (Figure 9).

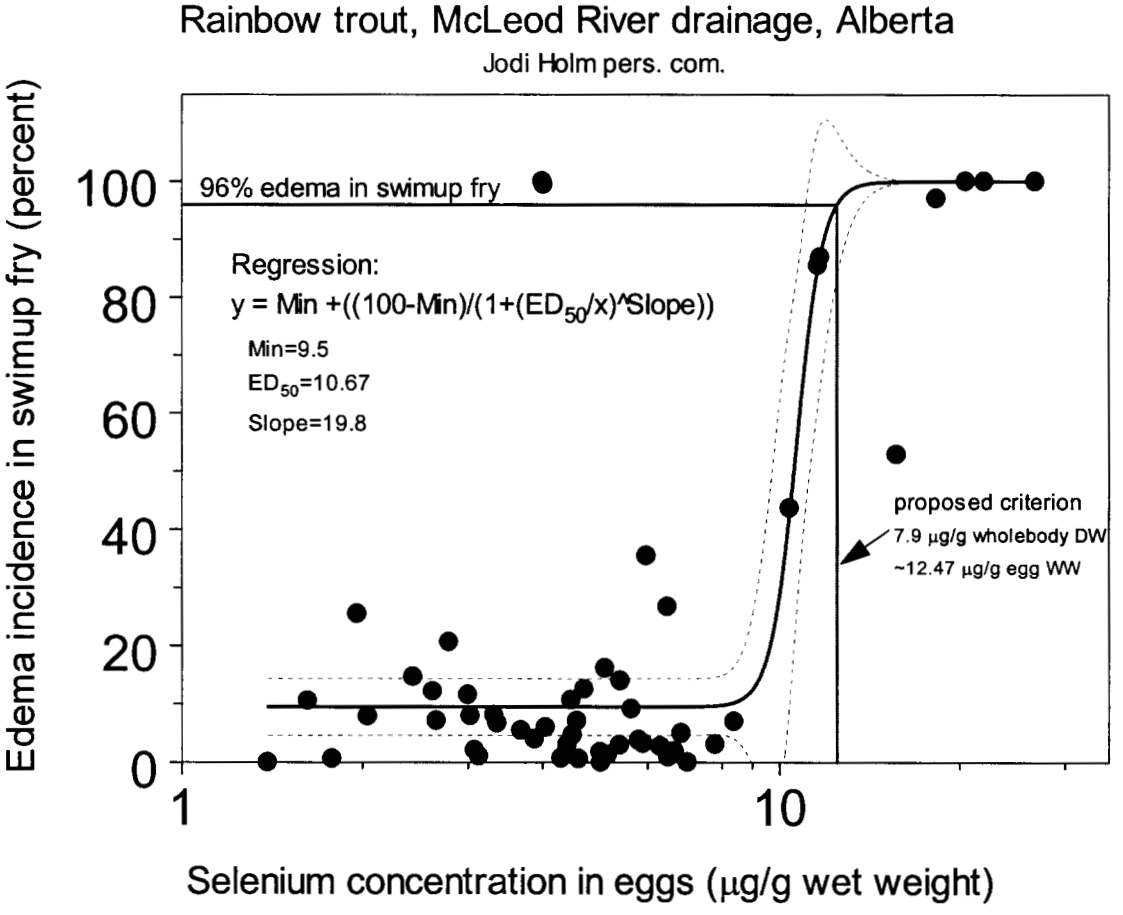

Figure 9. Relationship between selenium in rainbow trout eggs and edema in surviving swimup fry. Data from the years 2000-2002.

Edema is only one of a number of gross defects caused by selenium and measured in this study. For example, 42 percent of the surviving fry will have craniofacial deformities, 32 percent attributable to selenium (Figure 10, Holm 2002, Holm et al. 2003, Holm pers.com.).

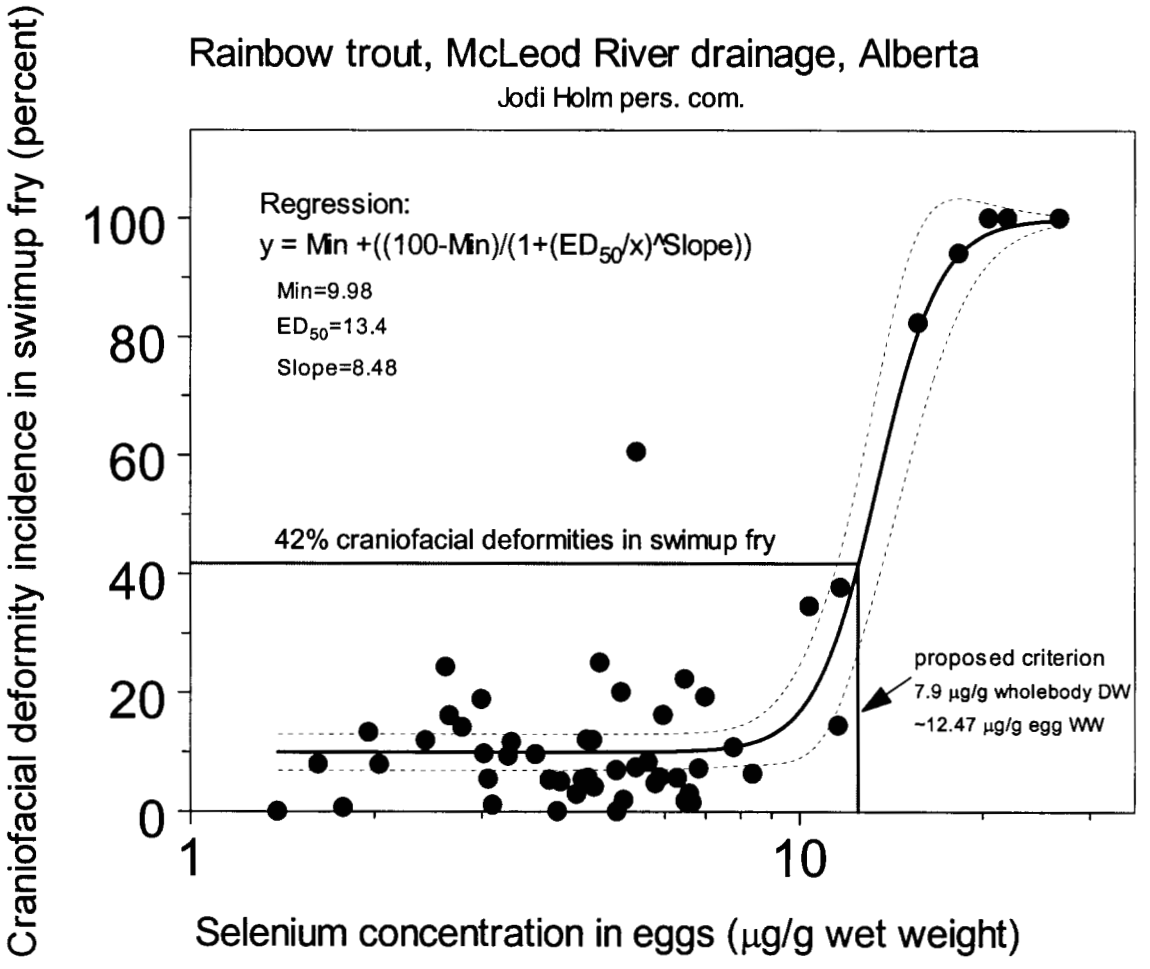

Figure 10. Relationship between selenium in rainbow trout eggs and craniofacial deformities in surviving swimup fry. Data from the years 2000-2002.

Furthermore, all these defects could only be assessed in the fry that survived to reach the swimup stage. Analysis of mortality data from the same study indicates rainbow trout would produce eggs experiencing 44.2 percent mortality at swimup stage with the proposed criterion concentration (7.91 µg/g whole-body dry weight), (Figure 11).

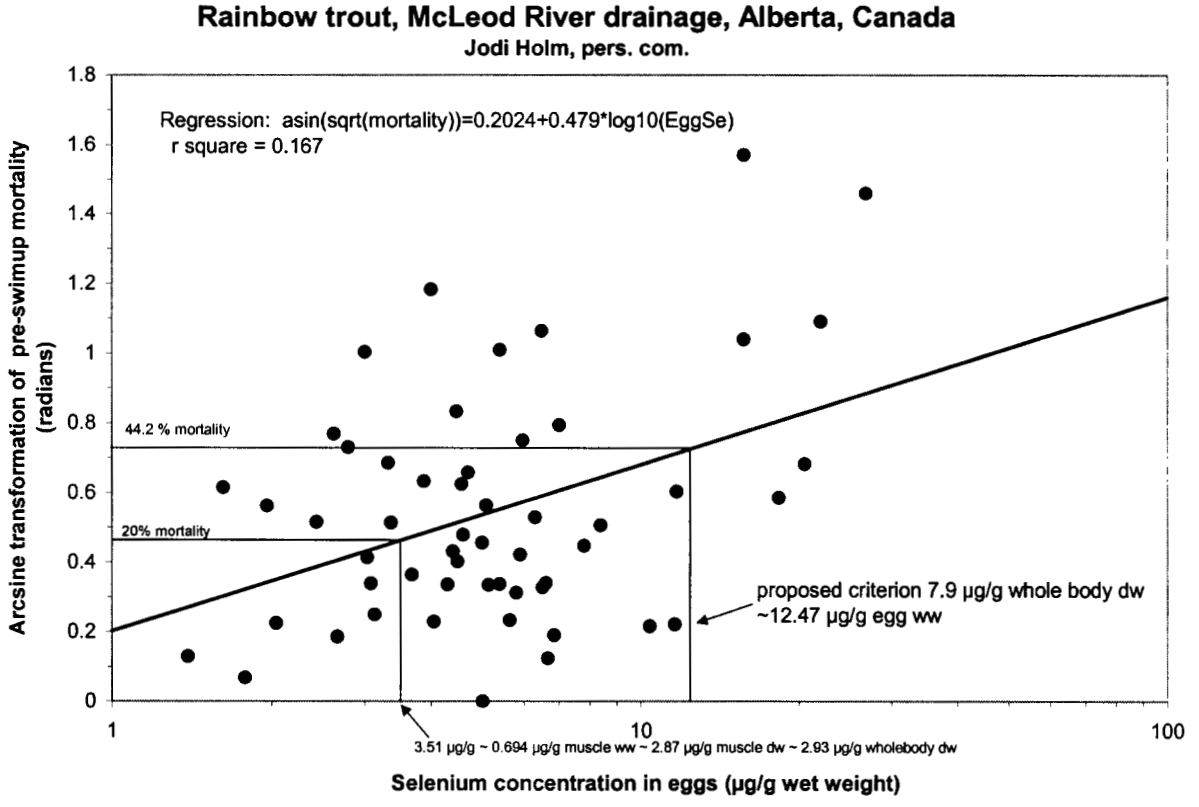

Figure 11. Relationship between selenium in rainbow trout eggs and mortality of eggs and fry by swimup stage. The arcsine transformation is applied to mortality data, as appropriate for linear regressions with percents or proportions (Sokol and Rohlf 1981). Data from the years 2000-2002.

Applying the EC_{20} benchmark is used in the Draft Criterion Document (EPA 2004) for regression analyses, and using the regression in Figure 7, the rainbow trout mortality data from this study yield a species maximum chronic value of 2.93 µg/g (Figure 11).

3. Laboratory exposure of juvenile rainbow trout to sodium selenite-spiked diet: (Hilton et al. 1980, cited in EPA 2004: Appendix I-14)

This experiment indicates the proposed chronic criterion in young rainbow trout will impair growth by at least 86 percent. The proposed criterion in prey of young rainbow trout will impair growth by 34 percent. Because the form of selenium used in this feeding experiment was inorganic selenium (rather than organo-selenium), the Hilton et al. (1980) data are not suitable for deriving a chronic criterion (cf., Heinz et al. 1987); but these data do provide an excellent example of the hormetic nature of selenium.

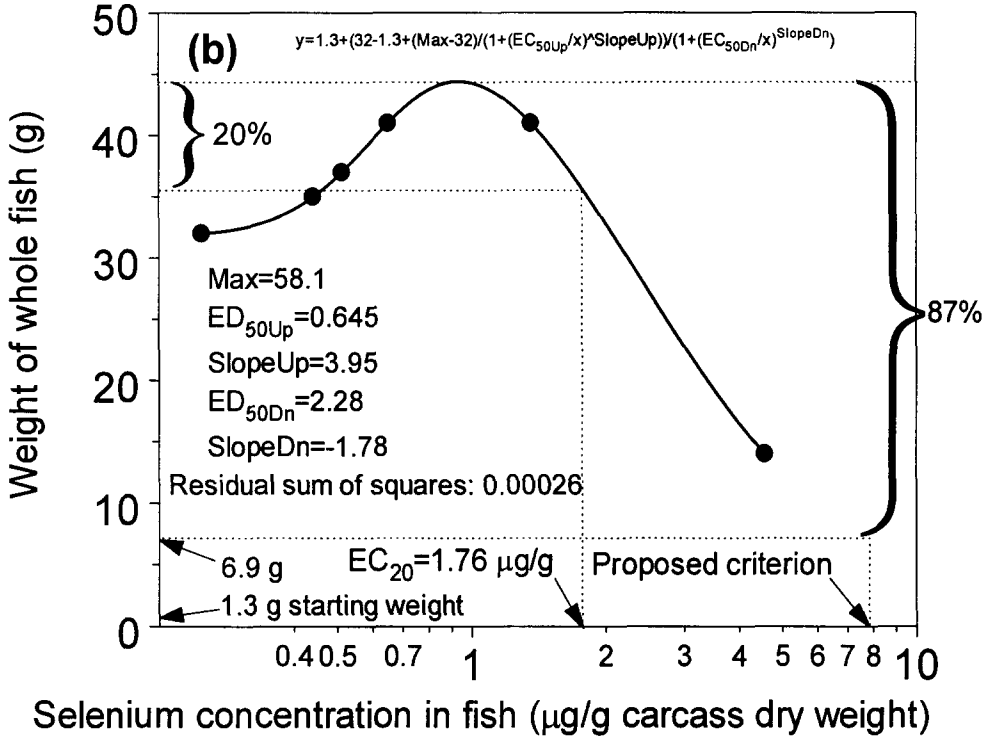

Figure 12. Average weights of juvenile rainbow trout after 20 weeks of exposure to diets spiked with sodium selenite (Hilton et al. 1980), with least squares regression using (a) a standard hormetic model (Brain and Cousens 1989)[, and (b) an improved general hormetic model (Beckon et al. In Prep.) with the assumption the baseline weight was the weight measured at the lowest treatment concentration of selenium. In both models it was assumed at very high selenium concentrations, the fish would have remained at the initial average weight of 1.3 g. Carcass concentrations are from Fig. 2 of Hilton et al. 1980.

The Draft Criteria Document (EPA 2004) selected only data for liver concentrations of the rainbow trout rather than the carcass selenium concentrations for their analysis of selenium in the experiment. The liver concentrations were converted to whole-body concentrations using a linear regression based on bluegill data (EPA 2004: 57-58, I-14). The liver-carcass data from this experiment compared to the bluegill liver-whole body data used by the Draft Criteria Document (EPA 2004) show elevated exposures to selenium. Rainbow trout sequester selenium in their livers to a greater extent than bluegill sunfish by an order of magnitude. Therefore, the bluegill sunfish-based conversion is inappropriate. Each carcass selenium concentration reported in this experiment is a combined value from three to six whole fish and three to four fish from which the liver (about 1% of body weight) and kidneys had been removed. Therefore, carcass selenium concentrations are a good approximation of whole-body concentrations. Using the carcass data from Fig. 2 of Hilton et al. (1980), this experiment indicates juvenile rainbow trout that reach the proposed criterion concentration by exposure for 20 weeks to dietary selenium in the form of sodium selenite will

experience at least an 86 percent reduction in weight relative to the weight they would gain if their exposure were optimal (Figure 12). Applying EPA's EC_{20} procedure to a hormetic model of these data yields a chronic value of 1.98 or 1.76 µg/g (Figure 12). These are the best data presently available for rainbow trout.

The Federal Register notice for the Draft Criterion Document (Federal Register 69(242):75541-75546) states that EPA took into consideration dietary exposure for aquatic life. This should include the effect of selenium concentrations in prey tissue on aquatic predators, e.g. the effect of selenium concentrations in small fish on the bigger fish that eat the small fish. However, the Draft Criterion Document (EPA 2004) does not include such analysis. Analysis of the data included in Draft Criteria Document (EPA 2004: I-14) for effects of selenium in the diet of juvenile rainbow trout on their weight (Hilton et al. 1980) indicates that if these fish feed on tissue at the criterion level (in the form of sodium selenite), they will suffer a reduction in growth of about 34 percent (Figure 13). Because the form of selenium administered to the fish in this experiment was sodium selenite, this analysis may underestimate the adverse effects of the more bioavailable organic forms of selenium.

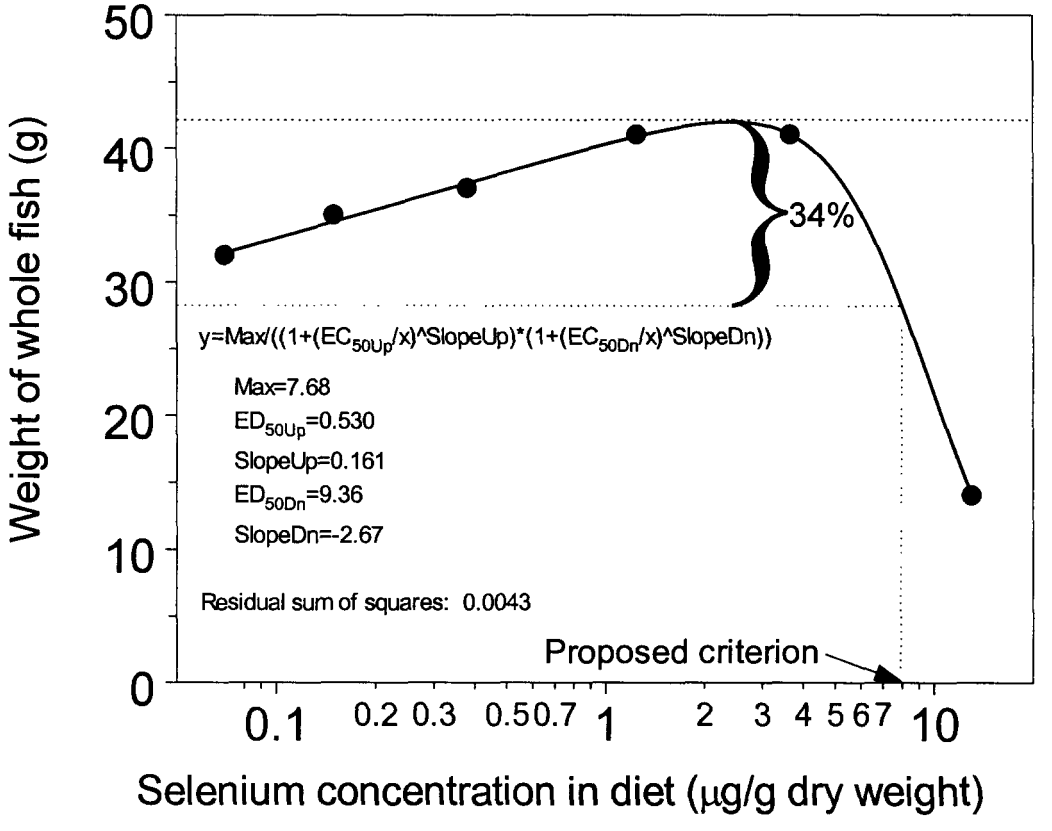

Figure 13. Average weights of juvenile rainbow trout after 20 weeks dietary exposure to sodium selenite (Hilton et al. 1980). A hormetic model is fitted to the data by least squares non-linear regression (Beckon et al. In Prep.).

Wet Weight/Dry Weight Conversions: Most of the wet weight to dry weight conversions for tissue concentrations of selenium are calculated with inappropriate estimates of percent moisture. For example, much of the most crucial analyses are focused on data from studies of bluegill, yet bluegill whole-body tissue was assumed to contain 80% moisture based on a single twenty-year-old Federal

Register citation (EPA 1985) which was meant to apply to edible filet tissue (not whole-body tissue) and is not taxon-specific for bluegill. However, taxon-specific data for percent moisture in bluegill whole-body tissue have been published (e.g., Saiki and May 1988:73.0% moisture; Saiki et al. 1992:74.7% moisture; Welsh and Maughan 1994:74.3%) and are consistent to an applied value of 75% moisture for fish whole-body tissue (e.g., Lemly 1996; Swift 2002; Holm et al. 2003; Hamilton 2004). In addition, national databases support the application of 75% moisture for fish whole-body tissue in the absence of taxon-specific information. National Contaminant Biomonitoring Program (NCBP) data (Walsh et al. 1977; May and McKinney 1981; Lowe et al. 1985; Schmitt and Brumbaugh 1990) yielded pooled estimates of 72% percent moisture in fish whole-body tissue (n=591 samples) in 1978-1981 and 74% (n=315 samples) in 1984. Data from the National Irrigation Water Quality Program for 57 species of freshwater fish revealed median percent moisture for whole-body tissue of 74.5% (Attachment 4).

The use of 80% moisture introduces a systematic 25% bias in the direction of overestimating species chronic values. For example, simply by using the appropriate percent moisture, the species chronic value EPA estimated from the intensely examined and re-analyzed study of bluegill by Hermanutz et al. (1996:Study II) would change downward from 12.12 ug/g to 9.70 ug/g (EPA 2004:81). Additionally, the tissue to tissue translation regressions are affected similarly which widely propagates inaccuracies related to percent moisture through much of the Draft Criteria Document's (EPA 2004) analyses.

7. *Significant New Data*: USFWS recently discovered a significant body of relevant data that we were previously unaware of and that we believe EPA has also not previously considered. The Department of Animal Science at the University of California-Davis conducted studies on the reproductive toxicity of selenium to Channel Catfish (*Ictalurus punctatus*) and bluegill (*Lepomis macrochirus*) for the California State Water Resources Control Board. The studies and their results are documented extensively in a final report to the State Water Board titled, "*Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River*" and is co-authored by Serge Doroshov, Joel Van Eenennaam, Christine Alexander, Erik Hallen, Howard Bailey, Kevin Kroll, and Camilo Restrepo (i.e., Doroshov et al. 1992; we provide a copy as Attachment 5 with the permission of Dr. Doroshov).

Channel Catfish Study: "Bioaccumulation of Selenium in Broodstock of Channel Catfish (*Ictalurus punctatus*) and its Effect on Reproduction" -- The objective of the channel catfish study was... "*...to determine effect of selenium bioaccumulation and yolkborne selenium concentration on the reproductive performance of broodstock and survival of resulting progeny.*" In summary selenium treatments did not affect vitellogenesis and ovarian development. Liver tissues exhibited rapid bioaccumulation and ovary tissue exhibited more delayed bioaccumulation of selenium. Eggs produced by treated females averaged 3.17 to 17.40 ug/g Se (dry wgt.) as compared to 2.85 ug/g Se in control eggs. Spawning response of experimental groups ranged from 23 to 40% with no statistically significant dose trend. Similarly, no significant differences were found for the endpoints of weight and relative weight of spawned egg masses. Fertilization success estimated at 48 hours was similar in all experimental groups.

Pre-hatch embryo mortality was significantly elevated in the two highest treatment groups of eggs which averaged 6.34 and 17.40 ug/g Se (dry wgt.). These two treatment groups exhibited 32% and

96% embryo mortality compared to 10% in controls and 5% (consistent with hormesis) in the lowest treatment group. Morphometrics of embryos that hatched did not differ between treatments. In the high treatment group only one of three egg batches produced hatchable embryos and most of those died between hatching and swim-up stage. The reported NOEC for egg selenium was 3.2 ug/g (dry wgt.) and the reported LOEC was 6.3 ug/g (dry wgt.). The LOEC on a control-adjusted basis was equivalent to about a 25% level of adverse effect (embryo death).

If these results are taken at face value they would indicate that Channel Catfish are more sensitive to selenium than any of the species currently included in EPA's database. Direct interpretation, for EPA's purposes, is complicated by two factors. First, whole-body selenium concentrations were not measured. Employing EPA's translation equation for ovary tissue, and the LOEC concentration of 7 ug/g for ovary tissue in this study, yields a whole-body equivalency value of 3.26 ug/g Se. EPA's translation equation is based on bluegill data with unknown relevance to channel catfish. However, EPA does not confine its use of translation equations to the taxa whose data are the basis for the equations (EPA 2004).

Secondly, Se treatments in this study consisted of exposure to seleno-L-methionine via injection directly into the bloodstream of broodstock fish rather than via dietary exposure. The authors contend that with respect to deposition of selenium into adult tissues and eggs, the only difference between this exposure route and a dietary exposure route would be the bypassing of gut transmission of selenium to the bloodstream. If the authors' contention is correct, then the form of selenium in the catfish eggs would be no different than studies based on dietary exposure to seleno-L-methionine.

Independent of potential uncertainty related to route of exposure, and more important from USFWS' perspective, is the fact that significant pre-hatch embryo mortality occurred in this experiment. This is unusual for fish studies. Doroshov et al. (1992) point out that catfish produce relatively large and yolky eggs. Therefore, unlike most other fish taxa, catfish embryos complete major organogenesis and draw more substantively upon yolkborne selenoproteins **before** they hatch. Dr. Doroshov suggests (Pers. comm.) that this different progression for selenium exposure is categorically more sensitive. With that very plausible possibility in mind, USFWS notes that EPA's database does not include reproductive endpoint data for any taxon reproductively comparable to catfish (i.e., that produce catfish-like eggs). Given the small number of fish species tested to date, and the possibility that the most sensitive reproductive category of fish is as yet unrepresented, we note that conservative treatments of the limited data we have would therefore be scientifically well justified.

Bluegill Study: "Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (*Lepomis macrochirus*)" -- The objective of the bluegill study was to..."*...determine tissue selenium concentrations in adult bluegill, Lepomis macrochirus Rafinesque, critical to normal reproduction.*" In summary, treatment diets contained measured concentrations of 5.5, 13.9, and 21.4 ug/g Se (as seleno-L-methionine; dry wgt.). Control diets with nominally paired additions of L-methionine for each Se-L-methionine treatment level contained measured concentrations of 1.1, 1.6, and 1.2 ug/g Se (dry wgt.).

No apparent differences were observed in fish behavior. No significant differences in fork length or body weight were measured. Testes accumulated less selenium compared to ovaries, but liver

accumulation was similar in both sexes. The dry matter content of ovaries was highly variable and related to the reproductive cycle. No histological differences were observed for post-spawning gonad tissues. No difference in fertilization success were measured.

Larval effects (edema) were observed for the 13.9 and 21.4 ug/g Se dietary treatments. For the 21.4 ug/g Se treatment more than 95% of larvae died before day 16 post-hatch. Low (5.5 ug/g Se) and medium (13.9 ug/g Se) treatments exhibited only slight increase in larval mortality to day 16. Overt larval abnormalities were observed, but were not clearly related to dose levels. For the endpoint of reproductive failure, and based on egg tissue, the reported NOEC and LOEC selenium concentrations were 8.3 and 19.5 ug/g Se (dry wgt.). That would yield a chronic value for this study of 12.7 ug/g.

Again, whole-body selenium concentrations were not measured. Ovary concentrations were measured, but only for stripped ovaries, which the authors report as likely lowering the selenium content of the ovaries. Thus, to translate the results of this study to a whole-body equivalency, 12.7 ug/g Se would have to be viewed as the best measure of selenium concentrations for unstripped ovaries. Using EPA's ovary translation equation, the whole-body equivalency chronic value would be 5.9 ug/g Se (dry wgt.). As presented earlier in these review comments, USFWS prefers to compare the egg chronic value from this study directly to data from Coyle et al. (1993) because EPA's translation regression is based largely on data from Hermanutz et al. (1996) for which there was no scientifically defendable basis for making wet weight to dry weight conversions. Based on data for bluegill presented by Coyle et al. (1993), who directly measured dry weight concentrations, a chronic value of 12.7 ug/g Se (dry wgt.) in eggs/ovary would be equivalent to a whole-body chronic value of about 4.9 ug/g Se (dry wgt.).

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ATTACHMENT 1.

LEMLY WINTER-STRESS STUDY
RAW DATA

Treatment Day	Cold +Se Fish at Start	Cold + Se Fish at End	Cold + Se Fish Exposure Days	Cold +Se Dead Fish	Daily Survival	Cumulative Mortality	Cold Control Fish at Start	Cold Control Fish at End	Cold Control Fish Exposure Days	Cd Control Dead Fish	Daily Survival	Cumulative Mortality
1	210	210	210	0	1	0	70	70	70	0	1	0
2	210	210	210	0	1	0	70	70	70	0	1	0
3	210	210	210	0	1	0	70	70	70	0	1	0
4	210	210	210	0	1	0	70	70	70	0	1	0
5	210	210	210	0	1	0	70	70	70	0	1	0
6	210	210	210	0	1	0	70	70	70	0	1	0
7	210	210	210	0	1	0	70	70	70	0	1	0
8	210	210	210	0	1	0	70	70	70	0	1	0
9	210	210	210	0	1	0	70	70	70	0	1	0
10	210	210	210	0	1	0	70	70	70	0	1	0
11	210	210	210	0	1	0	70	70	70	0	1	0
12	210	210	210	0	1	0	70	70	70	0	1	0
13	210	210	210	0	1	0	70	70	70	0	1	0
14	210	210	210	0	1	0	70	70	70	0	1	0
15	210	210	210	0	1	0	70	70	70	0	1	0
16	210	210	210	0	1	0	70	70	70	0	1	0
17	210	210	210	0	1	0	70	70	70	0	1	0
18	210	210	210	0	1	0	70	70	70	0	1	0
19	210	210	210	0	1	0	70	70	70	0	1	0
20	210	209	209.5	1	0.995238	0.004762	70	70	70	0	1	0
21	209	209	209	0	1	0.004762	70	70	70	0	1	0
22	209	209	209	0	1	0.004762	70	70	70	0	1	0
23	209	209	209	0	1	0.004762	70	70	70	0	1	0
24	209	209	209	0	1	0.004762	70	70	70	0	1	0
25	209	209	209	0	1	0.004762	70	70	70	0	1	0
26	209	209	209	0	1	0.004762	70	70	70	0	1	0
27	209	209	209	0	1	0.004762	70	70	70	0	1	0
28	209	209	209	0	1	0.004762	70	70	70	0	1	0
29	209	209	209	0	1	0.004762	70	70	70	0	1	0
30	209	209	209	0	1	0.004762	70	70	70	0	1	0
31	209	209	209	0	1	0.004762	70	70	70	0	1	0
32	209	209	209	0	1	0.004762	70	70	70	0	1	0
33	209	209	209	0	1	0.004762	70	70	70	0	1	0
34	209	208	208.5	1	0.995215	0.009524	70	70	70	0	1	0
35	208	208	208	0	1	0.009524	70	70	70	0	1	0
36	208	208	208	0	1	0.009524	70	70	70	0	1	0
37	208	208	208	0	1	0.009524	70	70	70	0	1	0
38	208	208	208	0	1	0.009524	70	70	70	0	1	0
39	208	208	208	0	1	0.009524	70	70	70	0	1	0
40	208	207	207.5	1	0.995192	0.014286	70	70	70	0	1	0
41	207	207	207	0	1	0.014286	70	70	70	0	1	0
42	207	207	207	0	1	0.014286	70	70	70	0	1	0
43	207	207	207	0	1	0.014286	70	70	70	0	1	0
44	207	207	207	0	1	0.014286	70	70	70	0	1	0
45	207	207	207	0	1	0.014286	70	70	70	0	1	0
46	207	206	206.5	1	0.995169	0.019048	70	70	70	0	1	0
47	206	205	205.5	1	0.995146	0.02381	70	70	70	0	1	0
48	205	205	205	0	1	0.02381	70	70	70	0	1	0
49	205	205	205	0	1	0.02381	70	70	70	0	1	0
50	205	204	204.5	1	0.995122	0.028572	70	70	70	0	1	0
51	204	204	204	0	1	0.028572	70	70	70	0	1	0

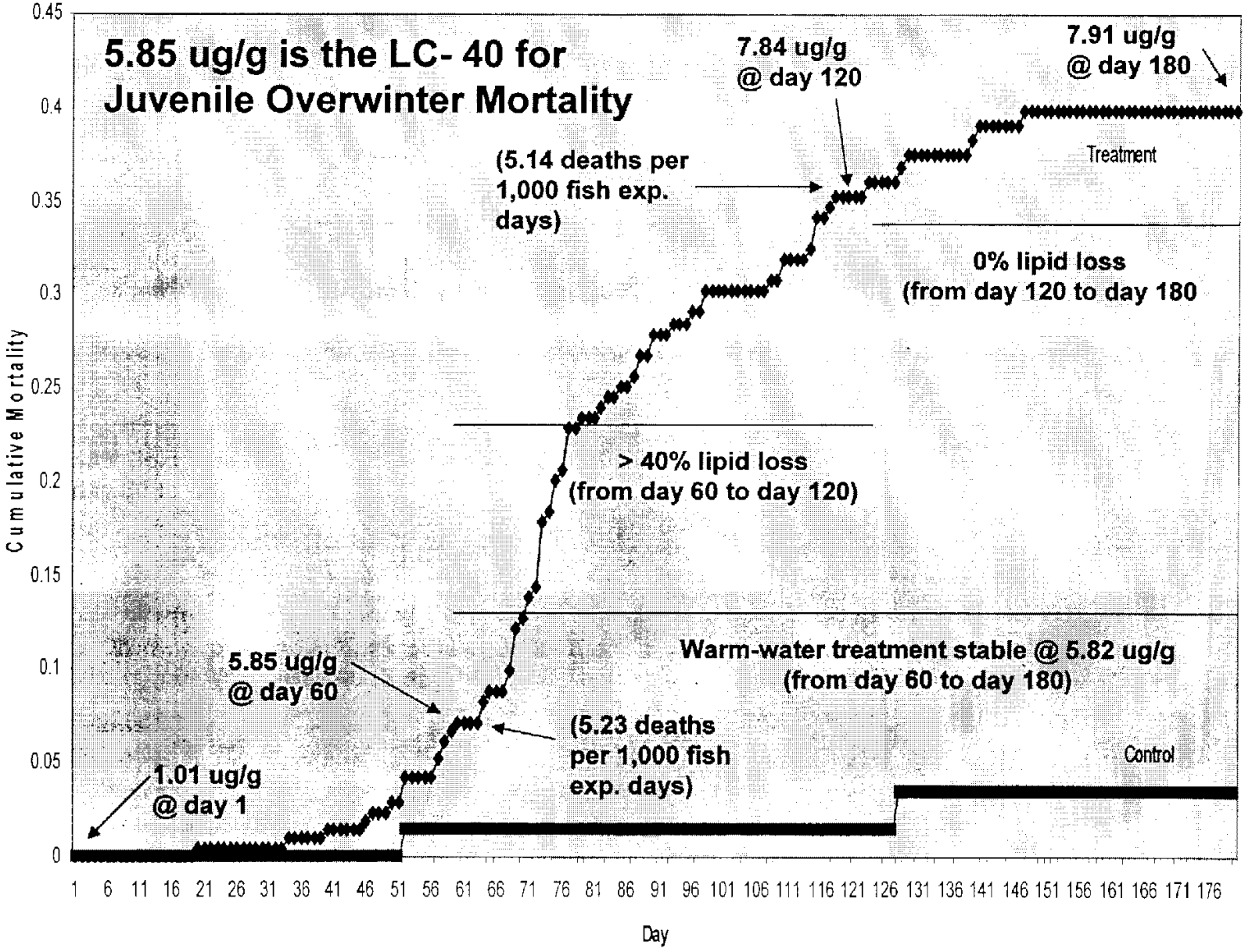
52	204	201	202.5	3	0.985294	0.042858	70	69	69.5	1	0.985714	0.014286
53	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
54	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
55	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
56	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
57	201	199	200	2	0.99005	0.052381	69	69	69	0	1	0.014286
58	199	197	198	2	0.98995	0.061905	69	69	69	0	1	0.014286
59	197	196	196.5	1	0.994924	0.066667	69	69	69	0	1	0.014286
60	196	195	195.5	1	0.994898	0.071429	69	69	69	0	1	0.014286
Whole-body Se = 5.85 ug/g			**30 fish removed**						**10 fish removed**			
61	165	165	165	0	1	0.071429	59	59	59	0	1	0.014286
62	165	165	165	0	1	0.071429	59	59	59	0	1	0.014286
63	165	165	165	0	1	0.071429	59	59	59	0	1	0.014286
64	165	163	164	2	0.987879	0.082684	59	59	59	0	1	0.014286
65	163	162	162.5	1	0.993865	0.088312	59	59	59	0	1	0.014286
66	162	162	162	0	1	0.088312	59	59	59	0	1	0.014286
67	162	162	162	0	1	0.088312	59	59	59	0	1	0.014286
68	162	160	161	2	0.987654	0.099567	59	59	59	0	1	0.014286
69	160	156	158	4	0.975	0.122078	59	59	59	0	1	0.014286
70	156	155	155.5	1	0.99359	0.127706	59	59	59	0	1	0.014286
			3825	20					1421.5	1		
			5.23 mortalities per 1,000 Exp. Days						**0.703 mortalities per 1,000 Exp. Days**			
71	155	153	154	2	0.987097	0.138961	59	59	59	0	1	0.014286
72	153	152	152.5	1	0.993464	0.144589	59	59	59	0	1	0.014286
73	152	146	149	6	0.960526	0.178355	59	59	59	0	1	0.014286
74	146	145	145.5	1	0.993151	0.183983	59	59	59	0	1	0.014286
75	145	142	143.5	3	0.97931	0.200866	59	59	59	0	1	0.014286
76	142	141	141.5	1	0.992958	0.206494	59	59	59	0	1	0.014286
77	141	137	139	4	0.971631	0.229005	59	59	59	0	1	0.014286
78	137	137	137	0	1	0.229005	59	59	59	0	1	0.014286
79	137	136	136.5	1	0.992701	0.234632	59	59	59	0	1	0.014286
80	136	136	136	0	1	0.234632	59	59	59	0	1	0.014286
81	136	136	136	0	1	0.234632	59	59	59	0	1	0.014286
82	136	135	135.5	1	0.992647	0.24026	59	59	59	0	1	0.014286
83	135	134	134.5	1	0.992593	0.245888	59	59	59	0	1	0.014286
84	134	134	134	0	1	0.245888	59	59	59	0	1	0.014286
85	134	133	133.5	1	0.992537	0.251515	59	59	59	0	1	0.014286
86	133	133	133	0	1	0.251515	59	59	59	0	1	0.014286
87	133	132	132.5	1	0.992481	0.257143	59	59	59	0	1	0.014286
88	132	130	131	2	0.984848	0.268399	59	59	59	0	1	0.014286
89	130	130	130	0	1	0.268399	59	59	59	0	1	0.014286
90	130	128	129	2	0.984615	0.279654	59	59	59	0	1	0.014286
91	128	128	128	0	1	0.279654	59	59	59	0	1	0.014286
92	128	128	128	0	1	0.279654	59	59	59	0	1	0.014286
93	128	127	127.5	1	0.992188	0.285282	59	59	59	0	1	0.014286
94	127	127	127	0	1	0.285282	59	59	59	0	1	0.014286
95	127	127	127	0	1	0.285282	59	59	59	0	1	0.014286
96	127	126	126.5	1	0.992126	0.290909	59	59	59	0	1	0.014286
97	126	126	126	0	1	0.290909	59	59	59	0	1	0.014286
98	126	124	125	2	0.984127	0.302165	59	59	59	0	1	0.014286
99	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
100	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
101	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
102	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
103	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
104	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286

105	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
106	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
107	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
108	124	123	123.5	1	0.991935	0.307792	59	59	59	0	1	0.014286
109	123	123	123	0	1	0.307792	59	59	59	0	1	0.014286
110	123	121	122	2	0.98374	0.319048	59	59	59	0	1	0.014286
111	121	121	121	0	1	0.319048	59	59	59	0	1	0.014286
112	121	121	121	0	1	0.319048	59	59	59	0	1	0.014286
113	121	121	121	0	1	0.319048	59	59	59	0	1	0.014286
114	121	120	120.5	1	0.991736	0.324676	59	59	59	0	1	0.014286
115	120	117	118.5	3	0.975	0.341559	59	59	59	0	1	0.014286
116	117	117	117	0	1	0.341559	59	59	59	0	1	0.014286
117	117	116	116.5	1	0.991453	0.347186	59	59	59	0	1	0.014286
118	116	115	115.5	1	0.991379	0.352814	59	59	59	0	1	0.014286
119	115	115	115	0	1	0.352814	59	59	59	0	1	0.014286
120	115	115	115	0	1	0.352814	59	59	59	0	1	0.014286
Whole-body Se = 7.84 ug/g			**30 fish removed**						**10 fish removed**			
121	85	85	85	0	1	0.352814	49	49	49	0	1	0.014286
122	85	85	85	0	1	0.352814	49	49	49	0	1	0.014286
123	85	84	84.5	1	0.988235	0.360428	49	49	49	0	1	0.014286
124	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
125	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
126	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
127	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
128	84	83	83.5	1	0.988095	0.368042	49	48	48.5	1	0.979592	0.034403
129	83	82	82.5	1	0.987952	0.375656	48	48	48	0	1	0.034403
130	82	82	82	0	1	0.375656	48	48	48	0	0.034403	0.034403
			2141.5	**11**					**1136.5**	**1**		
			5.14 mortalities per	**1,000 Exp. Days**					**0.88 mortalities per**	**1,000 Exp. Days**		
131	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
132	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
133	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
134	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
135	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
136	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
137	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
138	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
139	82	81	81.5	1	0.987805	0.38327	48	48	48	0	1	0.034403
140	81	80	80.5	1	0.987654	0.390884	48	48	48	0	1	0.034403
141	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
142	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
143	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
144	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
145	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
146	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
147	80	79	79.5	1	0.9875	0.398498	48	48	48	0	1	0.034403
148	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
149	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
150	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
151	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
152	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
153	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
154	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
155	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
156	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
157	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403

158	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
159	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
160	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
161	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
162	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
163	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
164	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
165	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
166	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
167	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
168	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
169	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
170	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
171	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
172	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
173	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
174	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
175	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
176	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
177	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
178	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
179	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
180	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
Whole-body Se = 7.91 ug/g			**30 fish removed**						**10 fish removed**			
TOTALS			**25321.5**	**71**		**0.398498**			**10,619.0**	**2**		**0.034403**

Note: Exposure Days were quantified based on the assumption that fish died at mid-day.

Attachment 2: Lemly winter-stress results



ATTACHMENT 3.

MATCHED SAMPLES OF FISH AND AQUATIC INVERTEBRATES FROM SAMPLING SITES WHERE THE FISH TISSUE SAMPLES AVERAGED 5-10 ug/g SELENIUM, DRY WEIGHT

Location	Invertebrate Selenium	Fish Selenium	Implied Concentration Factor
Colorado	4.8 ug/g	5.3 ug/g	1.10
Utah	4.4	6.0	1.36
Utah	4.4	5.2	1.18
Utah	8.2	10	1.22
Utah	8.4	9.4	1.12
Utah	7.6	5.7	0.75
Utah	6.9	6.7	0.97
Montana	4.8	6.1	1.32
Montana	9.2	5.3	0.67
Median Concentration Factor			1.12
Average Concentration Factor			1.08

Source: National Irrigation Water Qualtiy Program biota database (4, 76)

ATTACHMENT 4.
Summary of Percent Moisture Data From NIWQP Database for Fish Whole-body Samples (asce

Fish Species Common Name	Percent Moisture	No. of Samples
Tahoe Sucker	68.4	1
Goldeye	69.3	25
Cutthroat Trout	69.7	5
Flannelmouth Sucker	70.3	175
Longnose Sucker	70.4	39
Mountain Whitefish	70.6	6
Shorthead Redhorse	70.9	74
Utah Chub	71	11
Speckled Dace	71	193
Gizzard Shad	71.3	9
Sauger	71.4	5
Longnose Dace	71.4	8
Bluehead Sucker	71.8	54
Squawfish	72	1
River Carpsucker	72.5	9
Sacramento Perch	72.6	2
Flathead Chub	72.9	32
Northern Squawfish	73.1	1
Brown Trout	73.2	51
Redear Sunfish	73.8	1
Hitch	73.8	7
Bairdiella	74	5
Utah Sucker	74.1	6
Sailfin Molly	74.1	6
Rainbow Trout	74.2	39
Yellow Perch	74.3	50
Channel Catfish	74.4	78
White Sucker	74.5	82
Walleye	74.5	15
Smallmouth Bass	74.6	21
White Bass	74.6	6
Tui Chub	74.8	36
Sunfish	75	3
Pumpkinseed	75.2	1
Green Sunfish	75.2	60
Red Shiner	75.2	14
Largemouth Bass	75.4	14
Common Carp	75.8	165
Mottled Sculpin	75.8	34
Roundtail Chub	75.9	100
Stonecat	76	4
Redside Shiner	76	1
White Crappie	76.1	7
Plains Killifish	76.1	6
Bluegill	76.1	5
Black Crappie	76.2	23

Brook Stickleback	76.6	13
Brassy Minnow	76.8	4
Fathead Minnow	77.6	97
Mosquitofish	77.6	54
Bullhead	78.3	23
Northern Pike	78.4	7
Sacramento Blackfish	79	2
Longjaw Mudsucker	80.2	1
Black Bullhead	80.8	75
Brown Bullhead	81.6	5
Northern Redbelly Dace	82.9	1

nding order)

Notes

25th Percentile Value = 72.25
25th Percentile Value = 72.25

Genus Lepomis

50th Percentile Value

Genus Lepomis
Genus Lepomis
Genus Lepomis

75th Percentile Value
75th Percentile Value
Genus Lepomis

EPA uses 80% moisture for all species!!

[ATTACHMENT 5]

FINAL REPORT (DRAFT)

Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River.

for

State Water Resources Control Board

State of California

Contract Number 7-197-250-0

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September, 1992

PART I. Bioaccumulation of Selenium in Broodstock of Channel Catfish (Ictalurus punctatus) and its effect on reproduction.

TABLE OF CONTENTS: PART I

A. INTRODUCTION

Selenium has been implicated in reproductive failure of fish and waterfowl in polluted aquatic systems. In particular, certain areas of lower San Joaquin River were severely affected by selenium pollution. State Board Order 85-1 addressed waterfowl problems at Kesterson Reservoir resulting from selenium laden water discharged to the facility. State Board staff and San Joaquin River Basin Technical Committee developed water quality criteria for nine constituents and proposed objectives for three of them, selenium, boron, and molybdenum. The toxicity data upon which the criteria and objectives are based are not adequate in that site-specific toxicity data were generally not available. The objective of this contract is to provide additional selenium toxicity data for two resident species of fish, channel catfish and bluegill.

Species investigated in the first part of the study is channel catfish, Ictalurus punctatus. The objective was to determine effect of selenium bioaccumulation and yolkborne selenium concentration on the reproductive performance of broodstock and survival of resulting progeny. Commonly, in experiments with small size laboratory fish, selenium bioaccumulation is induced by dietary treatment. However, due to the large size of catfish broodstock and the prolonged period required for ovarian bioaccumulation we utilized an alternative selenium delivery method: by repeated injections of selenoaminoacid. Introduction of exogenous selenium directly into the blood stream bypasses the assimilation via gut absorption.

B. MATERIALS AND METHODS

1. Broodstock

Channel catfish broodstock were obtained from farm ponds (Fishery Inc., Galt, California) in January-March 1989. Fish were visually sexed and females 3-4 year old, ranged in body weight from 1.1 to 3.4 kg, average weight 2 kg. During the experimental treatment before spawning, the fish were held in six foot diameter (1400 L volume) fiberglass tanks located outside. The initial stocking density was 60 fish (120 kg) per tank. Tanks were supplied with flow-through water with temperature ranging 13-21°C. Water quality parameters are summarized in Appendix 1. Fish were fed Silvercup trout diets (Murray Elevators, Utah), 1 to 2 % body weight per day. Separate tanks were used for each experimental treatment. The additional stock of sexed males was obtained before the spawning season, in May. Males were not treated and were kept in separate tanks.

2. Experimental Protocol.

2.1. Range Finding

This study was conducted in January-February 1989. Three untreated females were sampled on Day 0 (muscle, liver, ovaries, and blood plasma). The remaining fish were injected with L-methionine (L-Met) or seleno-L-methionine (Se-L-Met, Sigma Chemical) at dose levels 0.25, 2.5, and 25 mg/kg body weight. On Day 14, one fish from each control dose and 2 fish from each selenium treatment were sampled. Half of the remaining fish (3 in control and 2 in each selenium treatment) were injected a second time, similar to the first dose. The other half did not receive a second injection. All females were sampled on Day 28, half were injected once (Day 0) and the other half were injected twice (Days 0 and 14). Tissue samples were analyzed for selenium concentrations.

2.2. Bioaccumulation Treatment

Treatment by intramuscular injections was initiated on March 14 (Day 0). Populations were randomly assigned to six tanks. Six untreated females were sacrificed and sampled on Day 0. All remaining females received biweekly injections of L-Met and Se-L-Met at doses 0.02, 0.2, and 2.0 mg/kg body weight. Six injections total were given in each treatment on Days 0, 14, 28, 42, 56, and 70 (Table 1). On each day 2 fish from each control and 3 fish from each selenium treatment were randomly sampled for ovarian and liver selenium burden (actual number of sampled fish or analyzed tissue varied from 1 to 4). The last sampling was conducted on Day 84 (June 5).

2.3. Spawning

The remaining females (all injected 6 times) were used for spawning with untreated males. Spawning trials were conducted from June 5 to June 24 in five consecutive sessions, with 12 randomly chosen females (3 control and 3 in each selenium treatment dose) in each session (Table 1). Spawned females were sacrificed and sampled for tissue selenium analysis. The egg masses were weighed, treated in iodophore (10%) to prevent fungal infection and placed in catfish hatchery incubators. A core sample was taken for selenium analysis. At 48 hours after fertilization, two additional samples were taken from each egg mass: one for microscopic examination of fertilization success, and another for the embryo-larval bioassay. To separate the eggs from their adhesive matrix, samples were bathed in a 1% sodium sulfite solution. Bioassays

were conducted with two replications for each progeny (except for one with no replication) for 28 days. Survived fry were counted, weighed and measured. A more detailed description of each procedure follows.

Table 1. Treatment/spawning schedules, and number of sampled catfish females. Step: INJ - sampling and injection of remaining fish with treatment dose; SPAWN - spawning and sampling.

DATE	DAY	STEP	CONTROL L-Met mg/kg			TREATMENT Se-L-Met mg/kg		
			0.02	0.2	2.0	0.02	0.2	2.0
Mar 14	0	INJ	6 (untreated)					
Mar 28	14	INJ	2	2	2	3	3	2
Apr 11	28	INJ	2	2	2	3	3	2
Apr 25	42	INJ	2	2	2	3	3	3
May 9	56	INJ	2	2	1	3	3	2
May 23	70	INJ	2	2	2	1	1	1
Jun 5	84	-	2	4	3	2	2	1
Jun 7	86	SPAWN	1	1	1	3	3	2
Jun 11	90	SPAWN	1	1	1	3	3	3
Jun 15	94	SPAWN	1	1	1	3	3	3
Jun 19	98	SPAWN	1	1	1	3	3	3
Jun 24	103	SPAWN	1	1	1	3	3	3
		Total:	17	19	17	30	30	25

2.4. Tissue Sampling and Selenium Analysis

Fish were selected by random numbers. Approximately 15 ml of blood was collected by vacutainer, plasma was separated by centrifugation at 3000 rpm, distributed in plastic vials and stored frozen at -20°C. Fish was sacrificed with a blow to the head, weighed and measured. Tissue samples were divided into two subsamples, weighed, rinsed in distilled water, and frozen in plastic bags for selenium analysis. White muscle samples were collected from the filet on the left side of the fish (skin and red muscle tissue were removed). Duplicate tissue samples were used for selenium analysis on a wet weight basis, and for dessication (lyophilization) to determine dry matter content for

conversion of selenium concentrations to dry weight (both wet and dry weight data were used for data analysis). All selenium analyses were conducted by California Veterinary Diagnostic Laboratory System, Veterinary Medicine, UC Davis. Samples were analyzed by the ICP atomic emission, using hydride generation. Analytical detection limit of method is 0.005 µg/g for tissue selenium. The details of this method and the quality control protocol are described in the report of CVDLS (Ardans et al.,1988).

2.5. Plasma Protein Phosphorus Analysis

Plasma alkali-labile protein phosphorus (ALPP) measures relative concentration of plasma yolk precursor, vitellogenin. The technique is based on precipitation of plasma proteins, liberation of protein (mainly vitellogenin) -bound phosphorus, and measuring phosphorus concentrations by colorimetry. Plasma ALPP concentrations increase with progression of vitellogenesis (synthesis of vitellogenin by liver and deposition of proteins into the egg yolk) and decrease around spawning time. With some minor modifications, we used the technique described by Wallace and Jared (1968) and de Vlaming et al.(1984).

2.6. Spawning Induction Procedure

Fish were injected IM with human chorionic gonadotropin (hCG), females with 1800 IU and males with 600 IU per kg body weight. Each pair was put in a rectangular 6 x 2 x 2 foot tank supplied with flow-through water at a constant temperature 26°C. Each tank had a spawning container. Fish were allowed to spawn for 72 hours, and containers were observed at regular intervals for mating and spawning. Date and time of oviposition were recorded. The egg mass was weighed and placed in a water bath of 10% iodophore solution for 2 minutes and then put in a wire basket in a standard paddlewheel catfish egg incubator.

2.7. Embryo-Larval Bioassay

Two replicate samples of thirty eggs from each egg mass were placed into a glass petri dish (100x15mm) and submerged into a 21 L rectangular glass aquaria, supplied with constant flow of underground water from a campus well (Hardness 225-300 mg/L $CaCO_3$). Temperature was maintained constant within the range 24 - 26°C. Other water quality parameters are summarized in the Appendix 2. Incubation of eggs and rearing of fry continued for 28 days. The aquaria were examined daily and mortalities were removed and recorded. Starting from the completion of yolk sac absorption and swimup stage (Days 10-11), fry were fed ad libitum a

commercial salmon diet (Biodiet, Bio-Products Inc). The fry were starved for 24 hours before final sampling on Day 28. Wet body weight was measured on an electronic balance (0.01 mg) and total length was measured on a measuring board (1 mm).

3. Data Analysis

Relative weight of ovaries and liver were expressed as gonadosomatic (GSI) or hepatosomatic (HSI) indices, in percent of whole body weight. All proportion data were transformed into the arcsine-roots before statistical analysis. Selenium concentrations were transformed into $\log_{10}$ values.

Differences between control groups, and between pooled control and treatment groups were tested by one-way analysis of variance and Dunnett's procedure, at the probability level 95%. Linear regression analysis was used to examine relationships between selenium concentrations in different tissues. For the estimation of LC_{50}, we used the trimmed Spearman-Karber method.

C. RESULTS

1. Range Finding

The females injected with 25 mg Se-L-Met/kg BW died from acute selenosis (edema, paralysis, and strong odor) within four hours after injection. Controls, 0.25 and 2.5 mg/kg selenium treatments were not affected: they survived to Day 28 and were sampled on Days 14 and 28. Extra fish injected with intermediate Se-L-Met doses (6.2; 10.0, and 17.5 mg/kg) survived for 8 days, 27 hours, and 18 hours after injection, respectively. These fish also suffered acute selenosis. A single female injected with dose 4.4 mg/kg died in 6 days after injection, with extensive hemorrhages of fins and skin. Death of this fish might have been caused by transportation and handling.

Tissue selenium concentrations of sampled fish are shown in Table 2. There was no significant effect of control treatment (carrier amino acid) on tissue selenium level. Concentrations of selenium in liver, muscle and plasma of fish sampled on Day 14 exhibited significant increase after one injection of 2.5 mg/kg Se-L-Met. Liver and muscle selenium concentrations in this treatment group remained significantly elevated on Day 28. Fish that received two consecutive injections of 2.5 mg/kg Se-L-Met exhibited significantly elevated selenium levels in all four tissues sampled, including the ovaries. Selenium treatment dose 0.25 mg/kg did not result in bioaccumulation, although plasma

selenium concentration was slightly elevated compared with control. We concluded from the range-finding experiment that Se-L-Met injections at doses higher than 2.5 mg/kg produce acute effect on catfish broodstock, and the selenium bioaccumulation is likely to occur at the dose range 0.25-2.5 mg/kg.

2. Reproductive Indices and Plasma Protein Phosphorus (ALPP)

Data on GSI and HSI in fish sampled biweekly during the 84 day period of treatment are shown in Table 3. The GSI increased from 6% at Day 0 (March) to 7-12% on Day 84 (June). The HSI exhibited some increase during the sampling period (Days 28-56) and decreased before spawning (Day 84). Overall changes in GSI and HSI reflect normal reproductive profile of channel catfish female. No significant differences were detected between control and treatment groups, for both GSI and HSI (analysis of variance). However, there was substantial individual variability in the ovarian growth (characteristic of farmed catfish broodstock), and small sample size may not be adequate to detect the effect of selenium treatment.

Samples of plasma ALPP included more fish, particularly in control (Table 4). Data show increase in plasma vitellogenin level during April-May (Days 28-56), with no significant differences between control and treatment groups. In summary, observations on GSI, HSI, and plasma protein phosphorus suggest that selenium treatment did not affect vitellogenesis and ovarian development.

Table 2. Tissue selenium concentrations (µg/g, wet weight) in range-finding experiment with channel catfish. Fish were injected once, on Day 0 (x1), or twice, on Day 0 and Day 14 (x2). Data are means and SEM. Asterisks indicate treatments significantly different from their respective controls (L-Met). All control doses (0.25-25 ppm) are pooled).

TREATMENT	GONADS	LIVER	MUSCLE	PLASMA
	Sampled on Day 0:			
Untreated (n=3)	1.65 ±0.13	1.45 ±0.05	0.15 ±0.01	0.22 ±0.02
	Sampled on Day 14:			
L-Met (n=3)	1.76 ±0.20	1.95 ±0.25	0.12 ±0.01	0.26 ±0.01
Se-L-Met 0.25 (n=2)	1.93 ±0.21	2.25 ±0.08	0.15 ±0.01	0.26 ±0.02
Se-L-Met 2.5 ±0.07 (n=2)	3.13 ±0.96	5.17 ±0.38 *	0.66 ±0.06 *	0.78 *
	Sampled on Day 28:			
L-Met (n=6)	2.02 ±0.46	1.92 ±0.20	0.14 ±0.01	0.22 ±0.01
Se-L-Met 0.25 (x1) (n=2)	0.92 [1)]	2.59 ±0.08	0.15 ±0.01	0.28 ±0.01
Se-L-Met 0.25 (x2) (n=2)	1.79 ±0.15	3.02 [1)]	0.20 ±0.01	0.32 ±0.01
Se-L-Met 2.5	2.41 ±0.04	4.87 ±0.15 *	0.67 ±0.07 *	0.53 ±0.01

(x1) (n=2)				
Se-L-Met 2.5 (x2) (n=2)	4.79 ±1.27 *	9.55 ±0.51 *	1.59 ±0.12 *	1.27 ±0.36 *

1) n=1

Table 3. Gonadosomatic (GSI) and hepatosomatic (HSI) indices of channel catfish. Data are x ± s.e.m., sample size in parentheses.

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
		GSI		
0	5.9 ±1.0 (6)	- sample on Day 0, untreated fish		
14	5.3 ±1.5 (6)	3.6 ±1.0 (3)	2.5 ±1.8 (3)	6.9 ±0.5 (2)
28	N/A	1.6 ±0.9 (2)	6.2 ±0.7 (3)	3.8 ±2.4 (2)
42	5.4 ±1.4 (6)	6.3 ±0.8 (3)	5.4 ±1.6 (3)	3.3 ±1.5 (3)
56	6.7 ±1.5 (5)	5.4 ±1.7 (3)	7.5 ±1.4 (3)	1.6 ±0.7 (2)
70	7.3 ±0.7 (6)	6.5 (1)	10.0 (1)	5.2 (1)
84	8.1 ±1.6 (9)	8.1 ±1.3 (2)	11.9±1.0 (2)	7.0 (1)
		HSI		
0	0.9 ±0.1 (6)	- sample on Day 0, untreated fish		
14	1.0 ±0.1 (6)	1.1 ±0.1 (3)	0.8 ±0.1 (3)	1.1 ±0.3 (2)
28	N/A	1.1 ±0.2 (2)	1.9 ±0.2 (3)	1.1 ±0.2 (2)
42	1.2 ±0.1 (6)	1.2 ±0.1 (3)	1.4 ±0.2 (3)	1.2 ±0.1 (3)
56	1.0 ±0.1 (5)	1.3 ±0.1 (3)	1.4 ±0.2 (3)	0.9 ±0.1 (3)

70	1.2 ±0.1 (6)	1.0 (1)	1.2 (1)	1.1 (1)
84	1.1 ±0.1 (9)	1.1 ±0.1 (2)	1.1 ±0.1 (2)	1.1 (1)

Table 4. Plasma protein phosphorus (ALPP, µg/ml) of channel catfish. Data are x ± s.e.m., sample size in parentheses.

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
0	14 ± 3 (6)	- sample on Day 0, untreated fish		
14	18 ± 2 (9)	19 ± 3 (3)	9 ± 4 (3)	24 ± 13 (2)
28	58 ± 4 (9)	26 ±10 (2)	77 ±16 (3)	26 ± 16 (2)
42	64 ± 6 (9)	61 ± 7 (3)	51 ±10 (3)	58 ± 11 (3)
56	32 ± 5 (7)	34 ± 8 (3)	48 ± 4 (3)	34 ± 10 (2)
70	33 ± 6 (9)	42 (1)	21 (1)	11 (1)
84	41 ± 7 (9)	19 ± 2 (2)	37 ± 7 (2)	30 (1)

3. Tissue Selenium in Bioaccumulation Treatments

Ovarian and liver selenium concentrations are shown in Tables 5 and 6. Treatment 2.0 mg/kg Se-L-Met resulted in significant selenium bioaccumulation in the ovarian tissue after two injections (Day 28), and in the liver after the first injection (Day 14). Treatment 0.2 mg/kg resulted in significant increase

of liver selenium level after 6 weeks and 3 injections (Day 42). Data for the ovary in this treatment were less consistent: significantly elevated ovarian selenium levels were observed on Days 28, 56, and 84 for the wet weight tissue, and only on Day 84 for the dry weight. The discrepancies may relate to procedural errors, but most likely they resulted from individual variation in stages of gonadal development and different dry matter content, associated with vitellogenesis.

Treatment 0.02 mg/kg produced no significant effect on selenium bioaccumulation, although sample means were consistently higher than in control, and in one case (liver, Day 28) there was detectable ($P<0.05$) difference between control and treatment. Average concentrations of bioaccumulated selenium were similar between ovarian and hepatic tissues (Table 5 and 6). In summary, data indicate that repeated injections of 0.2 and 2.0 mg/kg Se-L-Met elicited rapid bioaccumulation response in liver, and delayed response in ovary. In this respect, the results were similar with observations in the range-finding experiment (see Table 2).

Table 5. Selenium content of catfish ovaries (µg/g). Data are x ± s.e.m., sample size in parentheses. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test).

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
		Wet Weight		
0	1.59±0.14(6)	- sample on Day 0, untreated fish		
14	1.50±0.14(6)	1.55±0.05(3)	1.32±0.10(3)	1.80±0.06(2)
28	1.33±0.06(6)	1.37±0.04(3)	1.94±0.17(3)*	5.40±0.80(2)*
42	1.45±0.14(6)	1.64±0.10(3)	2.26±0.15(3)*	9.19±1.87(3)*
56	1.48±0.07(5)	1.82±0.13(3)	2.13±0.10(3)	12.45±0.88(2)
70	1.42±0.07(6)	1.56 (1)	2.14 (1)*	8.20 (1)
84	1.23±0.10(3)	1.62±0.03(2)	2.46±0.07(2)	9.74 (1)

Dry Weight

0	3.99±0.42(6)	- sample on Day 0, untreated fish		
14	4.43±0.81(6)	4.38±0.27(3)	7.00±1.85(3)	4.03±0.01 (2) *
28	3.29±0.17(6)	5.70±1.83(3)	4.88±0.44(3)	29.66±13.74(2) *
42	4.32±0.76(6)	4.05±0.30(3)	6.01±0.70(3)	31.04±8.06 (3) *
56	3.96±0.34(5)	4.72±0.53(3)	5.25±0.29(3)	59.80±19.51(3)
70	3.47±0.18(6)	3.76 (1)	4.95 (1) *	22.40 (1)
84	3.00±0.28(3)	3.93±0.14(2)	5.80±0.22(2)	23.58 (1)

Table 6. Selenium content of catfish liver (µg/g). Data are x ± s.e.m., sample size in parentheses. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test).

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
		Wet Weight		
0	2.15±0.16(6)	- sample on Day 0, untreated fish		
14	1.63±0.22(6)	1.60±0.05(3) *	2.48±0.34(3)	3.90±0.32(2) * *
28	1.11±0.09(6)	1.84±0.33(3)	1.40±0.18(3) *	6.11±0.71(2) *
42	1.39±0.12(6)	1.65±0.25(3)	1.94±0.06(3) *	8.14±0.23(3) *
56	1.29±0.06(5)	1.59±0.37(3)	1.87±0.05(3)	10.75±0.25(2)
70	1.29±0.06(6)	1.29 (1)	1.29 (1) *	9.37 (1)
84	1.29±0.17(3)	1.54±0.05(2)	2.10±0.08(2)	10.30 (1)

Dry Weight

0	9.17±0.72(6)	- sample on Day 0, untreated fish		
14	6.57±1.04(6)	5.97±0.27(3) *	10.60±1.49(3)	16.56±2.36(2) *
28	4.15±0.40(6)	7.22±1.47(3)	5.55±0.80(3) *	25.90±3.07(2) *
42	5.45±0.50(6)	6.83±1.22(3)	7.93±0.31(3) *	34.53±1.29(3) *
56	4.85±0.23(5)	5.75±1.29(3)	7.07±0.19(3)	43.37±0.37(3)
70	5.22±0.24(6)	5.17 (1)	5.12 (1) *	41.67 (1)
84	5.73±0.81(3)	6.01±0.15(2)	8.86±0.25(2)	39.78 (1)

4. Selenium in Eggs and Tissues of Spawned Females

Spawning trials were conducted in five consecutive sessions, during the interval of time 16 to 33 days after the last, sixth, injection (see Table 1). The analysis of variance revealed that ovarian and liver selenium concentrations did not exhibit significant changes over time, e.g. there was no detectable tissue depuration during overall spawning. Therefore, observations for all female tissues and fertilized eggs were pooled within each treatment. Data analysis shows significantly elevated selenium concentrations in 0.2 and 2.0 mg/kg Se-L-Met treatments, for all sampled tissues (Table 7). Treatment 2.0 mg/kg exhibited 5 times higher selenium concentrations compared with control. No detectable effect was observed in 0.02 mg/kg treatment. Concentrations of selenium were highest in liver, intermediate in the ovary, and lowest in fertilized eggs, with significant linear relationships between different tissues. Linear regressions for the ovarian/liver and egg/liver selenium residues are shown in Figure 1.

Table 7. Selenium content of spawned catfish females and fertilized eggs (µg/g). Data are x ± s.e.m., sample size in parentheses. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test). First and second rows for each tissue are concentrations on wet and dry weight bases.

TREATMENT			
L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
Liver			
1.63±0.13 (15)	1.93±0.15 (15)	3.08±0.26 (15)*	8.80±0.79 (13)*
6.79±0.43 (15)	7.34±0.69 (15)	12.54±0.94 (15)*	34.30±3.61 (13)*
Ovary			
0.99±0.10 (15)	1.31±0.18 (15)	1.91±0.17 (15)*	5.56±0.62 (13)*

5.72±0.81 (15)	5.65±1.14 (15)	7.02±0.37* (15)	25.97±3.08* (13)

Eggs

0.46±0.04 (6)	0.55±0.04 (6)	0.99±0.04* (5)	2.96±0.31* (3)
2.85±0.15 (6)	3.16±0.17 (6)	6.34±0.35* (5)	17.40±1.61* (3)

5. Spawning Performance

Fifteen females were used for spawning trials in the pooled control and each of the 0.02 and 0.2 mg/kg treatments, and 13 females were used in 2.0 µg/kg treatment (one female jumped out of tank and was lost for spawning). Spawning response ranged from 23-40% (Table 8). There was no statistically significant difference in spawning response between control and selenium treatments (Fisher's Test, P>0.05). The trend of decreasing spawning response in higher dose selenium treatments, seen in Table 8, may be due to a higher proportions of fish with underdeveloped ovaries in the 2.0 mg/kg treatment group. No significant differences in weight and relative weight of spawned egg masses were detected between control and treatment groups. Fertilization success estimated at 48 hours was similar in all groups (Table 8).

Table 8. Spawning performance of channel catfish. Data are x ± s.d. (sample size is number of spawned females). L-Met and Se-dose are control and treatment.

	TREATMENT			
	L-Met	Se-0.02	Se-0.2	Se-2.0
Number of spawned females	6	6	5	3
Spawning response (%)	40	40	33	23
Weight of egg mass (g)	409 ±131	372 ±46	474 ±91	600 ±14

Weight of egg mass (%-body weight)	18 ± 5	24 ± 2	24 ± 5	28 ± 1
Fertilization success (%)	49 ±19	69 ±14	60 ±12	61 ±19

6. Embryo-Larval Survival and Growth

Average cumulative mortalities for each treatment are shown in Figure 1. Major mortalities were observed during the first week of bioassays. Hatching in all treatments occurred on Day 6 after fertilization, and the swimup stage (onset of exogenous feeding) on Days 11-12. High mortalities (>90%) were observed in 2.0 mg/kg selenium treatment before hatching. Only one out of three egg batches in this treatment produced hatchable embryos, and most of them died between hatching and swim-up stage. Embryos and newly emerged larvae had pale yellow color, contrasting with orange-red coloration of normal embryos (possibly, circulatory system or blood pigments were affected, but no microscopic examination was conducted). Treatment 0.2 mg/kg Se-L-Met also exhibited substantial mortality before hatching and some additional die-off during the swimup stage. Lowest mortality was observed in 0.02 mg/kg selenium treatment.

The analysis of survival was conducted for three intervals: from Day 0 to hatching, from hatching to Day 28, and from fertilization to Day 28 (Table 9). Selenium treatment 2.0 mg/kg exhibited significantly lower survival in each interval, compared with control. Survival in treatment 0.2 mg/kg was significantly different from control only for the interval between hatching and Day 28. Length and body weight of fry sampled on Day 28 did not differ between control and treatments. Substantial differences in survival and tank densities between control and two highest selenium treatments might have affected growth end points (Table 9). No differences between treatments were observed in weight-length relationship. Observations from all treatments fitted the common linear regression: Log(Weight) = 0.438*(Length) + 0.966 (R^2=0.933, N=660).

Table 9. Survival and body size of catfish embryos and fry in bioassays with progenies of treated females. Data are x ± s.e.m. for pooled observations on each progeny. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test).

	TREATMENT			
	L-Met	Se-0.02	Se-0.2	Se-2.0
Number of tested progenies	5	6	4	3
Survival (%):				
Fertilization - hatching	86 ± 5	95 ± 2	74 ± 10	7 ± 5 *
Hatching - 28 days	90 ± 3	95 ± 1	68 ± 8 *	4 ± 3 *
Fertilization - 28 days	78 ± 7	90 ± 2	63 ± 10	2 ± 1 *
Body size (28 d):				
	(n=230)	(n=296)	(n=146)	(n=3)
Length (mm)	30.0 ±0.1	30.0 ±0.1	29.8 ±0.1	30.3 ±0.1
Wet weight (mg)	200 ± 24	197 ± 21	194 ± 17	204 ± 28

7. Relationship between Maternal Selenium and Survival of Progenies.

Data used for the analysis of LC_{50} are shown in Table 10 and in Figure 3. In general, bioassay mortality rates were in good correspondence with tissue selenium levels. LC_{50}'s for liver and fertilized egg selenium were 11.5 and 6.3 µg/g (dry weight), respectively. Based on observed responses in three selenium treatments, average tissue concentrations in treatments 0.02 and 0.2 mg/kg may approximate empirical NOEC and LOEC values. Maximum acceptable range and LC_{50}' for selenium residues in different tissues are summarized in Table 11.

Table 10. Maternal tissue selenium concentrations (µg/g, d.w.) and mortality in bioassays, from fertilization to 28 days (data

used for LC_{50} analysis). N - number of live embryos on Day 0, r - mortality on Day 28, p - proportions.

Female/progeny identification	Selenium concentration		Mortality		
	Liver	Eggs	N	r	P
L-Met-0.02	4.47	2.49	60	2	0.033
Se-0.02	4.50	3.02	30	2	0.067
Se-0.02	5.42	2.64	60	13	0.217
Se-0.02	5.94	3.25	60	7	0.117
Se-0.02	6.77	3.70	60	5	0.083
Se-0.02	7.44	3.52	60	2	0.033
Se-0.02	7.88	2.82	60	5	0.083
Se-0.20	9.52	6.60	60	9	0.150
Se-0.20	10.13	6.82	60	15	0.250
Se-0.20	13.02	6.06	60	41	0.683
Se-2.00	12.50	19.06	60	57	0.950
Se-2.00	31.70	18.96	60	60	1.000
Se-2.00	33.96	14.19	60	60	1.000

Table 11. Maximum acceptable tissue selenium concentrations and LC_{50} selenium concentrations in maternal tissues, for 28 day survival of progeny. Selenium concentrations are µg/g, d.w.

TISSUE	MATC RANGE		LC_{50} (95% CL)
	NOEC (Se-0.02)	LOEC (Se-0.2)	
Liver	7.3	12.5	11.5 (10.9-12.1)

Eggs (fert)	3.2	6.3	7.7 (7.2-8.4)
Ovary	5.6	7.0	N/A

D. DISCUSSION AND RECOMMENDATIONS

The effect of accumulated ovarian selenium on reproduction has been investigated in bluegill (see Part II of this report). Information on the selenium effect on catfish is limited to field observations and experimental works on nutrition (Gatlin and Wilson, 1984), pathology (Ellis et al., 1937), and mercury metabolism (Jorgensen and Heisinger, 1987). This report provides the first experimental evidence for reproductive effect of elevated tissue selenium in channel catfish female. Bioaccumulation of selenium in broodstock tissue after six consecutive injections of 0.2 and 2.0 mg/kg Se-L-Met did not appear to affect gonadal development, spawning and egg fertility. However, fertilized eggs had significantly elevated selenium burden and survival of embryos before and soon after hatching was significantly reduced. The injection dose 2.0 mg/kg was lethal for the offsprings, and a dose 0.2 mg/kg reduced fry survival.

Catfish produce relatively large (3-3.4 mm) and yolky eggs. Their embryos complete major organogenesis during a relatively long period of embryonic development before hatching (Armstrong, 1962). High mortalities observed in the selenium treatments may be associated with utilization of yolkborne selenoproteins during the embryonic growth and the excessive selenium in embryonic circulation. Early life stages of fish appear to be much more sensitive to selenium, compared with adults. The LC_{50} 8 ppm was reported for newly hatched larvae of zebrafish exposed to waterborne inorganic selenium (Niimi and LaHam, 1975, 1976).

The tissues of channel catfish and closely related species were analyzed for selenium content in several selenium-polluted areas. Sager and Cofield (1984) and Woock and Summers (1984) reported selenium concentrations 12 µg/g in liver and 9-10 µg/g in the ovaries (wet weight) of channel catfish sampled in Hyco Reservoir, North Carolina. These concentrations are similar with 2.0 mg/kg Se-L-Met treatment in our study, which produced a lethal effect on the catfish embryos. Even higher selenium concentrations (26 µg/g, wet weight) have been found in muscle tissue of catfish in Belew Lake (Cumbie and Van Horn, 1978). Field data collected by Lemly (1985) indicate that channel catfish were not found in the lake after 1977, suggesting complete reproductive failure.

Muscle tissue from limited number of channel catfish from selenium-polluted areas of San Joaquin River had low selenium concentrations, 0.26-0.52 µg/g wet weight (CDFG, 1987, 1988). However, the catfish livers collected from the same areas and analyzed by California Veterinary Diagnostic Laboratory, UC Davis, ranged in selenium level 4-24 µg/g dry weight (Ardans et al.1988), with about 30 percent of samples above LC_{50} value estimated by our study.

The acute effect of yolkborne selenium on the offsprings of channel catfish indicates that biomonitoring program in selenium-polluted areas of San Joaquin River (such as Mud Slough North, Salt Slough, and confluence with the Merced River) should be focussed on reproduction; the most sensitive to selenium, part of the life cycle. In sampling programs, the seasonality of the ovarian cycle and vitellogenesis in catfish should be considered to obtain reliable information on the potential effect of yolkborne selenium level. Catfish initiate vitellogenesis in early fall, but the ovarian growth and vitellogenin synthesis are particularly intense in early spring. Vitellogenesis is completed by mid or late spring, and spawning takes place in late spring or early summer. Females with GSI 9-10 % have completed or are close to completion of vitellogenesis and should be most suitable for sampling.

E. ACKNOWLEDGEMENTS

We appreciate cooperation and assistance of California Veterinary Diagnostic Laboratory System in the analyses of tissue selenium. Catfish broodstock was supplied by the Fishery, Inc. Aquaculture and Fishery Program, UC Davis provided important support in aquatic facility management and animal maintenance. State Board Staff, Laura Valoppi, Nancy Richard, and Victor de Vlaming contributed to this study by providing helpful advice and suggestions.

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G. LIST OF FIGURES

Figure 1. Top: Relationship between selenium concentrations of ovary and liver in catfish females. Data are log-transformed values. Regression equation: $Y = 0.923*X - 0.067$ ($R^2=0.810$, d.f.=52). Outliers are marked by "x".
Bottom: Relationship between selenium concentrations of fertilized eggs and livers of spawned catfish females. Regression equation: $Y = 0.647*X - 0.057$ ($R^2=0.895$, d.f.=16). Outliers are marked by "x".

Figure 2. Average cumulative mortalities in embryo-larval bioassays with progenies of catfish females from different treatments. Control is pooled L-Met treatments, Se is Se-L-Met treatment with respective dose (injections mg/kg body weight).

Figure 3. Relationships between mortalities of catfish in bioassay (from fertilization to Day 28) and selenium concentrations
(ppm, d.w., log) in fertilized eggs (top) and livers of treated females (bottom). C is control with highest selenium concentrations, L - treatment Se-0.02, M - Se-0.2, H - Se-2.

H. APPENDICES

Appendix 1. Water Quality Parameters in Broodstock Tanks (data are averages and ranges in 9 tanks).

Date	T°C	D.O.mg/L	pH	TAN mg/L
4-4-89	19.8	7.3	7.9	0.3
	17.0 - 23.0	4.9 - 8.0	7.6 - 8.1	0.2 - 0.5
4-5-89	19.9	7.1	8.0	0.3
	18.5 - 22.0	6.7 - 7.6	8.0 - 8.1	0.2 - 0.4
4-6-89	20.5	7.2	7.9	0.4
	18.0 - 23.0	6.4 - 7.5	7.8 - 7.9	0.3 - 0.4
4-7-89	19.6	7.6	7.6	0.0
	18.0 - 22.0	7.2 - 7.9	7.6 - 7.6	0.0 - 0.2
4-8-89	19.5	8.3	7.7	0.3
	18.0 - 21.0	7.5 - 9.9	7.7 - 7.8	0.3 - 0.4
4-10-89	19.7	7.8	N/A	N/A
	18.0 - 25.0	7.6 - 8.0		
4-14-89	18.8	8.1	7.7	0.3
	13.0 - 21.0	7.8 - 8.6	7.8 - 8.7	0.3 - 0.4
4-17-89	19.2	8.1	N/A	N/A
	16.0 - 21.0	7.8 - 8.5		
4-21-89	21.3	8.1	7.7	0.3
	17.0 - 20.0	7.5 - 8.5	7.6 - 7.7	0.2 - 0.3
4-24-89	17.8	8.0	N/A	N/A
	15.0 - 20.0	7.6 - 8.3		
4-28-89	18.5	8.3	7.6	0.4
	17.0 - 20.0	8.1 - 8.5	7.6 - 7.7	0.3 - 0.4
5-1-89	19.2	8.2	N/A	N/A
	17.5 - 21.5	7.9 - 8.5		
5-5-89	20.2	7.8	7.7	0.4
	19.0 - 23.0	7.4 - 8.1	7.6 - 7.7	0.3 - 0.4
5-8-89	19.7	8.4	N/A	N/A
	18.0 - 22.0	8.1 - 8.6		
5-12-89	18.4	8.3	7.7	0.2

Appendix 1 (Continued)

	14.0 - 21.0	7.5 - 8.7	7.6 - 7.7	0.1 - 0.4
5-15-92	19.5	7.9	N/A	N/A
	18.0 - 21.0	7.5 - 8.6		
5-19-89	18.8	8.5	7.8	0.3
	15.0 - 20.5	8.1 - 8.8	7.7 - 7.8	0.2 - 0.4
5-22-89	18.9	8.5	N/A	N/A
	18.0 - 20.0	8.3 - 8.8		
5-26-89	N/A	8.1	7.7	0.2
		8.0 - 8.3	7.6 - 7.8	0.1 - 0.3
5-30-89	N/A	8.0	N/A	N/A
		7.7 - 8.3		
6-9-89	N/A	6.6	7.9	0.4
		5.7 - 7.4	7.8 - 7.9	0.2 - 0.5
6-12-89	N/A	7.0	N/A	N/A
		6.5 - 7.8		
6-15-89	N/A	7.2	8.1	0.4
		6.2 - 7.8	8.0 - 8.1	0.3 - 0.5
6-19-89	N/A	7.1	N/A	0.3
		6.9 - 7.3		0.2 - 0.4

Appendix 2. Water Quality Parameters in Larval Bioassay System (data are averages and ranges of 30 aquaria).

Date	T°C	D.O.mg/L	pH	TAN mg/L
6-27-89	25±1 through all period	7.5 7.3 - 7.6	8.2 8.2 - 8.3	0.3 0.1 - 0.4
6-30-89		7.7 7.5 - 7.8	8.2 8.2 - 8.4	0.3 0.3 - 0.4
7-3-89		7.7 7.4 - 7.9	8.2 8.2 - 8.5	0.3 0.3 -0.4
7-6-89		7.5 7.4 - 7.8	8.2 8.2 - 8.3	0.3 0.2 - 0.4
7-11-89		7.4 6.8 - 7.7	8.2 8.1 - 8.2	0.3 0.3 - 0.4
7-15-89		7.7 7.7 - 7.6	8.1 8.0 - 8.1	0.3 0.3 - 0.4
7-18-89		7.7 7.2 - 7.8	8.2 8.1 - 8.3	0.3 0.3 - 0.4

Appendix 3. A. Dry matter content of channel catfish tissues (observed in broodstock females).

TISSUE	DRY MATTER (%) x ± s.e.m. (n)	Coeff. of variation (%)
Liver	24.75 ± 0.16 (135)	7.5
Ovary	34.79 ± 0.83 (114)	25.6
Egg mass	16.56 ± 0.44 (20)	11.8

B. Conversion factors for selenium content from wet (X) to dry weight (Y). Data are from regression equations with zero intercepts.

LIVER (n=134): $Y = 4.124*X$ (R^2=0.994, S.ERR.= 0.843)

EGG MASS (n=20): $Y = 5.923*X$ (R^2=0.981, S.ERR.= 0.711)

OVARY: (all fish with weight of ovaries < 50g are deleted)

During vitellogenesis (n=58) $Y = 2.498*X$ (R^2=0.993, S.ERR.= 0.310)

Before spawning (n=21) $Y = 2.851*X$ (R^2=0.991, S.ERR.= 0.646)

PART II. Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (Lepomis macrochirus).

TABLE OF CONTENTS: PART II

A. INTRODUCTION

State Board Order 85-1 addressed waterfowl problems at Kesterson Reservoir resulting from selenium laden water discharged to the facility and directed the formation of the San Joaquin River Basin Technical Committee. One of the tasks of this committee was to develop proposed water quality objectives for constituents of agricultural drainage. State Board staff developed water quality criteria for nine constituents based on available toxicity data. The Technical Committee proposed objectives for three of these constituents, selenium, boron and molybdenum. The toxicity data upon which the criteria and objectives are based are not adequate in that site-specific toxicity data were generally not available. As a result, the Technical Committee recommended that additional site-specific toxicity data be developed to refine these water quality criteria and objectives. The objective of this contract is to provide additional selenium toxicity data to be used to refine the criteria and objectives already developed.

The aim of this study was to determine tissue selenium concentrations in adult bluegill, Lepomis macrochirus Rafinesque, critical to normal reproduction. Bioaccumulation of selenium was induced by dietary selenomethionine treatments applied during the periods of gonadal growth and spawning. We examined the effect of selenium on gonadal development, fertilization, early development and survival of progeny to age 30 days. Two studies were conducted: 1) a selenium bioaccumulation study aimed to evaluate the effect of dietary treatments on tissue selenium burden and gonadal development, and 2) a reproductive performance study aimed at evaluating the effect of tissue selenium concentrations, on the survival of offspring. Both experiments contained six groups of fish: three control groups (diet supplemented with L-Methionine), and three treatment groups (diet supplemented with Se-L-Methionine). The experiments were started in the fall 1990 and completed during the summer 1991. Work was conducted at UC Davis Aquatic Center (Aquaculture and Fisheries Program) and in the Department of Animal Science.

Previous work indicates that bioaccumulation of environmental selenium in reproductive tissue, not detrimental (at least, not lethal) to adult fish, may cause reproductive failure. Reduced population recruitment in selenium-contaminated Belews Lake was documented by sampling at several locations in 1974 through 1977

(Cumbie and Horn, 1979). By the end of sampling period, virtually no juveniles of several species, including bluegill, were found in the polluted locations. Muscle selenium concentrations in adult fish from polluted areas ranged from 10 to 50 ppm compared to a 0.5 to 7.0 ppm (wet weight) from non-polluted locations. Changes in selenium tissue burden of adults were consistent with the disappearance of juveniles.

Sorensen et al (1982, 1983, 1984) investigated the histopathology of Centrarchids collected from the selenium-polluted lakes. The authors described some atretic changes in the vitellogenic oocyte, but the major pathological changes were observed in the kidney, liver and pancreatic tissues of the adults.

Gillespie and Baumann (1986) presented strong evidence for associating the potential route of selenium reproductive effects with maternal egg yolk. They conducted artificial cross-inseminations of wild bluegill collected from lakes with high and low waterborne selenium. Crosses were performed between the parents that had high and low tissue selenium burdens, corresponding to waterborne selenium. The tissue selenium of males did not affect survival of progeny, but selenium levels of females did correlate with larval survival. The ovarian selenium concentrations 7-8 ppm (wet weight) resulted in high larval mortality.

Woock et al (1987) investigated the effects of bluegill broodstock exposure to dietary and waterborne selenium on the survival of progenies. Dietary treatment with up to 30 ppm selenomethionine had no effect on spawning and hatching success; however, elevated or complete larval mortality were observed in 13 and 30 ppm selenomethionine treatments, respectively. This reproductive effect was clearly confirmed by a recent study of the National Fisheries Contaminant Research Center, Missouri (Lemly, 1990). In the waterborne (inorganic) and dietary (organic) selenium treatments, the tissue selenium burden of the broodstock and fertilized eggs, exhibited correlations with dose-dependent increases. High selenium concentrations did not affect gonadal development and natural spawning of treated broodstocks, but all larvae hatched in the high-dose selenium treatment, died before exogenous feeding.

These studies suggest that detrimental effects of selenium on reproduction is, most likely, due to its bioaccumulation in the oocyte yolk during vitellogenesis, and utilization of selenium-saturated yolk during embryonic and early larval development. The effect of selenium bioaccumulation in the egg yolk was experimentally confirmed in our study with channel catfish

broodstock injected with selenoaminoacid during vitellogenesis (Part I of this report).

B. MATERIALS AND METHODS

1. Source of fish

Two different populations of bluegill were used for observations on selenium bioaccumulation (A) and spawning performance (B). Population A included 250 fish obtained from Rainbow Ranch Fish Farm, Kelseyville, California. These fish were held in 1400 L and 6400 L, flow-through outdoor tanks, at water temperatures ranging from 18° to 22°C. The fish were fed Silver Cup #4 trout diet (Murray Elevators, Utah) ad libitum three times daily. After a 32 day weaning period, 194 fish were transferred into the laboratory tanks. The average body weight was 113 g (range 30-220 g). Due to large variation in individual size and poor expression of secondary sex characters, mixed sex-cohorts were used in the experiments, and the sex ratio was assumed to be 1:1.

Population B included 45 females and 50 males obtained from Chico Game Fish Farm. Females averaged 106 g in body weight (range 65-250 g), and males 164 g (range 80-289 g). These fish were reliably sexed (McComish, 1968), and maintained in outdoor 1400 L foot flow-through tanks for 56 days before initiation of the experiments under similar to population A conditions, but in a different tank.

2. Experimental Design

Population A and fin-clipped females of population B were moved into indoor facilities in November, 1989 and randomly assigned to 6 tanks, each receiving one of the following nominal dietary treatments through the end of the experiment: L-Met-8, 18, and 28 ppm (controls); Se-L-Met-8, 18, and 28 ppm (3 selenium treatments). Males from population B were held in separate indoor tanks and received untreated diets until the start of the spawning season.

Population A was randomly sampled on Days 0, 30, 58, 86, and 114. Sampling from each tank was done by sacrificing fish and examining gonads until 3 females were sampled (the number of males varied). After the last sampling, on Day 114, all remaining fish of population A were removed to outdoor tanks, fed with untreated diets, and sacrificed on Day 144, to examine tissue depuration.

In March (day 120 from the initiation of treatment) treated females and untreated males of population B were paired in tanks to obtain natural spawning. These trials had limited success, and natural spawning was replaced with hormonal induction of ovulation and fertilization *in vitro*.

Fish were maintained in treatment tanks, and both females and males were fed treatment diets. In May-July females were examined by *in vivo* ovarian catheterization for ripeness, and ready-to-spawn fish were induced to ovulate, stripped of ova, and their eggs were fertilized *in vitro* by semen from two randomly chosen males from the same treatment tank (males exhibited natural spermiation). Spawned females were necropsied within one hour after stripping. Males were kept in tanks for repeated spawning, and were necropsied at the end of the spawning season.

Fertilized eggs from each individual mating were sampled for fertilization success, selenium content, and two live subsamples were randomly removed: one for the embryo-larval 30-day bioassay, and another for observations on larval development during the first 5 days after hatching. More detailed descriptions are provided in further sections.

3. Feed Preparation

Trout chow was supplemented with either L-methionine or seleno-L-methionine to achieve nominal selenium concentrations of 8, 18, and 28 mg/kg in the diets. The purity of both the L-Methionine and the Se-L-Methionine was 99% (Sigma Chemical Company, St. Louis, Missouri). The Silver Cup mash contained minimum 38 % crude protein and 10 % fat, and maximum 4 % crude fiber and 12 % ash. The dietary mash contained 2.93 ppm residual selenium (ICP-atomic emission, Veterinary Diagnostic Laboratory, UC Davis). Experimental diets were prepared by mixing premix containing supplemented amino acids, cellulose, and dietary mash, with water, herring oil, and dietary mash. 150 g of cellulose and the appropriate amount of Se-L-met (calculated based on the proportion of selenium in the molecular weight of the amino acid) were placed into a vortex mixer and mixed for 20 minutes. The cellulose mixture was combined with 600 g of mash, mixed for 20 minutes. 13.5 kg of mash was combined with premix and mixed for twenty minutes. 750 g of herring oil was added and mixing continued for 20 more minutes. 1800 ml of distilled water was added and mixed for 5 more minutes. Diets were cold extruded to form one eighth inch pellets and dried overnight in a forced air drier. Prepared diets were sealed in plastic bags and stored at -20°C. Three samples were collected during the experiment (11/9/89, 12/5/89, 2/27/90) and analyzed for selenium content.

Observed selenium concentrations were approximately 25% lower than targeted concentrations. The selenium concentrations in three control diets ranged from 1.2 to 1.6 μg/g, and the treatment diets had a selenium content of 5.5, 13.9, and 21.4 μg/g (Table 1).

Table 1. Selenium concentrations and moisture content of the experimental diets.

Nominal Concentration of Selenium (μg/g)		Moisture (percent)	Actual Selenium concentration (dry weight) (μg/g) mean ± sd (n=3)
	-	14.32	1.17 ± 0.25
L-met	-	16.33	1.60 ± 0.49
(control)	-	15.59	1.23 ± 0.19
	8	13.03	5.52 ± 0.75
Se-L-met	18	16.52	13.93 ± 1.55
(treatment)	28	15.11	21.41 ± 1.92

4. Experimental Protocol

4.1. Rearing and sampling

Fish were put into the experimental tanks on November 6, 1989 at an initial stocking density of 31 per tank (round fiberglass tanks, diameter 130 cm, water volume 355 L). Tanks were supplied with flow-through well water, at a rate of 7-8 L/min. Water was degassed and aerated in a stripping column, and distributed to rearing tanks by gravity from a holding container supplied with a quartz water heater. The water source was an underground well which had the following water quality parameters: pH = 7.6, Alkalinity = 220 mg/L $CaCO_3$, Hardness = 140 mg/L $CaCO_3$, Sulfate = 40 mg/L, Nitrate = 2 mg/L, Boron = 0.46 mg/L, Calcium = 35 mg/L, Sodium = 31 mg/L. Tanks were housed in a room with luminescent light. The initial photoperiod was 10 L: 13 D, with a water temperature of 19 °C. Daily photoperiod was gradually increased from December through February to 15 L, with a concomitant increase of water temperature to 26°C (Figure 1). The adjustments

were made to stimulate the bluegill gonadal cycle, based on information of Kaya and Hasler (1972). Fish were fed ad libitum three times a day, at a daily rate of 1-3 percent of their total body weight, depending on temperature. Bioaccumulation (Population A) fish were sampled in November, December, January, February, and March. With few exceptions, each treatment sample included 3 females and 1-3 males. On Day 114, the last sampling, only two remaining females were sampled in the 8 ppm L-methionine control and the 13.9 ppm selenium treatment, where the proportions of males were higher than expected. Population A fish, remaining in tanks after Day 114 sampling (3 females in pooled control, and 6 females and 5 males in 5.5 and 21.4 ppm selenium treatments) were removed for depuration sampling.

Population B fish were not sampled during the experimental treatment before spawning, but a Day 0 sample was collected in November. Starting in March, photoperiod was increased to 16 L, and maintained through the spawning season. Water quality parameters were recorded throughout the period of rearing and spawning, and are provided in Appendix 1.

4.2. Spawning

Paired broodfish for natural spawning were placed in rectangular tanks (122cm x 64cm x 58cm) constructed from marine plywood and supplied with water from the same system as the rearing tanks. Water flow rates were 1.0 L/min and water depth was maintained at 46cm. After about one month, there had been no natural spawning. Literature data indicated that our 26°C spawning temperature was at the lower level of the optimum range 26-30 C° (Kaya and Hasler, 1972; Banner and Hyatt, 1975). Banner and Hyatt (1975) also reported that daily temperature cycling was favorable for final gonadal maturation and spawning of bluegill.

We began a daily temperature cycle in April, continuing for eight weeks. The spawning tanks were allowed to cool down during the evening and night to 20° C and then warmed up during the day to 27-28° (by turning the heaters on at 8 am). Since no spawning was observed after two weeks of temperature cycle, we decided to catheterize females to determine the stage of ovarian maturity. A polyethylene tubing (1.14 mm ID) was inserted into the females oviduct, and the ovarian eggs were removed by aspiration. Collected eggs were examined under a dissecting scope and egg diameter was measured in each sample. In all females, we observed 4 to 5 distinct clutches of vitellogenic follicles, and all females had clutches of mature follicles, recognized by the coalesced oil globule and transparent yolk (Figure 2). It was also apparent that the most advanced clutches periodically undergo atretic changes, without ovulation and oviposition. The

administration of exogenous hormone was used to induce spawning. Six females were injected IM with 0.03 µg/kg body weight of synthetic mammalian LHRH analog (des Gly 10, D-Ala 6, LHRH ethylamide), dissolved in a physiological saline carrier. After 48 hours only one female spawned. All males were milting. Catheterization of the remaining five females revealed that they had ovulated. In fact, two of the females had freely flowing eggs when netted and removed from the tank for catheterization. About 10 mls of eggs from one female were collected in a petri dish and inseminated by hand-stripped semen, to examine egg quality. At one hour postfertilization, fertilization success was 75 percent (16-32 cell stage). We concluded that broodfish were capable of normal ovulation and spermiation, but unknown environmental or behavioral factors inhibited spawning. Selenium treatments were not a factor, since the response was similar in both control and treatment groups.

Since reliable natural spawning was not available, we developed and applied a standard induced spawning procedure as follows. All females were catheterized weekly. A 1.14 mm (ID), 1.57 mm (OD) polyethylene tubing was inserted 2-3 cm into the female oviduct and the eggs were removed by aspiration. Eggs were placed in a petri dish containing Leibovitz L-glutamine cell culture media (Sigma Chemical Company, St. Louis, Missouri), and examined under a dissecting scope. Females with ripe eggs (Figure 2) were selected for spawning, whereas those with immature follicles or with ripe atretic follicles, were re-sampled weekly, until they were found in the proper ovarian stage. Ripe females were injected with 0.1 µg/ml LHRHa at approximately 8:00 am, and 24 hours later a second dose, of the same concentration, was given. They were examined every two hours, beginning 7 hours after the second injection. Ovulation was evident when freely flowing eggs were released upon gentle pressure to the lower abdomen. Naturally spermiating males were available during the entire spawning season. The following standard procedure of in vitro fertilization was performed at each spawning. About 10-15 ml of ova were stripped into a 100 x 15 mm plastic petri dish. Two males were removed, from the same treatment tank, and a single drop of milt from each male was stripped onto the top half of the petri dish. The milt was then rinsed off the petri dish into the dish containing the eggs, with 40 ml of the spawning tank inlet water. The mixture was gently stirred for two minutes, and then rinsed three times with clean water. Time of fertilization was recorded.

Fertilized eggs were placed in a Lab-Line Incubator at 26 ± 0.5°C in a 1.5 liter (21 cm x 15.5 cm x 5 cm) pyrex glass tray filled with 1 liter of water. The eggs were distributed over the bottom of the glass tray in a single layer using a gentle swirling motion. They quickly adhered to the bottom of the tray. At one hour postfertilization, a subsample of 100 eggs was collected by

placing a plastic grid containing 60 (2.6 cm x 2.1 cm) rectangles underneath the glass trays, and ten eggs from each of ten randomly selected rectangles were collected with a plastic pipet to examine fertilization success. Developing embryos (at 8-32 cell stage) were counted under a dissecting scope.

In 10-12 hours after fertilization (to allow completion of epiboly) approximately 400 eggs were removed from the tray and stocked in a 1 L beaker. Beakers were placed in the incubation cabinet, for observations on larval development. In addition, 90 eggs were transferred into the larval bioassay system. The remaining eggs were weighed and frozen for selenium analyses.

4.3 Larval Development

Four hundred eggs, placed in the incubation cabinet at 26°C, were used for observations on larval development. After hatching, 100 larvae were randomly transferred into a new 1 L beaker containing 500 ml of water. Dissolved oxygen concentrations were measured each day, followed by a renewal of 80% water. Samples were collected daily during the next 5 days. Ten larvae were randomly pipetted from the beaker, into a petri dish, and examined under a dissecting scope. The numbers of normal, abnormal and dead were recorded. The larvae were anesthetized, preserved in 10% phosphate buffered formalin, and later examined for total length, and the oil globule and yolk sac cross-sectional optical areas. Measurements were conducted by point-count image analysis, using a darkfield dissecting microscope with camera lucida, and a Nikon Microplan II image analyzer with microcomputer interface (accuracy 0.01mm).

In addition, approximately three thousand eggs from three females in one selenium treatment (Se-L-Met 28 ppm) and two females in the respective control were placed in separate 1.5 L beakers. Several thousand larvae were sampled at 4 days posthatch, from each female, and analyzed for selenium content.

4.4 Larval Bioassay

Ninety fertilized eggs from each female were placed in groups of approximately 30 eggs into three separate 1-L Nalgene Tri-pour beakers with screened windows (Nitex, 250 μm). Three beakers were suspended in a common 15 L circular fiberglass tank, housed in a recirculating system with temperature control and biological filtration. All components of the systems were made of either fiberglass or PVC. Temperature was maintained at 26° ± 0.5° C by quartz heaters and YSI thermostats. Water was supplied at the surface of each tank at a rate of 400-500 ml/min. Artificial

photoperiod was 14 L.

Hatching was observed in 24 hours, and feeding with rotifers was initiated at swim-up stage, 4 days after hatching. On Day 16, the survived larvae were counted in each beaker and released in the common tank, where rearing was continued until Day 30 on brine shrimp nauplii. On Day 30, fry were euthanized, counted, weighed (0.1 mg, Mettler AE-100) and measured (0.1 mm). Samples of fry were frozen for selenium analyses.

The following standard protocol was used for monitoring the larval bioassay. Beakers and tanks were checked three times daily for dead embryos and larvae. Beakers were removed one at a time, draining off approximately 2/3 the beaker's volume in the process. Care was taken not to impinge the larvae on the screen during draining. Beakers were placed on a light box, and sediments and dead animals were pipetted out and recorded. When all three beakers were on the light box, food was added. When the beakers were returned to the tank, their relative positions were shifted to randomize the effect of their location. During the period 4-12 days posthatch, each beaker received a 5 to 10 ml suspension of rotifers (concentration 900-1800/ml) three times a day (Appendix 2). The feeding rate was adjusted as necessary to maintain approximately ten rotifers per 1 ml of beaker volume. During the morning and evening feedings, the rotifers were supplemented with a 4 ml concentrate of Selenastrum capricornutum, in order to provide a diet for the uneaten rotifers remaining in the beaker. Once the larvae were large enough to consume brine shrimp nauplii, 8-12 drops of a freshly hatched Artemia suspension were added to each beaker at each feeding, starting on days 8-9 posthatch. Rate of additional feeding with nauplii was adjusted downwards if the uneaten nauplii were present on the bottom of the beaker, indicating that the feeding rate was too high.

Once all larvae were feeding on Artemia nauplii (12-14 days post hatch, or 14-16 days post fertilization) and they had been transferred from the beakers to the tank, they were no longer fed rotifers. In general, 25 ml of an Artemia suspension was given to each tank three times a day. Rate of feeding was adjusted in each tank to maintain an approximate concentration of 3 nauplii per 1 ml at 10 min after each feeding.

After the larvae were released into the tank, uneaten food and wastes were siphoned from the tanks daily. Daily records of embryonic and larval mortalities in beakers and tanks were used for estimation of cumulative mortalities to Day 30. However, small numbers of embryos and larvae were not accounted for by mortality records, and the final analysis of bioassay survival was based on counted numbers of eggs stocked, larvae survived to Day 16 in each beaker, and those survived to Day 30 in the common tank.

Water temperature was measured three times daily in one of the tanks of the system. Dissolved oxygen (YSI Model 58), electrical conductivity (YSI Model 33), hardness (Hach kit) and alkalinity (Hach kit) were measured in the system sump weekly. Total ammonia nitrogen (Hach kit) and Ph (Nestor probe) were measured weekly in one of the three beakers, tanks, and sump. When the conductivity of the water in the recirculating system increased to 800 μmos, water was siphoned out of the sump, allowing fresh well water in, for about 2 hours.

5. Laboratory Methods

5.1 Necropsy and Sample Preparation

All necropsied fish were measured for body and eviscerated carcass weight (accuracy 0.01 g), and the fork length (1 mm). Liver and gonads were dissected and weighed (0.01 g). Wet weight of organs was used to calculate hepatosomatic (HSI = 100 x liver weight/body weight) and gonadosomatic (GSI = 100 x gonads weight/body weight) indices. A strip of the dorsal muscle (4-6 cm length) was dissected and separated from the skin. Samples of liver, gonad, muscle, eggs, larvae, and 30-day old juveniles were weighed and frozen (-20 °C) in Whirl-Pak bags for subsequent selenium analysis. In addition, blood was collected from the caudal vein, with 22 gauge needles and heparinized vacutainers, plasma was separated by centrifugation and frozen (-20°C) in plastic vials for the analysis of protein phosphorus. Gonadal samples were preserved in 10% phosphate-buffered formalin for histological analysis.

5.2 Selenium Analysis

Total selenium was determined by fluorimetry, using methods described by Brown and Watkinson (1977), and Whetter and Ullrey (1978). Wet tissue was desicatted by lyophilization, and stored at -40°C.

Gonad and liver samples (0.1-0.3 g dry weight) were digested with 5 mL of concentrated HNO_3 and 2 mL $HClO_4$. Digestion was carried out at 150°C for 1.5 hr and then at 210°C for 1.25 hr. Reduction of selenate to selenite was accomplished by adding 3 mL of 6 N HCl to the cooled solution and returning to the digestion block for 7 min at 160°C (modification of A.Jacobson and R. Burau, UC Davis, pers.comm). The solution was cooled before adding 2.5 mL of EDTA (0.016 M). The resulting solution was adjusted to pH 1.0 using cresol red indicator, 10M NH_4OH, and 6 M HCl sequentially.

Samples were diluted to a final volume of 25 ml with 0.1 M HCl and compared to selenite liquid standards (0.001-0.010 ppm), a tissue standard (bovine 1577A, NIST, SRM), and blanks using a Perkin-Elmer 650-15 fluorimeter. All reagents were of analytical grade and selenium free. Replicate tissue samples and liquid standard measurements were within 10% accuracy, the recovery of liquid standards was 85-115%. The bovine liver standard (NIST standard 1577A = 0.71 ug/g was analyzed eight separate times, yielding a concentration of 0.739 ± 0.076 $\mu g/g$ (mean and s.d.). Inter-laboratory validation was performed at the end of the study on 30 randomly chosen tissue samples (liver and gonad) in the analytical laboratory of the Department of Fish and Game (Stockton, California) by hydride generation atomic absorption (HGAA). Selenium concentrations of samples, analyzed in the two laboratories, exhibited correlations of 0.966 and 0.912 for gonadal and liver samples, respectively. Data are provided in Apperdix 3.

5.3 ALPP (plasma protein phosphorus)

Plasma vitellogenin concentrations were evaluated by measurement of alkali-labile protein phosphorus (ALPP), which is an indirect but appropriate method to measure yolk precursor. Previous studies with numerous teleost species demonstrated that the plasma vitellogenin molecule contains nearly all plasma protein phosphorus, and ALPP profile correlates with vitellogenin secretion (Wallace and Jared, 1968; Emmersen and Petersen, 1976; Hori et al , 1979; Nath and Sundararaj, 1981). The laboratory procedure utilized in this study was similar to that described by Wallace and Jared (1968), and de Vlaming et al (1984).

5.4 Histology

Samples of gonadal tissue were dehydrated in a series of alcohol, cleared in xylenes, embedded in paraffin and sectioned at a thickness of 5 microns. Slides were stained with hematoxylin and eosin, and with periodic acid- Schiff stain (PAS), using procedures described in Shoehan and Hrapchak (1980). Slides were examined under a compound scope for the staging of development and atretic changes in the ovarian follicle.

6. Statistical Methods

Comparisons between control and treatments were conducted by Dunnett's procedure; one tailed test, at $\alpha = 0.05$ was used (Dunnett, 1955). Three control groups were pooled when the analysis of varience did not reveal significant differences

between all groups (P>0.05). Tissue selenium concentrations were transformed to $\log_{10}$ values before performing ANOVA tests. The proportional data (fertilization, survival, abnormalities) were transformed into the arcsine-roots. The relationships between selenium concentrations in different tissues were computed by linear regression analysis. Evaluation of larval bioassay results was based on the survival from egg stocking to 16 and 30 days after fertilization. Continuation of larval rearing in the common tank after their pooling on Day 16 affected final results in some trials due to beaker effect. The proportions of survived larvae in three beakers were compared post-factum by Chi-square analysis, and the assays with heterogenous beaker survival were deleted from the analysis of 30 day endpoint (Appendix 4 and 5). The LC_{50} of maternal tissue and egg yolk selenium for resulting progenies were computed by the Spearman-Karber method (Hamilton et al., 1977; Gelber et al., 1985). Log-transformed tissue selenium concentrations were used. Abbott's correction (Finney, 1971) for control mortality was utilized. The LC_{50} values give an approximate estimation of the acute effect of maternal tissue selenium, and are not intended for regulatory use.

C. RESULTS

1. Bioaccumulation (population A)

1.1 Survival and growth

Only two fish out of the total population 236 died during the course of the experiment (one in the medium selenium treatment and one in the control group).

No apparent difference was observed in fish behavior between the control and treatment groups. Most fish in all experimental treatments fed well on the prepared diets. Swimming activity, as well as feeding, increased as the temperature and day length increased.

Changes in fork length and body weight of both sexes are shown in Tables 2 through 5. No significant differences between control and treatment groups were observed at any sampling time. Control groups gained approximately 1-2 cm in fork length and 50 % in body weight. No such gain was observed in females from the medium and high dose selenium treatments, but statistical comparison of growth was not possible due to the small sample size.

Table 2. Fork length (cm) of females (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	15.80 ± 0.49 (4)			
30	16.08 ± 0.49 (9)	16.47 ± 1.16 (3)	14.63 ± 0.45 (3)	15.20 ± 0.55 (3)
58	16.56 ± 0.55 (9)	17.87 ± 0.81 (3)	14.23 ± 0.94 (3)	15.37 ± 0.39 (3)
86	14.75 ± 0.46 (6)	16.40 ± 0.44 (3)	15.60 ± 1.21 (3)	16.07 ± 1.09 (3)
114	17.45 ± 0.34 (8)	17.17 ± 0.68 (3)	15.70 ± 0.10 (2)	16.00 ± 0.93 (3)
Depuration				
142	17.17 ± 0.60 (3)	16.57 ± 0.38 (3)	N/A	16.10 ± 0.55 (3)

N/A = not available.

Table 3. Fork length (cm) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	18.27 ± 0.37 (6)			
30	18.25 ± 0.43 (6)	18.07 ± 0.64 (3)	15.20 ± n/a (1)	17.73 ± 0.93 (3)
58	18.30 ± 0.25 (5)	N/A	18.57 ± 0.07 (3)	17.40 ± n/a (1)
86	16.95 ± 0.53 (6)	18.37 ± 0.23 (3)	18.77 ± 0.86 (3)	19.30 ± 0.20 (2)
114	18.50 ± 0.49 (8)	16.50 ± n/a (1)	19.53 ± 0.15 (3)	17.35 ± 1.55 (2)
Depuration				
142	18.70 ± n/a	18.95 ± 0.18		18.93 ±

0.56	(1)	(2)	N/A	(3)

N/A = not available.

Table 4. Body weight (g) of females (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	80.05 ± 7.55 (4)			
30	88.51 ± 10.84 (9)	99.04 ± 27.43 (3)	64.25 ± 8.08 (3)	74.24 ± 9.87 (3)
58	98.47 ± 10.19 (9)	138.44 ± 23.11 (3)	61.90 ± 15.13 (3)	70.77 ± 6.94 (3)
86	68.82 ± 8.44 (6)	106.54 ± 14.89 (3)	85.07 ± 23.52 (3)	86.87 ± 16.51 (3)
114	131.93 ±	135.37 ±	96.15 ±	100.03 ±

	8.84 (8)	18.40 (3)	1.45 (2)	18.43 (3)
Depuration				
142	145.63 ± 14.18 (3)	113.45 ± 8.53 (3)	N/A	102.43 ± 11.67 (3)

N/A = not available.

Table 5. Body weight (g) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	149.08 ± 0.37 (6)			
30	140.56 ± 10.78 (6)	140.97 ± 15.94 (3)	71.02 ± n/a (1)	122.72 ± 16.99 (3)
58	134.00 ± 6.61 (5)	N/A	153.37 ± 4.46 (3)	131.65 ± N/A (1)
86	112.03 ± 12.34 (6)	148.47 ± 5.91 (3)	154.33 ± 19.86 (3)	182.55 ± 11.65 (2)

114	162.41 ± 13.43 (8)	121.70 ± n/a (1)	190.47 ± 5.54 (3)	136.35 ± 38.85 (2)
Depuration				
142	171.50 ± n/a (1)	190.10 ± 23.70 (2)	N/A	176.03 ± 16.28 (3)

N/A = not available.

1.2 GSI, HSI and Plasma ALPP

There was dramatic increase in GSI of broodstock, particularly in females, from February (Day 86) to March (Day 114) (Tables 6 and 7). This increase reflects rapid ovarian and testicular growth in response to elevated rearing temperature and increased photophase. No statistically significant differences were observed between selenium treatments and control.

Changes in female and male HSI were less evident but, in general, followed the same pattern as GSI (Tables 8 and 9). On Day 86, female HSI values in the 13.9 and 21.4 ppm selenium treatments were significantly higher than in control groups, and the mean values of HSI in these treatments were also higher on Day 114, although not at a significant level (Table 8).

Plasma protein phosphorus (ALPP) in females was elevated on Day 114 and 142 (Table 10). Two selenium treatments (5.5 and 13.9 ppm) exhibited significantly higher ALPP values on Days 86 and 114, compared with controls. However, there were no consistent trends, indicating effect of selenium treatment on vitellogenesis.

Bluegill males exhibited unusually high plasma ALPP concentrations (Table 11). In the majority of teleost fish plasma ALPP of males remains below 10 μg/ml throughout the reproductive cycle, whereas we observed concentrations above 50 μg/ml in some samples. However, the spontaneous synthesis of vitellogenin in male fish was reported in the literature.

Table 6. GSI (percent) of females (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	1.06 ± 0.04 (4)			
30	1.12 ± 0.06 (9)	1.06 ± 0.12 (3)	1.19 ± 0.09 (3)	1.26 ± 0.05 (3)
58	1.24 ± 0.04 (9)	1.11 ± 0.04 (3)	1.22 ± 0.10 (3)	1.16 ± 0.02 (3)

86	1.20 ± 0.05 (6)	1.20 ± 0.15 (3)	1.30 ± 0.05 (3)	1.29 ± 0.03 (3)
114	7.98 ± 0.42 (8)	7.32 ± 0.69 (3)	6.44 ± 0.42 (2)	9.06 ± 1.25 (3)
Depuration				
142	7.26 ± 0.51 (3)	8.01 ± 1.28 (3)	N/A	6.71 ± 0.64 (3)

Table 7. GSI (percent) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	0.34 ± 0.05 (6)			
30	0.39 ± 0.05 (6)	0.36 ± 0.13 (3)	0.25 ± (1)	0.22 ± 0.05 (3)
58	0.35 ± 0.05 (5)	N/A	0.37 ± 0.10 (3)	0.47 ± (1)

86	0.39 ± 0.08 (6)	0.45 ± 0.10 (3)	0.55 ± 0.17 (3)	0.68 ± 0.03 (2)
114	1.10 ± 0.09 (8)	0.60 ± 0.67 (1)	1.05 ± .09 (3)	0.99 ± (2)
Depuration				
142	1.48 ± 0.11 (1)	1.20 ± 0.21 (2)	N/A	1.10 ± (3)

Table 8. HSI (percent) of females (population A). Data are means ± s.e.m. (n). Asterisks denote significant differences between selenium treatments and control (Dunnett's test).

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	1.37 ± 0.23 (4)			
30	1.25 ± 0.10 (9)	1.48 ± 0.05 (3)	1.24 ± 0.13 (3)	1.42 ± 0.05 (3)
58	1.50 ± 0.12	1.87 ± 0.08	1.23 ± 0.28	1.26 ± 0.03

	(9)	(3)	(3)	(3)
86	1.01 ± 0.11 (6)	1.35 ± 0.05 (3)	1.39 ± 0.07* (3)	1.39 ± 0.06* (3)
114	1.59 ± 0.12 (8)	1.46 ± 0.08 (2)	1.72 ± 0.27 (3)	1.71 ± 0.10 (3)
Depuration				
142	1.45 ± 0.26 (3)	1.37 ± 0.11 (3)	N/A	1.52 ± 0.18 (3)

Table 9. HSI (percent) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	1.50 ± 0.14 (6)			
30	1.29 ± 0.13 (6)	1.13 ± 0.07 (3)	1.34 ± (1)	1.08 ± 0.13 (3)

58	1.44 ± 0.08 (5)	N/A	1.40 ± 0.08 (3)	2.61 ± (1)
86	1.10 ± 0.05 (6)	1.23 ± 0.08 (3)	1.30 ± 0.09 (3)	1.12 ± 0.10 (2)
114	1.36 ± 0.06 (8)	1.57 ± (1)	1.20 ± 0.05 (3)	1.47 ± 0.03 (2)
Depuration				
142	1.71 ± (1)	1.48 ± 0.05 (2)	N/A	1.25 ± 0.03 (3)

Table 10. Plasma ALPP (ug/ml) of females (population A). Data are means ± s.e.m. (n). Asterisks denote significant differences between the selenium treatments and control (Dunnett's test).

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	58.5 ± 7.9 (4)			
30	25.4 ± 6.3 (9)	16.0 ± 4.0 (3)	6.0 ± 0.0 (1)	20.7 ± 4.7 (3)
58	23.7 ± 2.8	33.0 ± 8.4	17.0 ± 3.1	16.0 ± 2.0

	(9)	(3)	(3)	(3)
86	16.5 ± 4.0 (6)	26.0 ± 1.5 (3)	41.7 ± 4.4* (3)	16.7 ± 2.4 (3)
114	40.3 ± 0.8 (8)	67.0 ± 11.7* (3)	41.0 ± 6.0 (2)	47.7 ± 5.0 (3)
Depuration				
142	64.0 ± 18.1 (3)	118.0 ± 16.7 (3)	N/A	49.3 ± 9.2 (3)

Table 11. Plasma ALPP (μg/ml) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	56.8 ± 9.7 (6)			
30	36.5 ± 7.4 (6)	25.3 ± 4.8 (3)	20.0 ± (1)	65.0 ± 5.0 (3)

58	28.0 ± 5.6 (5)		28.0 ± 6.7 (3)	6.0 ± (1)
86	10.8 ± 2.2 (6)	26.3 ± 8.8 (3)	23.3 ± 8.7 (3)	24.0 ± 1.0 (2)
114	37.8 ± 11.0 (8)	12.0 ± (1)	29.3 ± 7.9 (3)	58.0 ± 46.0 (2)
Depuration				
142	13.0 ± (1)	18.0 ± 9.0 (2)	N/A	13.2 ± 2.7 (3)

1.3 Gonadal development

Microscopic examination of the ovarian and testicular histological sections did not reveal abnormalities in any experimental treatments.

At the beginning of the experiment (in November), ovaries contained 50% immature and 50% previtellogenic follicles, approaching the onset of vitellogenesis (medium to large cytoplasmic vacuoles and chorion in the process of differentiation). These proportions were estimated by observing 10 separate fields of one slide at 20x.

During the next two samplings (12-5-89, 1-2-90), the proportion of previtellogenic and early vitellogenic follicles remained unchanged, but vitellogenic oocytes were gradually increasing in size.

During January (1-30-90), the proportion of early vitellogenic oocytes increased to 70-80 % , and an estimated 10-20% of the oocytes were in phase of active vitellogenesis (cytoplasm contained yolk globules).

In February (2-27-90 sample) vitellogenesis progressed rapidly. Over 90% of the ovarian follicles contained late vitellogenic oocytes that almost doubled in size and had cytoplasm filled with large yolk platelets, in some cases fused in yolk spheres.

At last sampling (3-27-90), the majority (90%) of the gonad contained large vitellogenic oocytes. In addition, there were about 10% degenerating follicles, recognized by the disintegration of yolk platelets and cytoplasm. These atretic follicles were observed in all control and treatment groups.

Spermatogenesis in the males followed a similar rate of development. During the first three months (11-7-89, 12-5-89, 1-2-90 samplings), testicular tissue contained 50-60% cysts with spermatogonia and the remaining cysts had spermatocytes in the early phase of meiotic proliferation. In sample 1-30-90, the more advanced meiotic stages (secondary spermatocytes, and spermatids) were found towards the main duct of the lobule testis. Cysts with gonial cells were still predominant around the periphery of the testis. During the next month (2-27-90) spermatogenesis accelerated, and testes contained large cysts with mature spermatozoa. At the end of March, testicular ducts were filled with free spermatozoa.

1.4 Tissue selenium concentrations

Practically all treatment groups of females at each sampling had significantly higher tissue selenium concentrations, compared with controls. Female gonads and livers exhibited a 5-20 times increase in selenium concentrations, reflecting dietary selenium dose and exposure time (Table 12). At each sampling, mean tissue selenium concentrations exhibited significant correlations ($r = 0.923$ to 0.999, $d.f.=2$) with dietary selenium levels. After one month depuration (feeding regular diet), the selenium concentrations in female gonads and liver decreased 20-40 percent, but remained at significantly higher levels, compared with the control group (Table 12). Testes appeared to accumulate less selenium, compared with
the ovaries, but liver accumulation was similar in both sexes. The selenium concentrations in male gonads and liver decreased after one month of depuration (Table 13).

Moisture content of gonadal and liver tissues is presented in Appendix 6, allowing the conversion of dry weight selenium values to wet weight. Liver dry matter content exhibited little change during different sampling times, however dry matter content of gonadal tissues changes with an increase of GSI: in the ovary, dry matter content increases during vitellogenesis; in the testis, dry matter decreases during spermatogenesis.

Table 12. Tissue selenium concentrations of bluegill females (μg/g, dry weight). Data are means, s.e.m., (n). Asterisks denote significant difference between control and treatments (Dunnett's test).

Days on Feed	Control (pooled)	DIETS Se-5.5	Se-13.9	Se-21.4
		OVARY		
0	1.88 ± .16 (4)	-	-	-
29	2.30 ± .45 (9)	3.91 ± .23 (3)	4.91 ± 1.22* (3)	10.30 ± 0.78* (3)
57	2.24 ± .13	5.55 ± .11*	8.06 ± 1.63*	19.24 ± 0.61*

	(9)	(3)	(2)	(3)
85	2.65 ± .24 (6)	7.07 ± .64* (3)	20.20 ± 3.50* (3)	31.83 ± 9.38* (3)
113	2.17 ± .05 (8)	10.89 ± 1.83* (3)	26.17 ± 0.07* (2)	40.32 ± 2.44* (3)
Depuration				
141	2.76 ± .10 (3)	6.31 ± 0.64* (3)	N/A	32.12 ± 2.20* (3)
		LIVER		
0	3.07 ± .40 (4)	-	-	-
29	3.23 ± .37 (9)	5.78 ± .38* (3)	8.19 ± 1.84* (3)	19.07 ± 0.41* (3)
57	2.64 ± .16 (9)	5.40 ± 2.69* (3)	18.92 ± 2.79* (3)	25.37 ± 2.57* (3)
85	3.75 ± .14 (6)	.10 ± 1.41* (3)	29.12 ± 3.47* (3)	36.10 ± 7.02* (3)
113	2.51 ± .32 (8)	NA[1]	22.75 ± 2.96*	40.68 ± 2.14* (2)
(3)				
Depuration				
141	3.15 ± .80 (3)	9.25 ± 1.24* (3)	N/A	27.24 ± 2.28* (3)

NA[1]) Concentration was 8.83 ± 1.09* (3). However, sample was analyzed 4 days after digestion.

Table 13. Tissue selenium concentrations of bluegill males (μg/g, dry weight). Data are means, s.e.m., (n). Asterisks denote significant difference between control and treatments (Dunnett's test).

Days on Feed	Control (pooled)	DIETS Se-5.5	Se-13.9	Se-21.4
		TESTIS		
0	2.78 ± .11 (6)	-	-	-

29	3.20 ± .36 (6)	6.07 ± 1.13 (3)	15.72 (1)	8.58 ± 1.53* (3)
57	3.26 ± .68 (5)	N/A	12.91 ± 2.61* (3)	7.19 (1)
85	4.43 ± .84	4.94 ± .59 (6)	15.24 ± 1.98* (3)	24.79 ± 6.52* (2)
113	2.65 ± .21	9.87 (8)	16.38 ± 0.71* (1)	29.70 ± 5.02* (2)
Depuration				
141	4.94 (1)	6.06 ± 0.28 (2)	N/A	18.70 ± 1.59 (3)
		LIVER		
0	3.80 ± .33 (6)	-	-	-
29	3.29 ± .48 (6)	5.64 ± .25 (3)	12.75 (1)	18.01 ± 3.82* (3)
57	2.76 ± .37 (5)	N/A	22.67 ± 3.67* (3)	41.56 (1)
85	4.68 ± .45 (6)	10.93 ± 1.46* (3)	21.68 ± 2.21* (3)	29.47 ± 7.66* (2)
113	4.10 ± .37 (8)	14.32 (1)	24.28 ± 4.54* (3)	52.47 ± 5.23* (2)
Depuration				
141	7.02 (1)	9.93 ± 0.40 (2)	N/A	25.69 ± 4.58 (3)

2. Spawning Performance (population B)

2.1 Gonadal Histology

Histological examination of the broodstock gonads after spawning did not reveal any differences between control and treatment groups. Ovaries of spawned females contained previtellogenic, vitellogenic, mature and post-spawned follicles. Testes contained mature spermatozoa, and the next generation of spermatogonia along the periphery of the testicular cross-section. There were no apparent atretic changes in the ovaries and testes, except for the advanced (overripe) ovarian follicles, usually observed in bluegill females.

2.2 Ovulatory Response and Fertilization Success

Individual ovulatory response, latency, and egg fertility are shown in Appendix 7. Adequate ovulatory response was scored "1", based on the observed quality of ovulation and egg fertility. Lack of ovulation (no eggs stripped) or poor ovulation (overripe eggs, or eggs with little ovarian fluid) were scored as "0". One female (13.9 ppm selenium treatment) did not respond to hormonal stimulation, but this fish also had the smallest gonads at necropsy. Two injected females and one non-injected female (all in the 28 L-methionine control group) spawned naturally. Only 7 females (1 or 2 in each treatment except the high selenium) were given an ovulation score of 0, and no larval bioassay was conducted for these females. There was no clear treatment effect to the poor ovulations: in fact, the high-dose selenium treatment exhibited all normal ovulations.

The proportions of ovulatory females with score "1" ranged from 50-86 percent in control groups and 71-100 percent in the selenium treatments, with no significant differences between control and treatment groups. Overall ovulatory success was 81.6% (Table 14). Latency time exhibited small variation (9-12 hours), and control and treatment groups did not significantly differ as well. Mean fertilization success was 71 % in control and 76 % in selenium treatment groups, with no significant difference between control and treatments (Table 14).

Table 14. Ovulatory response, latency, and egg fertility of bluegill females, administered LHRHa. Data are means and standard deviations.

Treatment	N	Ovulation (scored "1") (n/percent)	Latency (hours)	Fertilization (percent)
Control:				
08	6	5 / 83.3	8.8 ± 1.0	78 ± 16.6
18	7	6 / 85.7	10.2 ± 2.8	69 ± 15.9

28	4	2 / 50.0	10.6 ± 2.6	58 ± 16.7
Pooled:	17	13 / 76.5	9.7 ± 2.4	71 ± 17.4
Selenium Treatment:				
5.5	7	5 / 71.4	11.5 ± 1.9	76 ± 7.9
13.9	7	6 / 85.7	10.8 ± 2.2	75 ± 14.1
21.4	7	7 / 100	11.7 ± 3.3	76 ± 8.3
repPooled :	21	18 / 85.7	11.3 ± 2.7	76 ± 10.5
Control & Selenium Treatment Pooled:	38	31 / 81.6	10.7 ± 2.7	74 ± 14.1

2.3 Observations on Early Development

Observations on early development (from hatching to Day 5 posthatch) revealed a severe effect of the 21.4 ppm selenium treatment on larval development. Systemic edema and underdevelopment of the lower jaw were evident in all larvae from this treatment on Day 3 posthatch, and there was complete mortality by day 5, except for two progenies where 10% of the larvae appeared normal. These abnormalities were not observed in control groups and in the 5.5 pm maternal selenium treatment. However, 3 out of 6 progenies from 13.9 selenium treatment exhibited 10 to 20 percent larvae with abnormalities similar to high selenium treatment (Appendix 8). The average proportions of larvae with edema were 5 ± 2 percent in 13.9 ppm, and 95.7 ± 2.7 percent in 21.4 ppm treatment (x ± SD), significantly different from the pooled control and 5.5 ppm selenium treatment (Table 15).

Yolk sac and oil globule absorption were affected by selenium treatment (Table 16). Areas of larval yolk sac and oil globules were significantly larger on Days 3 and 4 in 13.9 ppm selenium treatment, and on Days 2, 3 and 4 in 21.4 ppm selenium treatments. These abnormalities are shown in microphotographs, taken during the four consecutive days of posthatch development (Figure 3).

Total length of larvae was similar in all experimental groups on Day 1 posthatch, but significantly smaller in all three

selenium treatments on Day 2 posthatch. During the Days 3 and 4 the significant difference in length was observed only between control and the 21.4 ppm selenium treatment (Table 17). The utilization of the yolk sac for embryonic and larval growth might have been influenced by all selenium treatments, but persistent effect was observed only at the high dose. Throughout the period of observation on early development, water temperature was constant (26 ± 0.5 °C), and the dissolved oxygen in beakers never fell below 5 ppm (Appendix 9).

Table 15. Proportions of 5 day-old larvae with edema in different treatments. "n" is a number of progenies examined. Asterisks denote significant difference between control and treatments (Dunnett's test).

TREATMENT	n	PROPORTIONS (x ± SD)
Control (pooled)	14	0.000 ±
Se - 5.5	5	0.000 ±
Se - 13.9	6	0.050 ± 0.020 *
Se - 21.4	7	0.957 ± 0.027 *

Table 16. Cross-sectional optical areas (mm^2) of the yolk sac and oil globules in larvae from pooled control and selenium treatments at Days 1 through 4 posthatch. Asterisks denote significant difference between each selenium treatment and pooled control (analysis of variance and Dunnett's test). Sample size for each progeny was 10 larvae. Data are mean ± s.d. Table "n" values show the number of examined progenies.

	YOLK AREA (mm^2)			
	Day 1	Day 2	Day 3	Day 4

Treatment				
Pooled Controls (n=14)	.460 ± .052	.315 ± .043	.105 ± .018	.060 ± .016
Se - 5.5 (n=5)	.450 ± .044	.324 ± .033	.103 ± .021	.061 ± .014
Se - 13.9 (n=6)	.464 ± .041	.324 ± .032	.145 ± .043*	.080 ± .021*
Se - 21.4 (n=7)	.457 ± .046	.425 ± .031*	.298 ± .040*	.137 ± .040*

OIL GLOBULE AREA (mm^2)

Treatment	Day 1	Day 2	Day 3	Day 4
Pooled Controls (n=14)	.097 ± .014	.068 ± .013	.030 ± .009	.010 ± .005
Se - 5.5 (n=5)	.096 ± .012	.065 ± .011	.030 ± .006	.010 ± .003
Se - 13.9 (n=6)	.093 ± .013	.070 ± .011	.037 ± .008*	.014 ± .006*
Se - 21.4 (n=7)	.096 ± .016	.076 ± .013*	.060 ± .012*	.031 ± .007*

Table 17. Total larval length in pooled control and selenium treatments, at Day 1 through 4 posthatch. Data are means ± s.d. Asterisks denote significant difference between each treatment and control (analysis of variance and Dunnett's test). Sample size is 10 larvae for each progeny. Table "n" show number of progenies examined.

Treatment	TOTAL LENGTH (mm) Day 1	Day 2	Day 3	Day 4
Pooled Controls (n=14)	4.09 ± .12	4.62 ± .14	5.06 ± .13	5.32 ± .14
Se - 5.5 (n=5)	4.10 ± .12	4.56 ± .15*	5.08 ± .11	5.29 ± .16
Se - 13.9 (n=6)	4.10 ± .13	4.56 ± .14*	5.06 ± .15	5.30 ± .19
Se - 21.4 (n=7)	4.05 ± .15	4.48 ± .13*	4.90 ± .14*	5.18 ± .10*

2.4 Survival and Growth of Larvae in Bioassays

Three bioassays in control and 5 in selenium treatments were excluded from the analysis of 30-day survival because of significant difference in the proportions of survived larvae in three beakers by Day 16 (Appendix 4). However, survival to Day 16 was analyzed for most of the progenies, by pooling data for two beakers and deleting one beaker with the significantly different survival rate. One control progeny, 18-3-C, was entirely omitted (Appendix 5).

Survival in the bioassays is summarized in Table 18. Control survival was 70-80 percent, and the analysis of variance and Dunnett's test did not reveal significant difference between three control groups. Mean survival in selenium treatments was lower than the control (range 2.5 - 65 percent), but statistically significant difference was observed only in the 21.4 ppm treatment, where more than 95 percent of larvae died before Day 16. Cumulative mortality curves for control and each treatment are shown in Figure 4. Major mortality occurred between days 5 and 8 after fertilization (Days 3-6 after hatching) in selenium treatment 21.4 ppm, with very few individuals survived to

metamorphosis. Low and medium selenium treatments exhibited only slight increase in larval mortality that was, in general, similar with control. A compressed developmental period of high mortality in the 21.4 ppm selenium treatment indicates acute effect of maternal selenium treatment on larval development during the endogenous feeding phase.

Data on fry body size and proportions of abnormalities at age 30-days, are summarized in Table 19. Both total length and body weight were significantly smaller in the 21.4 ppm selenium treatment, compared with the pooled control. The condition factor of fry ($100xBW/TL^3$) ranged from 1.612 to 1.659 and did not differ between control and treatment groups. The proportions of abnormal juveniles appeared to be elevated in low and high selenium treatments, but statistical analysis did not reveal significant differences between control and treatment groups. Abnormalities observed in 30-day old fry might have been caused by both developmental defects and environmental factors. In contrast to edema observed at yolk sac stage, they did not discriminate selenium treatments. Water quality parameters in the bioassay system are given in Appendix 10.

Table 18. Survival of bluegill larvae in the bioassays. "n" is the number of progenies tested in each treatment. Asterisks denote significant difference between control and treatment means (Dunnett's test).

Treatment	Proportion Alive		
	Mean	Std. Dev.	n
Control:			
Survival, Day 16			
08	0.753	0.100	5
18	0.711	0.085	5
28	0.805	0.107	5
Survival, Day 30			
08	0.730	0.110	5
18	0.688	0.087	5
28	0.715	0.045	3
Control and treatments:			
Survival, Day 16			
Pooled control	0.756	0.099	15
Se-5.5	0.649	0.251	5
Se-13.9	0.626	0.235	6
Se-21.4	0.025*	0.035	7
Survival, Day 30			
Pooled control	0.710	0.085	13
Se-5.5	0.519	0.265	3
Se-13.9	0.644	0.034	3
Se-21.4	0.025*	0.035	7

Table 19. Body size of 30-day old bluegill juveniles and proportions of the individuals with spinal and head deformities, and non-inflated swimbladders. Data are means and standard deviations. "n" is the number of progenies tested in each

treatment. Asterisks denote significant differences between pooled control and selenium treatments (Dunnett's test).

Treatment (n)	Total Length (mm)	Weight (mg)	Abnormalities (percent)
Control:			
08 (5)	19.2 ± 0.6	116 ± 14	6.0 ± 8.6
18 (6)	19.2 ± 0.9	119 ± 15	8.8 ± 8.7
28 (5)	18.8 ± 1.7	106 ± 37	3.5 ± 4.8
Control and treatments:			
Pool.Cont.(16)	19.1 ± 1.2	114 ± 24	6.3 ± 7.9
Se-5.5 (5)	19.9 ± 1.2	133 ± 27	15.0 ± 5.8
Se-13.9 (6)	19.3 ± 0.8	119 ± 16	7.2 ± 3.1
Se-21.4 (4)	16.6 ± 2.5*	81 ± 37*	25.0 ± 43.3

2.5 Tissue Selenium Concentrations

Tissue selenium concentrations of the broodfish and resulting progenies are shown in Table 20. Concentrations in female livers were similar with those of fish from population A on Day 113 (Table 12). Concentrations of postspawned (stripped) ovaries were lower, compared with maturing ovaries, indicating that some selenium is lost with the ovulated eggs. Selenium concentrations of somatic and reproductive tissues were significantly elevated in all treatments, compared with controls, except for the male liver in the 5.5 ppm treatment (Table 20). Highest levels were observed in female liver, and the lowest in female muscle and testes.

Selenium concentrations of eggs, sampled 24 hours after fertilization, were lower than those in the liver, but higher than concentrations in the muscle and stripped ovary. Only three progenies (all from 21.4 ppm selenium treatment) were sampled at the yolk sac stage. Their selenium concentrations were high and similar to the concentration in fertilized eggs. However, 30 day old juveniles exhibited low (not different from control) selenium concentrations in all maternal treatments, indicating that

selenium was metabolized during a period between the end of endogenous feeding and metamorphosis (Table 20). Rotifers and brine shrimp nauplii, used as larval feed, had low selenium concentrations (0.5 μg/g, dry weight).

Egg selenium concentrations exhibited highly significant correlations with those of liver, muscle, and ovary in spawned females ($r = 0.947$, 0.947, and 0.957, respectively, DF=36). The regression analysis with log-transformed data revealed significant ($P<0.05$) relationships between selenium concentrations in fertilized eggs and those in liver, muscle and ovary of respective mothers (Figures 5a, 5b, 6a). Female liver, muscle, ovary, and fertilized eggs selenium levels exhibited significant linear relationships with dietary selenium (Figure 6b, equations are based on mean values, reported in Table 20).

Table 20. Tissue selenium concentrations in broodstock bluegill and their progenies (μg/g, dry weight). Data are means ± s.e.m. (n). Asterisks denote significant difference between control and treatment (Dunnett's test).

TISSUE	CONTROL (pooled)	Se-5.5	Se-13.9	Se-21.4
		TREATMENTS		
		MALES		
LIVER	4.07 ± 0.23 (9)	6.94 ± 1.58 (3)	20.46 ± 3.46* (3)	31.63 ± 1.75* (3)
TESTIS	1.87 ± 0.11 (9)	3.64 ± 0.47* (3)	9.96 ± 0.45* (3)	15.25 ± 0.45* (3)
		FEMALES		
LIVER	4.00 ± 0.26 (20)	12.33 ± 1.09* (7)	25.98 ± 4.28* (7)	47.60 ± 4.11* (7)
MUSCLE	1.47 ± 0.14 (20)	5.80 ± 0.79* (7)	10.41 ± 2.02* (7)	23.64 ± 2.04* (6)
OVARY	2.23 ± 0.11 (20)	6.34 ± 0.47* (7)	14.10 ± 2.62* (7)	30.63 ± 3.23* (7)
		PROGENIES		
EGGS[1]	2.81 ± 0.14 (19)	8.33 ± 0.63* (7)	19.46 ± 3.83* (6)	38.39 ± 3.14* (7)
LARVAE[2]	N/A	N/A	N/A	35.30 ± 4.16 (3)
FRY[3]	1.48 ± 0.11 (16)	1.25 ± 0.02 (5)	1.37 ± 0.06 (5)	1.46 ± 0.03 (2)

1) 24 hours after fertilization.
2) 4 days after fertilization.
3) 30 days after fertilization.

2.6 The Effect of Maternal Tissue Selenium on Larval Mortality in Bioassays

The proportions of larvae with edema and delayed resorption of the yolk sac indicates a significant negative effect of maternal selenium treatment 13.9 ppm on larval development. The effect was not observed in the 5.5 ppm treatment. Therefore, dietary selenium concentrations 5.5 and 13.9 ppm, and their respective mean selenium concentrations in female tissues and fertilized eggs can be treated as NOEC and LOEC values for reproductive failure. Data in Table 20 show the intervals of maximum acceptable tissue selenium concentrations.

Nonparametric Spearman-Karber method was used for all estimations of LC_{50} since the tolerance distribution substantially deviated from normal (due to high mortality in one observation from 5.5 ppm selenium treatment and low mortalities in two observations with 13.9 ppm, data in Table 21).

The resulting NOEC, LOEC and LC_{50} values for maternal tissue selenium are given in Table 22. LC_{50}'s for all tissues are within their observed MATC ranges. Muscle tissue has the lowest LC_{50} and LOEC values, and liver tissue exhibits the highest values. Data in Appendix 6 can be used to convert these values from dry to wet weight of tissue.

Table 21. Thirty day bioassay mortalities and tissue selenium concentrations in respective females. "n" is number of eggs on Day 0, "r" is mortality on Day 30, "p" is proportions. One control treatment with highest selenium concentration was used to estimate LC_{50} for respective tissue.

				Selenium, µg/g, dry weight (female)			
Progenies	n	r	p	Ovary	Liver	Muscle	Eggs
08-2C	89	17	0.191	1.95	4.04	2.25	3.54
18-4C	85	17	0.200	2.38	5.03	0.95	3.25
5.5-1S	85	64	0.753	7.72	14.89	7.07	11.49
5.5-2S	90	42	0.467	5.55	7.06	5.80	8.31
5.5-6S	85	19	0.224	4.06	10.49	1.41	6.18
13.9-1S	90	29	0.322	3.94	7.54	2.75	8.55
13.9-3S	87	34	0.391	21.82	34.74	15.44	22.06
13.9-6S	87	31	0.356	20.40	36.82	16.58	30.20
21.4-1S	88	87	0.989	29.90	38.02	NA	44.02
21.4-2S	90	89	0.989	45.82	33.96	31.10	36.31
21.4-3S	86	79	0.919	27.24	59.01	17.28	25.21
21.4-4S	88	88	1.000	23.18	62.71	27.40	52.18
21.4-5S	90	90	1.000	32.64	55.25	24.00	42.40
21.4-6S	86	86	1.000	37.63	48.14	24.66	38.47
21.4-7S	88	82	0.932	18.02	36.10	17.42	30.12

Table 22. MATC and LC_{50} (95% CL) tissue selenium concentrations for reproductive success of bluegill.

TISSUE	Selenium, µg/g, dry weight		
	NOEC	LOEC	LC_{50}
Liver	12.3	26.0	17.4 (15.7-19.4)
Eggs	8.3	19.5	16.4 (15.0-18.0)
Ovary (stripped)	6.3	14.1	10.8 (9.7-12.1)
Muscle	5.8	10.4	10.1 (9.0-11.3)

D. DISCUSSION

1. Bioaccumulation of selenium in somatic and reproductive

tissue.

Previous observations on bioaccumulation of dietary selenomethionine in fish tissue indicate that organic selenium is readily incorporated in the somatic and reproductive tissues, and has a long retention time (Hilton et al., 1980; 1982; Hodson and Hilton, 1983; Kleinow and Brooks, 1986; 1986a). Tissue saturation in the fathead minnow and bluegill was observed during a period of exposure from 2 weeks to 3 months (Bertram and Brooks, 1986; Lemly, 1982; Ogle and Knight, 1989). Our data are in general agreement with these reports. Several field observations indicate that the ovarian and testicular tissues may reach selenium concentrations similar with liver, and higher than those in the muscle or carcass (Cumbie and Van Horn, 1978; Sager and Cofield, 1984; Gillespie and Baumann, 1986; Sorenson, 1991).

Bioaccumulation of selenium in the gonads of fish depends on stage of gonadal development e.g. proliferation of germ cells in males and vitellogenesis in females. In addition, gonadal tissue of fish undergoes substantial changes in dry matter content during the reproductive cycle. Absence of information on stage of gonadal development (GSI) or dry matter content makes it difficult to interpret many field observations.

Bioaccumulation of selenium in the carcass, ovary and testis of bluegill broodstock, experimentally exposed to dietary selenomethionine and waterborne inorganic selenium, was recently reported by National Fisheries Contaminant Research Center, Missouri (Lemly, 1990). Ovaries and testes concentrated more selenium, compared with the carcass, but the final ovarian concentrations were higher compared with testes. Higher concentration of selenium in the egg yolk of vitellogenic oocytes could account for differences in gonadal bioaccumulation between the two sexes.

High concentration of selenium in maturing ovary and significant relationships between selenium bioaccumulation in liver, ovary and freshly spawned eggs suggest that selenium is stored in the egg yolk proteins, as was indicated by the analyses of yolk protein fractions in white sturgeon, Acipenser transmontanus from the Sacramento - San Joaquin Estuary (Kroll and Doroshov, 1991). The potential routes of selenium transfer may include vitellogenin synthesis in the liver, selenium-binding proteins, and incorporation of free selenium forms into the oocyte during the pre-ovulatory egg hydration phase.

2. Effect of Tissue Selenium on Reproductive Performance

Inspite of high selenium concentrations in somatic and reproductive tissues of bluegill from dietary treatments, and

potential subtle effects on growth and health of the broodstock in the high selenium treatment, we did not observe negative influence of selenium treatment on gametogenesis. The vitellogenic phase of the ovarian development was similar in all treatments, characterized by the increases of GSI analogous to the reproductive profile of wild bluegill stock (James, 1946). Natural spawning in our trials was adversely affected by conditions in the water system, but females in all treatments responded to hormonal injection with ovulation and produced eggs of high fertility; males were spermiating naturally throughout period of spawning. The researchers of NFCRC (Lemly, 1990) did not observe inhibition of natural spawning in bluegill in the high-dose (33.3 μg/g) dietary selenomethionine treatment. Other studies did not reveal negative effects of chronic dietary or waterborne selenium treatments on gonadal development, natural spawning, fertility, and hatchability of bluegill or fathead minnow (Gillespie and Baumann, 1986; Woock et al, 1987; Ogle and Knight, 1989; Schultz and Hermanutz, 1990; Lemly, 1990).

The experiments of Gillespie and Baumann (1986), Woock et al (1987), and Lemly (1990) indicated that the early larval mortality of bluegill correlates with selenium accumulated in spawned eggs. Freshly spawned eggs of bluegill in this study had selenium concentrations proportional to other maternal tissues and the dietary treatment dose. Furthermore, the newly hatched yolk sac larvae from the high selenium treatment had high selenium concentrations, similar to freshly spawned eggs, whereas the same progenies survived to metamorphosis exhibited low selenium concentrations, similar to the control. Therefore, the selenium stored in the egg yolk should be metabolized during yolk sac absorption.

The greatest impact of bioaccumulated selenium on embryonic and larval development can be anticipated in late embryonic or early larval stages, when the rates of yolk sac utilization increase compared to early embryonic development. Toetz (1966) provides detailed information on the utilization of yolk by the bluegill embryos. He indicates that only a small amount of yolk, mainly non-proteins are utilized before hatching, and 90 percent of the yolk proteins are utilized by larvae between hatching and 4th day posthatch, concomitant with observed abnormalities and larval mortality in selenium treatments. It appears that selenium-enriched yolk does not affect early phase of embryonic development, but produces major impact during yolk sac utilization for growth.

We observed almost 100% bioassay mortalities in the high selenium treatment, with maternal selenium concentrations of 48 μg/g in liver, 24 μg/g in muscle, and 38 μg/g in fertilized eggs. The effects of low and medium selenium treatments were more variable:

the average survival was less than in the control, but this decrease was not statistically significant. However, larvae in medium selenium treatment exhibited edema and delay of yolk sac resorption. This sublethal effect was observed at selenium tissue concentrations ranged from 10 to 26 µg/g (muscle and liver, respectively).

Similar acute effect of tissue selenium on bluegill larvae at high concentrations was reported by NFCRC (Lemly, 1990). However, sublethal concentrations of tissue selenium were not elucidated. Lemly and Smith (1987) proposed a value 12 µg/g (dry weight) of whole body selenium residue as a threshold level for reproductive failure in centrarchids, which approximately corresponds to LOEC values in this study. Ogle and Knight (1989) did not observe reproductive failure in the fathead minnow at the concentrations of selenium 8 µg/g in female's muscle and 11 µg/g in the ovaries (dry weight). Schultz and Hermanutz (1990) reported distinct reproductive failure in the same species at ovarian concentrations of 6 µg/g (wet weight), which corresponds to 23-26 µg/g selenium on a dry weight basis. Literature data supports a sublethal selenium concentration of maternal tissues to be within the range of 15-20 µg/g (dry weight).

The negative effect of yolk selenium on offspring can be manifested differently by fish species with different size of eggs and different rates of embryonic development. For example, our data for channel catfish indicate a significantly lower LC_{50} value for broodstock liver and spawned eggs, compared with the bluegill. However, even at twice lower selenium concentration in the yolk of channel catfish, the total selenium burden of one egg in this species is approximately 20 times greater due to the dramatic difference in egg size. In addition, the lethal effect of yolk selenium is expressed in catfish mainly before hatching due to the prolonged period of embryonic development and greater utilization of yolk by the embryo. It suggests the LC_{50} for maternal tissue selenium may be species-specific, reflecting different reproductive strategies.

The pathological mechanism of selenium effect on embryos and larvae is not clear. Larval abnormalities in bluegill appeared to be a result of functional disorders, rather than developmental defects. The most characteristic symptom was systemic edema, and an enlarged yolk sac. The inferior growth of fry survived to metamorphosis in the high selenium treatment may reflect an inadequate nutrient supply during endogenous feeding.

Similar reproductive failures have been observed in birds. Olson (1986) induced embryonic defects in chicken by selenium injections. Heinz et al (1987) have shown that dietary selenomethionine at concentrations of 10 µg/g or higher impairs

hatching success in mallards. Olendorf et al (1986) and Hoffman et al (1988) observed selenium-induced embryonic deformities in aquatic birds at concentrations above 28 μg/g in the mother's liver and above 9 μg/g in the eggs.

Data of NFCRC (Lemly, 1990) and our study confirm dietary dose-dependent bioaccumulation of selenium in broodstock gonadal tissue. We examined the relationship between dietary selenium levels and average selenium residues of fertilized eggs in our and NFCRC studies (estimated from graphs of Lemly, 1990). Data fit the same linear regression, where dietary selenium concentrations from 6 to 15 μg/g correspond to 95% CL of LC_{50} in fertilized eggs. Thus, continuous exposure of bluegill females to dietary selenium levels above 6 ppm (dry weight) during vitellogenesis can impair reproduction. For comparison, normal dietary selenium concentrations for livestock and cultured fish do not exceed 1-2 ppm, and selenium concentrations about 3-4 are considered dangerous (NRC, 1983). Lemly (1990) reports field sampling of bluegill and food organisms from the San Joaquin River basin. In several selenium-polluted areas, concentrations of selenium in bluegill carcasses ranged from 3-8 μg/g, and in food (chironomid larvae, amphipods, detritus) 3-22 μg/g (dry weight).

E. RECOMMENDATIONS FOR FURTHER STUDIES AND A BIOMONITORING PROGRAM

This study, as well as others, have utilized large intervals between selenium treatment doses because the information on selenium bioaccumulation in maturing ovary and critical levels in the egg yolk was absent. As a result, NOEC and LOEC values within the range of sublethal effect can be further narrowed. For example, it may be useful to conduct experiments using dietary selenium ranging from 5 - 15 ppm with smaller intervals between the doses. The 30 day larval bioassay may be unnecessary. Sensitive endpoints may be achieved by examining proportions of yolk-sac larvae with edema and evaluating survival, larval defects and body length at two weeks after hatching. Histopathological examination of larvae may add significant resolution to these end points.

Bioaccumulation and critical concentrations of selenium in the ovary and resulting eggs may differ in different species of fish, depending on vitellogenesis, egg size and rates of early development. Pilot studies with other key species affected by selenium may be necessary. The experimental findings should be validated by field sampling, taking into consideration the reproductive biology of species (seasonality and duration of vitellogenesis, gonadosomatic index, and spawning time). Some of these sampling programs have already been conducted in the San Joaquin River (Saiki and May, 1988).

To utilize tissue selenium concentrations in a biomonitoring program with bluegill, fish should be sampled at the end of gonadal growth or at the start of the spawning season. For bluegill stocks in San Joaquin River basin, optimal sampling time will be during March or April, or when water temperature reaches approximately 20-22°C (see James, 1946, for detailed description of bluegill gonadal cycle). The GSI higher than 8 % indicates completion of gonadal growth in bluegill female. Only adult mature fish will be important to sample. Fork length and body weight of mature females in our study were 17 cm (range 15-22 cm) and 123 g (range 74-266 g). Average wet weight of liver (whole organ), muscle and ovary samples were 1.1, 6.2, and 8.1 g, respectively. Liver may be a less convenient tissue to sample in bluegill, because of the small size of this organ. Muscle is easy to dissect, and this sample is adequate for several analyses.

F. ACKNOWLEDGMENTS

We would like to thank A. Dennis Lemly and Jim Coyle of U.S. Fish and Wildlife Service for their helpful advises during the course of this study. We also thank Stephan W. Woock, Silas S.O. Hung and Paul B. Lutes for their assistance in the diet preparations. Two commercial farms provided bluegill stocks for this study: Chico Game and Fish Farm (Chico, California), and Rainbow Ranch (Kelseyville, California). The Aquaculture and Fisheries Program, University of California, Davis, provided assistance in animal care and maintenance of the animals, and Dr. Richard Burau provided helpful advice for the selenium analytical methodology.

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H. LIST OF FIGURES

$$Y \text{ (eggs)} = -1.025 + 1.732 * X \quad (R^2=0.971)$$
$$Y \text{ (ovary)} = -1.140 + 1.369 * X \quad (R^2=0.951)$$
$$Y \text{ (muscle)} = -0.613 + 1.037 * X \quad (R^2=0.939)$$

I. APPENDICES

Appendix 1. Water quality parameters in the spawning system.

Means and ranges of total ammonia nitrogen values for the spawning tank system. All values are given as mg/L.

Dates	Mean	Range
11/6 - 11/27/89	0.46	0.43 - 0.49
11/28 - 12/18/89	0.41	0.38 - 0.45
12/19 - 1/8/90	0.65	0.54 - 0.73
1/9 - 1/29/90	0.56	0.44 - 0.68
1/30 - 2/19/90	0.62	0.58 - 0.69
2/20 - 3/12/90	0.63	0.49 - 0.72
3/13 - 4/2/90	0.62	0.58 - 0.70
4/3 - 4/20/90	0.63	0.43 - 0.71
4/21 - 5/11/90	0.45	0.40 - 0.55
5/12 - 6/1/90	0.48	0.42 - 0.54
6/2 - 6/22/90	0.40	0.35 - 0.45
6/23 - 7/13/90	0.41	0.35 - 0.46
7/14 - 8/6/90	0.38	0.35 - 0.42

Means and ranges of pH for the spawning tank system.

Dates	Mean	Range
11/6 - 11/27/89	8.3	8.2 - 8.6
11/28 - 12/18/89	8.2	8.1 - 8.4
12/19 - 1/8/90	8.4	8.2 - 8.6
1/9 - 1/29/90	8.5	8.2 - 8.7
1/30 - 2/19/90	8.3	8.1 - 8.5
2/20 - 3/12/90	8.4	8.2 - 8.6
3/13 - 4/2/90	8.3	8.2 - 8.4
4/3 - 4/20/90	8.3	8.2 - 8.6
4/21 - 5/11/90	8.5	8.3 - 8.8
5/12 - 6/1/90	8.2	8.1 - 8.5
6/2 - 6/22/90	8.4	8.3 - 8.5
6/23 - 7/13/90	8.5	8.4 - 8.7
7/14 - 8/6/90	8.7	8.5 - 9.2

Appendix 1. (continued)

Means and ranges of water temperatures for the spawning tank system. All values are given as ° C

Dates	Mean	Range
11/6 - 11/27/89	18.9	18.7 - 19.1
11/28 - 12/18/89	18.6	18.5 - 18.7
12/19 - 1/8/90	18.6	18.0 - 18.9
1/9 - 1/29/90	20.6	19.8 - 22.2
1/30 - 2/19/90	23.8	22.4 - 24.8
2/20 - 3/12/90	25.3	24.6 - 25.7
3/13 - 4/2/90	25.5	24.7 - 26.2
4/3 - 4/20/90	25.2	19.2 - 27.3
4/21 - 5/11/90	25.3	19.3 - 26.7
5/12 - 6/1/90	26.8	19.3 - 28.9
6/2 - 6/22/90	27.1	26.8 - 28.9
6/23 - 7/13/90	27.3	27.0 - 27.8
7/14 - 8/6/90	27.4	27.3 - 28.8

Means and ranges of dissolved oxygen for the spawning tank system. All values are given as mg/L.

Dates	Mean	Range
11/6 - 11/27/89	8.3	7.8 - 8.8
11/28 - 12/18/89	8.4	8.0 - 8.6
12/19 - 1/8/90	8.6	8.1 - 9.1
1/9 - 1/29/90	8.4	7.8 - 9.3
1/30 - 2/19/90	7.1	6.3 - 7.6
2/20 - 3/12/90	7.3	5.0 - 8.2
3/13 - 4/2/90	7.4	6.3 - 8.2
4/3 - 4/20/90	7.6	6.3 - 8.3
4/21 - 5/11/90	6.7	5.6 - 7.6
5/12 - 6/1/90	6.5	5.6 - 7.6
6/2 - 6/22/90	6.9	5.4 - 7.7
6/23 - 7/13/90	7.1	6.2 - 7.7
7/14 - 8/6/90	7.0	6.2 - 8.2

Appendix 2. Culture of Algae and Rotifers

Selenastrum capricornutum was raised in large batch cultures as a food source for rotifers. Glass water bottles (18.9 L) were used for the batch cultures. Heavy aeration kept the media mixed and the algal cells suspended. Fifteen liters of media (see table) were used in each bottle. Cool white fluorescent lights were placed above and next to the bottles. The remainder of the exposed surfaces in the batch culture area was covered in reflective metallic sheeting to maximize lighting efficiency. Temperatures ranged from 20 °C to 28 °C. The media was inoculated with S. capricornutum isolates (100 ml) that had been transferred under sterile conditions and reared in 500 ml Erlenmeyer flasks on a shaker table under continuous illumination (400 foot candles). Cultures were allowed to incubate for 5 to 7 days. After incubation, the algae was settled in large refrigerated vats. The resulting concentrated algae was used to feed the rotifers.

Brachionus sp. cysts (Florida Aqua Farms, Dade City, FL) were hatched in well water in 2 L aerated beakers. Six of these beakers were maintained as pure cultures and used to inoculate large batch cultures. The large cultures were grown in 18.9 L glass bottles containing 15 L of well water. Heavy aeration was used to keep the rotifers suspended in the water column. The rotifers were fed algae daily at a concentration of 100,000 to 300,000 cells/ml of rotifers. When the rotifer concentration reached 30 rotifers/ml, 5 L could be harvested per container per day. Five liters of well water plus the day's allotment of algae were added to replace the harvested rotifers. Approximately every 30 days the batch cultures were drained, the bottles washed and bleached, and new cultures started. This procedure was done to minimize contamination with fungi, ciliates and other zooplankton, and the accumulation of organic detritus and the buildup of algal growth on the sides of the containers.

Table. Algal media composition.

Ingredient	Amount used
Nusalts, Type 1 (Argent, Redmond, WA)*	3.75 g
Filtered Sea Water (0.45 micron)	1.5 L
Glass distilled water	13.5 L

*Guillard f2 medium

Appendix 3. Selenium Analyses Inter-Laboratory Validation
Inter-laboratory validation of selenium values for the same tissue samples, analyzed at UC Davis (fluorimetry) and the Department of Fish and Game (hydride generation atomic absorption). Values are ug/g dry weight.

Gonad Tissue		Liver Tissue	
DFG (Y)	UCD (X)	DFG (Y)	UCD (X)
1.9	2.1	4.3	1.0
28.0	26.1	25.0	26.9
31.0	45.8	41.0	34.0
10.0	15.0	11.0	10.7
2.8	2.3	3.0	1.8
17.0	20.4	32.0	45.2
39.0	38.4	16.0	13.8
2.9	2.5	12.0	12.9
2.6	2.3	18.0	18.6
6.5	8.0	14.0	2.1
30.0	32.6	2.8	4.5
6.5	8.6	30.0	28.7
25.0	26.3	16.0	14.9
5.4	5.5		
2.4	2.4		
Linear Correlations Between The Two Measurments:			
r = 0.966 (P<0.05)		r = 0.912 (P<0.05)	
Y = 0.603 + 0.848x		Y = 4.357 + 0.783x	

Figure 1. UC Davis and Fish & Game selenium analyses for gonad (top) and liver tissue (bottom). Data points are measured values and the regression line fits the equation from Appendix 4.

Appendix 4. Survival of bluegill larvae in three different beakers kept in one tank to Day 16 (before pooling). Asterisks denote heterogenous survival, deleted from the analysis of survival to Day 30. Progenies are coded by dose level (8,18,28) and female number.

		NUMBER ALIVE		SURVIVAL
Progenies	Beakers	Day 0	Day 16	(p)
CONTROLS:				
8-1	A	26	21	
	B	28	20	
	C	29	26	0.807
8-2	A	31	26	
	B	29	21	
	C	29	25	0.809
8-3	A	30	21	
	B	29	18	
	C	29	23	0.705
8-5	A	30	25	
	B	29	25	
	C	30	25	0.843
8-6	A	30	19	
	B	30	16	
	C	30	19	0.600
18-1	A	28	12	
	B	29	19	
	C	28	19	0.588
18-2	A	30	21	
	B	30	24	
	C	29	22	0.753
18-3	A	30	18	
	B	33	9	0.429 *
18-4	A	28	21	
	B	29	22	
	C	28	26	0.812

18-5	A	31	23	
	B	31	20	
	C	29	18	0.670

Appendix 4. (continued).

Progenies	Beakers	NUMBER ALIVE Day 0	Day 16	SURVIVAL (p)
CONTROLS:				
18-6	A	30	25	
	B	29	21	
	C	30	19	0.730
28-2	A	30	10	
	B	30	25	
	C	30	25	0.667 *
28-3	A	25	19	
	B	22	14	
	C	23	17	0.714
28-5	A	25	20	
	B	27	22	
	C	27	25	0.848
28-7	A	31	19	
	B	28	27	
	C	28	26	0.828 *
28-8	A	30	20	
	B	29	22	
	C	29	18	0.682
Se TREATMENTS				
8-1	A	23	9	
	B	18	4	
	C	44	11	0.282
8-2	A	30	15	
	B	30	18	
	C	30	16	0.544
8-3	A	29	16	
	B	31	27	

	C	29	28	0.798 *
8-4	A	29	21	
	B	28	4	
	C	31	19	0.500 *

Appendix 4 (continued).

Progenies	Beakers	NUMBER ALIVE Day 0	Day 16	SURVIVAL (p)
8-5	A	27	24	
	B	29	21	
	C	29	26	0.835
18-1	A	30	18	
	B	30	26	
	C	30	21	0.722
18-2	A	30	24	
	B	30	24	
	C	30	16	0.711 *
18-3	A	29	19	
	B	30	19	
	C	28	19	0.655
18-4	A	28	20	
	B	27	14	
	C	30	0	0.400 *
18-5	A	30	3	
	B	29	7	
	C	27	15	0.291 *
18-6	A	29	23	
	B	29	24	
	C	29	22	0.793
28-1	A	29	0	
	B	29	0	
	C	30	1	0.011
28-2	A	30	0	
	B	30	1	
	C	30	0	0.011
28-3	A	28	0	

	B	29	5	
	C	29	2	0.081
28-4	A	27	0	
	B	30	0	
	C	31	0	0.000

Appendix 4. (continued).

Progenies	Beakers	NUMBER ALIVE Day 0	SURVIVAL Day 16	(p)
28-5	A	30	0	
	B	30	0	
	C	30	0	0.000
28-6	A	29	0	
	B	29	0	
	C	28	0	0.000
28-7	A	30	1	
	B	30	2	
	C	28	3	0.068

Appendix 5. Pooled survival of bluegill larvae in bioassays to Days 16 and 30. Significantly different beakers were removed from Day 16 end-point.

PROGENIES	NUMBER ALIVE Day 0	Day 16	Day 30	SURVIVAL (p) Day 16	Day 30
CONTROL:					
8-1	83	67	67	0.807	0.807
8-2	89	72	72	0.809	0.809
8-3	88	62	57	0.705	0.648
8-5	89	75	72	0.843	0.809
8-6	90	54	52	0.600	0.578
18-1	85	50	50	0.588	0.588
18-2	89	67	67	0.753	0.753
18-4	85	69	68	0.812	0.800
18-5	91	61	61	0.670	0.670
18-6	89	65	56	0.730	0.629
28-2	60	50	1)	0.833	1)
28-3	70	50	50	0.714	0.714
28-5	79	67	60	0.848	0.759
28-7	56	53	1)	0.946	1)
28-8	88	60	59	0.682	0.670
SELENIUM TREATMENTS:					
8-1	85	24	21	0.282	0.247
8-2	90	49	48	0.544	0.533
8-3	60	55	1)	0.917	1)
8-4	60	40	1)	0.667	1)
8-6	85	71	66	0.835	0.776
18-1	90	65	61	0.722	0.678
18-2	60	48	1)	0.800	1)
18-3	87	57	53	0.655	0.609
18-4	55	34	1)	0.618	1)
18-5	59	10	1)	0.169	1)
18-6	87	69	56	0.793	0.644
28-1	88	1	1	0.011	0.011
28-2	90	1	1	0.011	0.011

28-3	86	7	7	0.081	0.081
28-4	88	0	0	0.000	0.000
28-5	90	0	0	0.000	0.000
28-6	86	0	0	0.000	0.000
28-7	88	6	6	0.068	0.068

1) due to different survival in three pseudoreplications (beakers) before pooling survived larvae in one tank, data for 30 day survival were discarded. The survival to 16 days in beakers is based on two beakers.

Appendix 6. Percent dry weight in female and male tissue analyzed for selenium content. Data are means and standard deviations.

	Bioaccumulation Study					
	Day 0	Day 29	Day 57	Day 85	Day 113	Day 141
FEMALE TISSUE						
Liver	30.5 ± 1.5	28.8 ± 2.3	29.8 ± 1.7	28.4 ± 1.7	30.1 ± 1.3	31.9 ± 1.1
Ovary	21.8 ± 1.1	19.9 ± 1.9	19.8 ± 1.3	21.0 ± 1.6	36.7 ± 0.7	40.4 ± 14.5
GSI(%)	1.06 ± .08	1.15 ± .16	1.20 ± .11	1.24 ± .13	7.86 ± 1.41	7.33 ± 1.35
MALE TISSUE						
Liver	31.4 ± 3.1	30.8 ± 3.5	30.1 ± 1.3	28.6 ± 1.9	30.1 ± 1.1	33.1 ± 1.2
Testis	21.3 ± 5.2	22.4 ± 6.8	15.5 ± 4.3	16.8 ± 2.9	18.0 ± 1.6	20.9 ± 1.9
GSI(%)	1.50 ± .35	0.33 ± .14	0.37 ± .12	0.48 ± .21	1.04 ± .35	1.20 ± .21

	Reproductive Study	
	Day 0	At Spawning
FEMALE TISSUE		
Muscle	-----	21.3 ± 2.7
Eggs (10-12 hours postfertilization)	-----	17.0 ± 1.8
Larvae (4 days	-----	17.6 ± 1.3

posthatch)

Liver	29.8 ± 1.5	26.1 ± 2.0
Ovary	36.2 ± 2.9	26.1 ± 4.8
GSI(%)	5.63 ± 2.79	6.35 ± 2.18
MALE TISSUE		
Liver	31.0 ± .87	30.1 ± 4.8
Testis	16.1 ± .34	15.2 ± .94
GSI(%)	1.16 ± .11	1.34 ± .31

Appendix 7. Latency (time from hormonal injection) to stripping, ovulation score and fertility at one hour postfertilization data for all control treatments.

ID	Latency (hrs)	Eggs Scored (n)	Fertility (%)	Ovulation Score (0/1)	Comments
08-1C	10.2	95	73.7	1	
08-2C	9.4	128	87.5	1	
08-3C	9.0	103	78.6	1	
08-4C	9.0	120	0.0	0	overripe eggs
08-5C	7.5	96	93.8	1	
08-6C	8.0	72	45.8	1	
18-1C	13.7	109	67.9	1	
18-2C	7.0	140	92.9	1	
18-3C	10.5	109	74.3	1	
18-4C	14.0	138	43.5	1	
18-5C	8.5	105	52.4	1	
18-6C	7.5	85	71.8	1	
18-7C	11.0	54	5.6	0	"dry ovulation"
28-1C	na	61	na	na	natural spawn, no bioassay
28-2C	na	130	na	na	injected & natural spawn
28-3C	na	143	na	na	natural spawn
28-4C	13.0	98	19.4	0	overripe eggs
28-5C	13.2	110	74.5	1	
28-6C	8.0	105	26.7	0	overripe eggs
28-7C	na	na	na	na	injected & natural spawn, siphoned out posthatch larvae for bioassay
28-8C	8.0	102	41.2	1	

na = not available

Appendix 7. (continued).

ID	Latency (hrs)	Eggs Scored (n)	Fertility (%)	Ovulation Score (0/1)	Comments
08-1	12.0	108	69.4	1	
08-2	10.0	112	70.5	1	
08-3	15.0	140	89.3	1	
08-4	10.4	132	68.2	1	
08-5	11.0	97	22.7	0	dry ovulation
08-6	10.0	90	77.8	1	
08-7	10.0	123	0.0	0	dry ovulation
18-1	14.0	122	68.9	1	
18-2	13.5	112	80.4	1	
18-3	10.0	121	58.7	1	
18-4	10.0	97	54.6	1	
18-5	8.0	126	95.2	1	
18-6	9.0	114	82.5	1	
18-7	na	na	na	0	no ovulation
28-1	10.0	101	80.2	1	
28-2	18.0	98	80.6	1	
28-3	14.4	100	81.0	1	
28-4	8.0	102	55.9	1	
28-5	13.0	118	76.3	1	
28-6	9.5	101	79.2	1	
28-7	9.0	67	73.1	1	

na = not available

Appendix 8. Percent abnormal (edema) out of ten larvae sampled each day.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
8-1C	0	0	0	0	0
8-2C	0	0	0	0	0
8-3C	0	0	0	0	0
8-5C	0	0	0	0	0
8-6C	0	0	0	0	0
18-1C	0	0	0	0	0
18-2C	0	0	0	0	0
18-3C	0	0	0	0	0
18-4C	0	0	0	0	0
18-5C	0	0	0	0	0
18-6C	0	0	0	0	0
28-5C	0	0	0	0	0
28-7C	0	0	0	0	0
28-8C	0	0	0	0	0
8-1	0	0	0	0	0
8-2	0	0	0	0	0
8-3	0	0	0	0	0
8-4	0	0	0	0	0
8-6	0	0	0	0	0
18-1	0	0	0	0	0
18-2	0	0	0	0	0
18-3	0	0	0	10	10
18-4	0	0	0	20	10
18-5	0	0	0	0	0
18-6	0	0	0	10	10
28-1	0	0	100	100	all dead

28-2	0	0	100	100	all dead
28-3	0	0	90	80	90% dead*
28-4	0	0	100	100	all dead
28-5	0	0	100	100	all dead
28-6	0	0	100	100	all dead
28-7	0	0	90	90	90% dead*

* 10% were normal swim-up stage.

Appendix 9. Dissolved oxygen data for the beakers in which the larvae were reared for five days and sampled daily for measurements of total length, oil and yolk areas. All values are given as mg/L.

Treatment	Posthatch	Day 1	Day 2	Day 3	Day 4	Day 5
8-1C	5.9	6.5	6.5	6.8	6.1	6.1
8-2C	6.1	5.9	6.5	5.9	5.9	6.4
8-3C	5.9	6.5	6.4	6.1	6.5	6.9
8-5C	7.0	6.9	7.2	7.0	7.4	7.1
8-6C	6.4	7.0	7.2	7.4	7.5	7.1
18-1C	5.9	6.5	6.1	5.9	6.5	7.1
18-2C	6.0	6.1	5.9	5.9	6.1	6.5
18-3C	5.9	6.1	5.8	6.4	7.0	6.9
18-4C	6.8	6.9	7.0	6.9	6.9	7.1
18-5C	6.8	7.1	7.1	6.8	7.0	7.0
18-6C	7.2	6.5	7.1	6.9	7.4	7.1
28-5C	6.1	5.9	6.6	7.0	6.2	5.9
28-7C	6.5	6.8	6.1	6.0	7.1	6.0
28-8C	7.6	6.9	7.1	6.9	7.0	7.1
8-1	6.5	5.8	6.2	6.2	5.8	6.9
8-2	5.9	6.7	7.0	6.6	6.5	6.9
8-3	6.8	5.9	6.5	6.1	6.5	7.1
8-4	6.8	6.1	5.8	6.9	6.5	6.1
8-6	5.9	6.5	7.1	6.5	6.9	7.0
18-1	6.9	6.5	6.9	6.2	6.0	6.9
18-2	6.5	5.9	5.9	6.4	7.1	7.0
18-3	5.9	6.1	5.8	6.1	5.9	6.5
18-4	5.9	6.1	6.4	7.1	6.9	6.5
18-5	7.1	7.4	6.9	7.2	7.1	7.1
18-6	7.4	6.8	7.1	7.2	7.5	7.0

28-1	5.9	6.1	6.4	6.4	7.0	7.1
28-2	6.0	6.2	5.9	6.4	6.1	6.9
28-3	6.1	6.5	6.4	5.9	6.4	6.1
28-4	5.9	6.5	5.9	6.7	6.9	7.0
28-5	7.2	6.9	7.1	7.0	6.8	7.0
28-6	6.9	7.4	7.1	6.9	7.4	7.2
28-7	6.9	7.2	7.1	7.1	6.8	7.5

Appendix 10. Weekly water quality parameters in the larval rearing system.

Daily Water Temperature (°C)

Dates	Mean	Range
4/11 - 5/2/90	25.5	24.8 - 25.9
5/3 - 5/10/90	25.3	24.3 - 26.2
5/11 - 5/22/90	25.5	24.6 - 26.6
5/23 - 6/7/90	25.5	24.5 - 26.5
6/8 - 6/20/90	25.7	24.8 - 26.7
6/21 -7/1/90	26.0	25.8 - 26.1
7/2 - 7/8/90	26.3	25.0 - 26.8
7/9 - 7/15/90	26.6	26.3 - 27.1
7/16 - 7/22/90	26.4	25.8 - 27.2
7/23 - 7/29/90	26.2	26.0 - 26.2
7/30 - 8/5/90	26.2	25.8 - 26.5
8/6 - 8/12/90	26.1	25.8 - 26.2
8/13 - 8/19/90	26.1	25.8 - 26.2
8/20 - 8/26/90	26.1	25.8 - 26.2
8/27 - 9/2/90	26.0	25.8 - 26.1
9/3 - 9/6/90	26.0	26.0 - 26.1

Appendix 10. (continued) Weekly water quality parameters in the larval rearing system.

Date	Conductivity (umos)	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)	Total Ammonia Nitrogen (mg/L)
4/11/90	470	8.0	9.2	273.6	239.4	0.51
4/18/90	590	8.0	9.1	273.6	290.7	0.50
4/25/90	640	7.7	8.9	290.7	290.7	0.62
5/2/90	600	6.9	8.8	na	342.0	0.63
5/10/90	720	7.4	8.4	na	342.0	0.55
5/11/90	800	7.8	8.8	273.6	393.3	0.62
5/12/90	650	7.7	8.6	256.5	256.5	0.54
6/6/90	720	7.4	9.0	290.7	342.0	0.45
6/20/90	800	7.8	9.1	na	393.3	0.46
6/27/90	650	7.7	9.0	256.5	256.5	0.47
7/4/90	720	7.7	9.0	273.6	307.8	0.29
7/11/90	650	7.7	9.0	222.3	290.7	0.33
7/18/90	750	7.7	8.9	239.4	273.6	0.38
7/24/90	700	8.5	8.9	239.4	273.6	0.45
7/31/90	750	8.6	8.8	239.4	239.4	0.50
8/7/90	800	8.6	8.9	239.4	239.4	0.44
8/15/90	550	8.5	8.6	239.4	256.5	0.50
8/24/90	550	8.5	8.8	239.4	273.6	0.43
8/31/90	800	8.4	8.8	239.4	273.6	0.40
9/6/90	780	8.4	8.7	239.4	273.6	0.38

Aquatic Toxicology 148 (2014) 65–73

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon

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ARTICLE INFO

Article history:
Received 14 October 2013
Received in revised form
27 December 2013
Accepted 29 December 2013

Keywords:
Selenomethionine
Selenium toxicity
Growth performance
Tissue burden
Histopathology
Green and white sturgeon

ABSTRACT

A comparative examination of potential differences in selenium (Se) sensitivity was conducted on two sturgeon species indigenous to the San Francisco Bay-Delta. Juvenile green (*Acipenser medirostris*), recently given a federally threatened status, and white sturgeon (*Acipenser transmontanus*) were exposed to one of four nominal concentrations of dietary L-selenomethionine (SeMet) (0 (control), 50, 100, or 200 mg SeMet/kg diet) for 8 weeks. Mortality, growth performance, whole body composition, histopathology, and Se burdens of the whole body, liver, kidneys, gills, heart, and white muscle were determined every 2 to 4 weeks. Significant ($p < 0.05$) mortality was observed in green sturgeon fed the highest SeMet diet after 2 weeks, whereas no mortality was observed in white sturgeon. Growth rates were significantly reduced in both species; however, green sturgeon was more adversely affected by the treatment. Dietary SeMet significantly affected whole body composition and most noticeably, in the decline of lipid contents in green sturgeon. Selenium accumulated significantly in all tissues relative to the control groups. After 4 and 8 weeks of exposure, marked abnormalities were observed in the kidneys and liver of both sturgeon species; however, green sturgeon was more susceptible to SeMet than white sturgeon at all dietary SeMet levels. Our results showed that a dietary Se concentration at 19.7 ± 0.6 mg Se/kg, which is in range with the reported Se concentrations of the benthic macro-vertebrate community of the San Francisco Bay, had adverse effects on both sturgeon species. However, the exposure had a more severe pathological effect on green sturgeon, suggesting that when implementing conservation measures, this federally listed threatened species should be monitored and managed independently from white sturgeon.

1. Introduction

Green (*Acipenser medirostris*) and white sturgeon (*Acipenser transmontanus*) are two sturgeon species native to the San Francisco Bay Delta (SFBD) and both have exceptional biological, commercial, and ecological values (Moyle, 2002). Their populations, however, have been in steady decline since the nineteenth century (Billard and Lecointre, 2001). Recently, green sturgeon was listed as a species of special concern by the State of California and a threatened species by the United States Federal Government (California Natural Diversity Database (CNDDB), 2006). Elevated concentrations of chemical contaminants found in their diets are considered one of the primary causes of their decline (National Marine Fisheries Service, 2006).

Selenium (Se) is a major water contaminant in SFBD. It is an essential micronutrient for all vertebrates (NRC, 2005), as it is a major component of glutathione peroxidase, an enzyme that protects lipid membranes from oxidative damages at the cellular and subcellular levels (Arteel and Sies, 2001). However, at a slightly higher concentration, dietary Se is toxic to many aquatic animals (Lemly, 2002, 1985; Skorupa, 1998; Steward et al., 2004). In SFBD, major Se inputs include waste discharges originating from petrochemical and industrial manufacturing operations. The most significant source, however, is from irrigated agricultural practices on the seleniferous soils of the Central Valley (Lemly, 2004).

Most field surveys on SFBD sturgeon populations have been conducted on white sturgeon due to their larger natural population. Several such reports have noted elevated tissue Se concentrations [Se]s (up to 30 μg/g dry weight (dw) in the liver and 15 μg/g dw in the muscle; Urquhart and Regalado, 1991; Linville et al., 2002) in these animals. Similar tissue Se levels have been reported to cause toxic effects in freshwater and anadromous fish (Lemly, 2002).

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http://dx.doi.org/10.1016/j.aquatox.2013.12.030

In contrast, very little is known about Se toxicity and tissue burden in green sturgeon. Although the two species are closely related, they exhibit different responses to environmental contaminants. Recent studies have demonstrated a higher sensitivity to dietary methylmercury (MeHg) in green sturgeon compared with white sturgeon (Lee et al., 2011, 2012). Therefore, information with regards to the physiological responses of green sturgeon to environmental contaminants, in general, should not be simply extrapolated from that of white sturgeon. The objective of our current study was to determine and compare the effects of elevated concentrations of dietary L-selenomethionine (SeMet) on the growth performance, tissue burden, and histopathology of juvenile green and white sturgeon.

2. Materials and methods

2.1. Diet preparation

Four isoenergetic and isonitrogenous purified diets were prepared according to Tashjian et al. (2006) and Lee et al. (2011). Different concentrations of L-selenomethionine (Fisher Scientific, Pittsburgh, PA) were added to a basal diet mixture to constitute the four nominal levels of 0 (control), 50, 100, and 200 mg SeMet/kg diet. These SeMet concentrations were chosen to span the range of projected dietary [Se]s in SFBD (Luoma and Presser, 2000) and the selenocompound was chosen as it is the predominant Se form found in natural diets of white sturgeon (Fan et al., 2002). Furthermore, previous studies have indicated that toxic responses in animals fed SeMet were similar to those fed diets containing naturally incorporated Se compounds (Hamilton, 2004).

2.2. Animal acquisition, experimental design, and animal maintenance

The acquisition, maintenance, handling, and sampling of animals were approved by the Campus Animal Care and Use Committee at the University of California, Davis and are as described by Lee et al. (2011). Due to the different spawning and hatching times of the two sturgeon species, the two experiments were conducted consecutively, with the green sturgeon experiment conducted between June 20th and August 8th, 2007, and the white sturgeon experiment between August 29th and October 17th, 2007. In brief, 300 juvenile sturgeon (mean weight of 30 ± 2 g) were used in each of the two experiments and they were randomly distributed into twelve 90-L tanks, resulting in 4 dietary groups with 3 replicate tanks per treatment. Daily rations of 3% body weight/day (BW/d) for the first 4 weeks and 2% BW/d for the second 4 weeks (Cui and Hung, 1995) were placed in an automatic feeder (Cui et al., 1996; Hung and Lutes, 1987) which dispensed feed continuously over 24 h. Water temperature, pH, and dissolved oxygen were maintained at 18–19 °C, 7–8, and 7–9 mg/L, respectively. The effluent water was sampled weekly and [Se] was determined by a certified laboratory (BSK Analytical Laboratory, Fresno, CA, using EPA 200.8 method) and ranged from undetectable to 4.2 μg/L.

2.3. Growth performance, tissue sampling, proximate composition and selenium analysis

Fish were batch weighed on a weekly basis and feed rations were adjusted accordingly. Growth performance, tissue sampling, and diet and tissue [Se]s were determined as previously described by Lee et al. (2011) and Huang et al. (2012). For [Se] analysis, each sample was analyzed in triplicates with one replicate spiked with a sodium selenate standard to verify Se recovery. Dolt-4 (National Research Council Canada) was analyzed simultaneously with the experimental samples and the observed concentration (6.89 mg Se/kg dw) was within the certified standard range (7.06 ± 0.48 mg Se/kg dw). The [Se]s determined in the 0, 50, 100, and 200 mg SeMet/kg diet were 2.2 ± 0.2, 19.7 ± 0.6, 40.1 ± 1.5, and 77.7 ± 3.6 mg Se/kg dw, respectively. Whole body samples were lyophilized and pulverized prior to proximate composition and energy content determinations, which were determined according to AOAC, 1984, 1995, respectively.

2.4. Tissue processing and light microscopy procedures

After 4 and 8 weeks of exposure, three fish from each tank were randomly captured and euthanized with an over-dose of tricaine methanesulfonate solution (1 g/L, Argent Chemical Laboratories, Redmond, WA). Gills, heart, liver, trunk kidneys, and skeletal muscle were surgically removed, fixed, sectioned, stained, examined, and photographed according to Lee et al. (2012).

2.5. Statistical analysis

Statistical analyses were conducted using JMP 7.0 statistical software program (SAS Institute, Cary, NC). A two-way analysis of variance with interactions was used to test for significant differences among the four dietary SeMet concentrations and between the two sturgeon species. The Tukey's honestly significant difference test was used for multiple comparisons among dietary SeMet concentrations and between the two species at each time point. Statistical significance was tested at the 0.05 probability level, and all values are presented as the mean ± standard error unless noted otherwise.

3. Results

3.1. Mortality and growth performance

Significant mortality was observed in green sturgeon fed the 200 mg SeMet/kg diet from week 2 and by week 8, mortality was also seen in the 100 SeMet/kg diet group (Table 1). At the end of the study, green sturgeon exhibited a mortality of 7.7% and 23% at the 100 and 200 mg SeMet/kg diet treatments, respectively. In contrast, no mortality was observed in the white sturgeon.

Growth rates (% BWI/d) were reduced significantly in both species. After 8 weeks, green sturgeon showed depressed growth rates in all the treatment groups, when compared with the control. In contrast, white sturgeon showed depressed growth rates only at the 100 and 200 mg SeMet/kg diet treatment groups. Although growth rate was significantly higher in the control green sturgeon group, compared with that of the white sturgeon, green sturgeon was more sensitive to SeMet than white sturgeon, at all dietary SeMet levels.

Similarly, by week 8, hepatosomatic index (HSI) of green sturgeon exposed to the two upper SeMet treatments was significantly decreased compared with the control. In contrast, dietary SeMet had no significant effect on the HSI in white sturgeon.

3.2. Whole body proximate composition

Significant increases in moisture content were observed in green sturgeon fed the two highest SeMet diets. Similarly, whole body crude protein, lipid and energy contents were also significantly reduced in these treatment groups (Table 2). In white sturgeon, significant increase, compared with the control, was observed in whole body moisture content in the 200 mg SeMet/kg diet group. Significant decreases were observed in lipid contents at the 100 and 200 mg SeMet/kg diet groups. Similar decrease in energy content was also observed at the 200 mg SeMet/kg diet group.

Table 1
Growth performances of green and white sturgeon exposed to different levels of dietary selenomethionine (SeMet) for 2, 4, 6, and 8 wk.

Parameters	mg SeMet/kg diet	2 wk		4 wk		6 wk		8 wk	
		Green	White	Green	White	Green	White	Green	White
Mortality (%)	(0) Control	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
	50	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
	100	0 b	0 b	0 b	0 b	0 b	0 b	7.7 ± 4.4 b	0 b
	200	5.3 ± 1.3 a	0 b	12.1 ± 1.5 a	0 b	16.7 ± 2.1 a	0 b	23.1 ± 4.4 a	0 b
% BWI/d[a]	(0) Control	4.5 ± 1.8 a	3.0 ± 2.1 cd	11.9 ± 6.1 a	7.1 ± 0.4 b	6.3 ± 15.9 a	3.7 ± 6.5 b	6.6 ± 14.9 a	4.2 ± 14.1 b
	50	3.8 ± 3.9 ab	3.6 ± 0.2 bc	6.8 ± 8.4 bc	7.8 ± 3.6 b	3.1 ± 14.8 bc	3.9 ± 10.5 b	2.6 ± 16.0 c	4.2 ± 22.5 b
	100	2.0 ± 3.2 ef	2.7 ± 1.2 de	3.2 ± 11.1 de	4.6 ± 4.4 cd	1.0 ± 8.7 d	2.5 ± 10.6 c	0.8 ± 4.1 de	2.8 ± 20.6 c
	200	0.7 ± 1.1 g	1.5 ± 3.2 fg	0.8 ± 7.6 f	1.9 ± 3.9 ef	-0.1 ± 3.7 d	0.9 ± 6.8 d	-0.1 ± 4.3 e	1.0 ± 11.0 d
HSI[b]	(0) Control	1.9 ± 0.1 c	3.2 ± 0.2 ab	2.0 ± 0.1 bc	3.5 ± 0.3 a	1.8 ± 0.3 c	3.0 ± 0.2 ab	2.0 ± 0.1 cd	2.6 ± 0.2 bc
	50	2.3 ± 0.2 bc	3.2 ± 0.2 ab	1.9 ± 0.2 bc	3.7 ± 0.2 a	1.4 ± 0.1 c	3.3 ± 0.3 a	1.3 ± 0.0 de	3.6 ± 0.2 a
	100	2.0 ± 0.2 c	3.4 ± 0.1 a	1.8 ± 0.3 bc	2.8 ± 0.2 ab	1.1 ± 0.2 c	3.2 ± 0.4 a	0.8 ± 0.2 e	3.0 ± 0.1 ab
	200	2.0 ± 0.4 c	3.3 ± 0.1 a	1.2 ± 0.1 c	2.7 ± 0.3 ab	0.8 ± 0.0 c	1.9 ± 0.1 bc	0.9 ± 0.1 e	2.2 ± 0.4 bc

Values represent the mean ± SE ($n = 3$), and different letters denote significant differences ($p < 0.05$) among treatments and between species within each exposure periods.
[a] Percent body weight increase per day (%BWI/d) = 100 × (final body weight − initial body weight)/(initial body weight)/number of days. Initial body weight of the sturgeon were 30 ± 2 g (mean ± SE).
[b] Hepatosomatic index (HSI) = 100 × liver weight/body weight.

Table 2
Whole body proximate composition (%) and selenium burden of green and white sturgeon exposed to different levels of dietary selenomethionine for 4 and 8 wk.

Parameters	mg SeMet/kg diet	4 wk		8 wk	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Moisture	(0) Control	82.9 ± 0.7 ab	78.4 ± 0.4 c	82.9 ± 0.5 b	76.7 ± 0.4 d
	50	82.4 ± 0.5 ab	77.1 ± 0.5 c	83.5 ± 0.6 b	77.5 ± 0.4 cd
	100	83.0 ± 0.7 ab	77.8 ± 0.3 c	86.5 ± 0.8 a	77.9 ± 0.1 cd
	200	85.3 ± 1.3 a	79.6 ± 1.0 bc	88.2 ± 0.2 a	79.5 ± 0.5 c
Crude Protein	(0) Control	10.2 ± 0.1 ab	11.5 ± 0.1 a	11.5 ± 0.3 a	11.6 ± 0.3 a
	50	10.6 ± 0.4 ab	11.4 ± 0.3 a	11.0 ± 0.3 a	11.4 ± 0.0 a
	100	10.5 ± 0.4 ab	11.6 ± 0.1 a	9.3 ± 0.5 b	11.7 ± 0.2 a
	200	9.4 ± 0.6 a	11.3 ± 0.4 a	7.8 ± 0.2 b	11.3 ± 0.5 a
Crude Lipid	(0) Control	2.9 ± 0.5 c	6.2 ± 0.3 ab	2.5 ± 0.4 d	7.9 ± 0.3 a
	50	2.1 ± 0.3 cd	7.7 ± 0.3 a	1.3 ± 0.1 de	6.8 ± 0.4 ab
	100	1.5 ± 0.3 cd	6.6 ± 0.3 ab	0.4 ± 0.1 e	6.1 ± 0.2 b
	200	0.7 ± 0.2 d	5.2 ± 0.9 b	0.2 ± 0.0 e	4.5 ± 0.3 c
Energy (kcal/g)	(0) Control	5.4 ± 0.1 b	6.4 ± 0.1 a	5.4 ± 0.1 c	6.6 ± 0.0 a
	50	5.1 ± 0.1 bc	6.7 ± 0.1 a	5.0 ± 0.0 d	6.5 ± 0.1 a
	100	4.9 ± 0.1 cd	6.5 ± 0.1 a	4.6 ± 0.0 e	6.4 ± 0.0 ab
	200	4.6 ± 0.1 d	6.3 ± 0.2 a	4.4 ± 0.1 e	6.1 ± 0.1 b
mg Se/kg dw	(0) Control	6.5 ± 0.9 e	7.3 ± 0.8 e	7.1 ± 0.9 d	5.6 ± 0.3 d
	50	21.7 ± 0.5 c	15.3 ± 1.6 d	22.8 ± 0.9 c	20.1 ± 0.5 c
	100	26.2 ± 1.2 bc	22.5 ± 0.9 c	27.8 ± 1.4 bc	31.8 ± 0.3 b
	200	30.6 ± 0.7 ab	34.3 ± 2.5 a	34.3 ± 0.3 b	47.1 ± 4.3 a

Values represent the mean ± SE ($n = 3$), and different letters denote significant differences ($p < 0.05$) among treatments and species within the exposure period. Initial body composition (%): Moisture 83.0 ± 0.6 and 80.2 ± 0.8, crude protein 10.5 ± 0.3 and 9.9 ± 0.4, lipid 1.8 ± 0.2 and 5.3 ± 0.2, energy (kcal/g) 5.1 ± 0.1 and 6.3 ± 0.1 in green sturgeon and white sturgeon, respectively. Initial whole body Se concentrations in green and white sturgeon were 7.2 ± 0.3 and 4.8 ± 0.5 mg Se/kg dry weight (dw), respectively.

Moisture, lipid, and energy contents of green sturgeon were significantly different from those of white sturgeon at all levels of dietary SeMet. Noticeably, crude protein contents of green sturgeon fed the 100 and 200 mg SeMet/kg diets were significantly lower than those of white sturgeon in the same treatment groups. However, the most significant differences were observed in crude lipid contents between the two species.

3.3. Se burden

Different patterns in whole body Se burden were also observed between the two species (Table 2). White sturgeon accumulated Se in a dose and duration-dependent manner. In contrast, whole body Se in green sturgeon did not increase much after week 4 and there was no obvious dose-dependent Se accumulation. Pattern of Se accumulation among tissues were also different between the two species (Tables 3a and 3b). Selenium levels in the gills and kidneys of green sturgeon showed little increase after week 2 and week 4, respectively. In the white muscle, however, [Se] was found to have increased in a dose dependent manner up to the 100 mg SeMet/kg diet level. Liver [Se] increased continuously throughout the 8 weeks, except in those fed the 200 mg SeMet/kg diet, where [Se] decreased after reaching a concentration asymptote at week 6. Similarly in the heart, [Se] plateaued after reaching a maximum concentration at week 4. In contrast, tissue Se burden of white sturgeon generally increased with increasing exposure duration. In the 200 mg SeMet/kg diet group, the highest Se levels were observed at week 6. The highest tissue Se levels in green sturgeon were observed in the liver, whereas the highest Se levels in white sturgeon were seen in the kidneys.

3.4. Histopathological alteration

Histological examination showed progressions of marked lesions in the kidneys and liver of both species after each sampling period (Tables 4 and 5 and Figs. 1 and 2). Mild histological changes

Table 3a
Selenium tissue burden (mg Se/kg dw) in green and white sturgeon exposed to different levels of dietary selenomethionine (SeMet) for 2 and 4 wk.

Tissues	mg SeMet/kg diet	2 wk		4 wk	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Kidney	(0) Control	ND	8.0 ± 1.5 a	10.7 ± 0.4 d	9.1 ± 1.6 d
	50	ND	18.1 ± 0.8 b	34.2 ± 0.3 bc	29.5 ± 1.0 cd
	100	ND	36.0 ± 0.5 c	53.1 ± 10.4 ab	50.7 ± 6.0 abc
	200	ND	54.3 ± 2.4 d	50.7 ± 1.8 abc	71.2 ± 2.2 a
Liver	(0) Control	6.1 ± 1.1 c	5.8 ± 1.4 c	4.2 ± 0.4 d	4.9 ± 0.7 d
	50	14.0 ± 1.3 bc	12.4 ± 1.2 bc	23.3 ± 3.2 bc	14.2 ± 1.1 cd
	100	25.6 ± 2.9 ab	16.1 ± 0.7 bc	31.4 ± 6.9 bc	20.9 ± 1.1 bcd
	200	39.5 ± 7.1 a	23.3 ± 0.8 b	65.6 ± 6.1 a	32.3 ± 1.2 b
Gill	(0) Control	6.6 ± 0.2 f	8.0 ± 1.6 ef	6.7 ± 0.2 e	7.0 ± 1.5 e
	50	23.2 ± 1.2 cde	17.5 ± 1.9 def	26.6 ± 0.2 d	25.3 ± 0.3 d
	100	32.5 ± 2.0 bcd	34.7 ± 2.6 bc	35.5 ± 0.6 cb	40.7 ± 3.6 c
	200	44.4 ± 4.4 ab	51.6 ± 6.5 a	48.1 ± 1.5 b	60.3 ± 2.7 a
Heart	(0) Control	9.1 ± 0.7 d	7.6 ± 1.0 d	7.6 ± 0.7 f	6.7 ± 1.1 f
	50	22.7 ± 1.3 bc	17.0 ± 0.4 cd	25.2 ± 0.8 e	26.8 ± 1.0 de
	100	28.8 ± 0.8 b	29.7 ± 1.5 b	34.9 ± 1.2 cd	42.0 ± 1.1 bc
	200	43.1 ± 3.8 a	42.0 ± 4.0 a	45.6 ± 1.2 ab	53.1 ± 4.2 a
White muscle	(0) Control	8.4 ± 0.6 e	11.7 ± 0.8 de	9.0 ± 0.2 d	9.5 ± 0.3 d
	50	20.4 ± 0.1 bc	17.6 ± 0.7 cd	25.6 ± 0.1 c	25.3 ± 0.3 c
	100	26.9 ± 0.3 ab	25.9 ± 1.3 a b	32.2 ± 1.2 b	29.5 ± 0.5 bc
	200	32.2 ± 3.6 a	33.2 ± 0.8 a	34.7 ± 2.6 ab	40.4 ± 2.3 a

Values represent mean ± SE ($n = 3$) and different letters denote significant differences ($p < 0.05$) among treatments and species within each exposure period and tissue type. Initial Se concentrations (mg Se/kg dw) in green and white sturgeon were as follows: gill 6.6 ± 0.1 and 4.8 ± 0.5; heart 6.3 ± 0.6 and 6.5 ± 1.3; liver 7.0 ± 1.0 and 3.1 ± 0.3; kidney ND and 6.3 ± 0.9; and white muscle 7.6 ± 0.2 and 8.94 ± 0.2, respectively. ND: not determined and dw: dry weight.

Table 3b
Selenium tissue burden (mg Se/kg dw) in green and white sturgeon exposed to different levels of dietary selenomethionine (SeMet) for 6 and 8 wk.

Tissue	mg SeMet/kg diet	6 wk		8 wk	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Kidney	(0) Control	9.1 ± 0.7 e	8.2 ± 1.3 e	8.5 ± 0.6 d	9.3 ± 0.9 d
	50	35.1 ± 1.0 cd	28.1 ± 1.8 de	33.3 ± 0.6 c	33.5 ± 0.3 c
	100	60.1 ± 12.6 b	54.8 ± 1.2 bc	53.0 ± 9.8 bc	54.5 ± 3.6 bc
	200	44.4 ± 1.3 bcd	127.6 ± 8.1 a	58.1 ± 2.6 b	93.3 ± 5.6 a
Liver	(0) Control	5.1 ± 0.8 c	4.7 ± 0.5 c	6.1 ± 0.3 c	4.2 ± 0.1 c
	50	32.6 ± 1.1 bc	16.0 ± 1.1 bc	34.4 ± 3.5 bc	28.0 ± 10.4 bc
	100	78.4 ± 10.5 a	26.6 ± 1.5 bc	86.1 ± 9.7 a	30.1 ± 1.0 bc
	200	106.5 ± 14.5 a	46.8 ± 2.6 b	87.0 ± 11.2 a	56.3 ± 2.6 ab
Gill	(0) Control	6.0 ± 0.2 e	6.6 ± 1.0 e	5.4 ± 0.3 e	7.6 ± 0.7 e
	50	29.3 ± 1.4 cd	20.7 ± 5.3 d	29.5 ± 0.6 d	26.7 ± 3.3 d
	100	34.1 ± 3.5 bc	45.2 ± 2.1 b	39.3 ± 0.6 c	46.4 ± 0.7 bc
	200	45.1 ± 1.6 b	60.6 ± 0.3 a	51.6 ± 1.6 b	69.5 ± 2.4 a
Heart	(0) Control	5.5 ± 0.5 d	6.4 ± 0.3 cd	5.3 ± 0.3 f	8.8 ± 0.5 f
	50	23.6 ± 0.9 bcd	26.0 ± 1.1 bcd	24.4 ± 0.3 e	28.9 ± 0.4 de
	100	29.5 ± 1.6 bc	41.0 ± 4.2 ab	33.0 ± 1.4 cd	45.8 ± 1.7 b
	200	35.5 ± 3.3 ab	58.2 ± 12.4 a	35.6 ± 2.1 c	70.6 ± 2.1 a
White muscle	(0) Control	10.0 ± 0.5 e	9.5 ± 0.3 e	8.4 ± 0.4 e	9.2 ± 0.7 e
	50	29.7 ± 1.0 cd	25.2 ± 0.6 d	31.1 ± 0.3 cd	27.0 ± 1.1 d
	100	31.4 ± 0.7 bcd	37.4 ± 3.4 ab	37.0 ± 0.3 bc	41.3 ± 0.6 b
	200	35.7 ± 1.9 abc	42.6 ± 1.1 a	36.8 ± 1.2 bc	57.9 ± 1.2 a

Note: See Table 3a.

were noted in the skeletal and heart muscles (results not shown). However, no prominent histological changes were observed in the gills of either species at all times.

3.4.1. Trunk kidney

After exposure to dietary SeMet, the kidneys of both sturgeon species exhibited marked histological changes, compared with the controls. These changes included increased tubular epithelium degeneration (TED), renal corpuscular disintegration (CD), and interstitial tissue degeneration (ITD) (Table 4 and Fig. 1c–h). Tubular epithelium degeneration was mainly characterized by hydropic degeneration, pyknosis, and cell necrosis (Fig. 1c, e, and h). Characterization of CD included the collapse of glomerular capillary loop, hypertrophy of mesangial cells, thickening of Bowman's capsule layers, and collapse or enlargement of Bowman's space (Fig. 1c, e, and h). Lastly, ITD was identified by necrotic area and loss of tissue (Fig. 1g and h). In general, pathological alterations of the kidneys were proportional to the dose and duration of SeMet exposure.

Compared with week 4, both species displayed a more severe and higher frequency of TED, CD, and ITD in the kidneys at week 8 (Table 4). The most serious damage occurred in the tubular epithelium as TED for both species (Table 4 and Fig. 1). Although some of the lesion scores were the same between the two species, green sturgeon exhibited more severe kidney pathology in all of the SeMet treatment groups (Table 4).

Table 4
Kidney histopathological alterations of green and white sturgeon exposed to a graded levels of dietary selenomethionine (SeMet) for 4 and 8 wk.

	mg SeMet/kg diet							
	Control		50		100		200	
	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
	Histopathology at 4 wk							
TED	0	0	++	+	+++	++	+++	+++
CD	0	0	0	0	+	++	++	++
ITD	0	0	0	0	+	+	+	+
	Histopathology at 8 wk							
TED	0	0	+++	++	+++	+++	+++	+++
CD	0	0	++	+	++	++	++	+++
ITD	0	0	0	0	++	+	+++	++

Lesion severity scoring: 0 = absent or rarely observed, + = mild (affected less than 10%), ++ = moderate (affected greater than 10% but less than 50%), and +++ = severe (affected greater than 50%). TED, tubular epithelium degeneration; CD, renal corpuscular disintegration; ITD, interstitial tissue degeneration. $N = 9$.

Table 5
Liver histopathological alternations of green and white sturgeon exposed to a graded levels of dietary selenomethionine (SeMet) for 4 and 8 wk.

	mg SeMet/kg diet							
	Control		50		100		200	
	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
	Histopathology at 4 wk							
GD	0	0	+	0	++	+	+++	+
VD	0	0	++	0	++	+	+++	+++
	Histopathology at 8 wk							
GD	0	0	++	0	+++	+	+++	++
VD	0	0	++	+	++	++	+++	++

Lesion severity scoring: 0 = absent or rarely observed, + = mild (affected less than 10%), ++ = moderate (affected greater than 10% but less than 50%), +++ = severe (affected greater than 50%). GD, glycogen depletion; VD, vacuolar degeneration including single cell necrosis. $N = 9$.

3.4.2. Liver

After 4 weeks, the livers of both species showed marked histological alterations, including glycogen depletion (GD) and vacuolar degeneration (VD) (Table 5 and Fig. 2). In both species, the progression of the aforementioned alterations was generally proportional to the dose and duration of exposure. However, between the two species, the green sturgeon livers exhibited more severe GD and VD (Table 5 and Fig. 2c–h).

4. Discussion

4.1. Mortality and growth depression

In the current study, green sturgeon exhibited significant higher mortalities at the highest SeMet treatment, which is equivalent to a 78 mg Se/kg diet. However, similar to Tashjian et al. (2006), who reported a mean survival rate of 99 ± 4% in white sturgeon exposed to diets containing up to 191 mg Se/kg for an 8 week period, no significant mortalities were observed among white sturgeon in the current study. Although green sturgeon appeared to be more sensitive to dietary Se, the mortality rate was still lower than that of other fish species. A mean mortality of 37.5% was observed in Chinook salmon parr (*Oncorhynchus tshawytscha*) after an 8.6-week exposure to a 35.4 mg Se/kg diet (Hamilton et al., 1990). Arshad et al. (2011) reported a mean mortality of 25% in juveniles of beluga sturgeon (*Huso huso*) exposed to dietary Se at levels between 1.26 and 20.26 mg/kg for 8 weeks.

Compared with white sturgeon, the significantly higher mortality in the green sturgeon may be a consequence of their higher initial growth. Deng et al. (2002) reported faster growth rates in juvenile green sturgeon when compared with white sturgeon of similar age. As faster growth rate reflects a higher energy demand, the green sturgeon may have been in an overall lower energy state, especially since the diets were provided in a fixed daily ration and adjusted on a weekly basis. The low HSI, whole body lipid and energy content, and glycogen storage in the hepatocytes are all indicative of the low energy reserves in the green sturgeon.

Compared with other fish species from similar studies, green sturgeon exhibited a more severe growth rate depression. At 8 weeks, green sturgeon fed the 50 and 100 mg SeMet/kg diets (equivalent to 19.7 and 40.1 mg Se/kg diet, respectively) had their average growth rates reduced to 39% and 12% of that of the controls, respectively. In contrast, growth rates of Chinook salmon parr were only reduced to 77.9% and 37.3%, when given an 18.2 and 35.4 mg Se/kg diet in the form of SeMet for 60 days (Hamilton et al., 1990). Interestingly, juvenile beluga sturgeon fed a 20.26 mg Se/kg diet, in the form of SeMet, for 8 weeks, exhibited increased growth rates (Arshad et al., 2011). The observed reduction in growth among the green sturgeon may be a combined physiological response to: (1) the higher energy demand during the rapid initial growth phase and (2) energy relocation/adaptation to chronic Se toxicity. Thus, reduced growth is likely a physiological tradeoff for achieving a comparatively lower Se-induced mortality, as to what were seen in the aforementioned studies.

4.2. Whole body proximate composition

Proximate analysis is a good indicator of the overall physiological condition of a fish (Ali et al., 2005). In the present study, changes in proximate composition, most notably the significant decreases in protein, lipid, and energy contents, indicated that both species were experiencing physiological stress induced by dietary SeMet exposure. However, the treatment effect was more severe on green sturgeon, as the white sturgeon seemed to be in an overall better

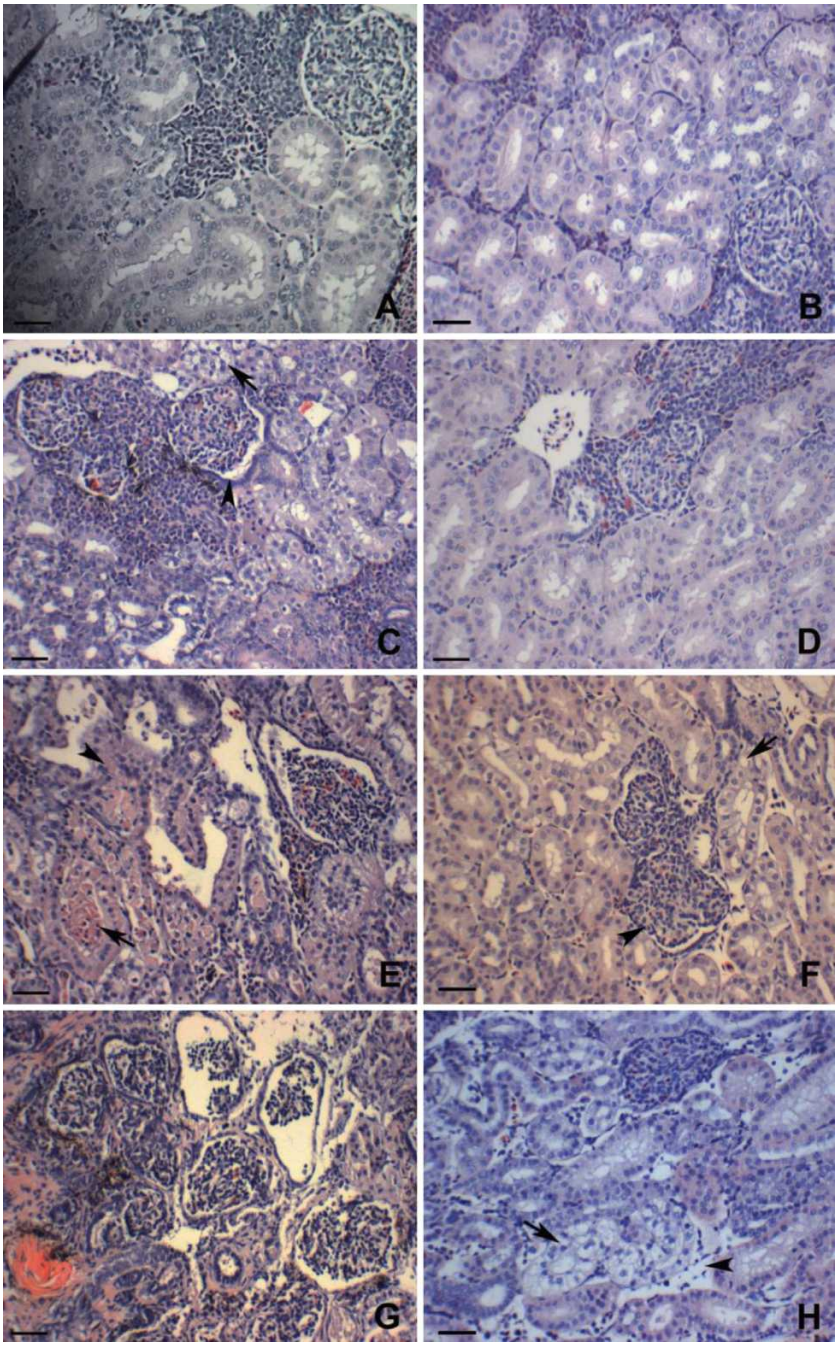

Fig. 1. The trunk kidney of *Acipenser medirostris* (left) and *A. transmontanus* (right) stained with hematoxylin/eosin: (A) and (B) kidneys of individuals from the control groups. (C) Kidney of *A. medirostris* exposed to 50 mg SeMet/kg diet for 8 weeks showing hydropic degeneration (arrow) and renal corpuscular disorganization (arrow head). (D) Kidney of *A. transmontanus* exposed to 50 mg SeMet/kg diet for 8 weeks showing slightly enlarged tubular cells. (E) Kidney of *A. medirostris* exposed to 100 mg SeMet/kg diet for 8 weeks showing severe tubular cell death (arrow head) and tubular inclusion (arrow), and renal corpuscular disintegration. (F) Kidney of *A. transmontanus* exposed to 100 mg SeMet/kg diet for 8 weeks showing moderate tubular hydropic degeneration (arrow) and collapse of glomerular capillary (arrow head). (G) Kidneys of *A. medirostris* exposed to 200 mg SeMet/kg diet for 8 weeks showing necrotic areas. (H) Kidney of *A. transmontanus* exposed to 200 mg SeMet/kg diet for 8 weeks showing severe tubular epithelium degeneration including hydropic degeneration (arrow) and loss of interstitial tissues (arrow head). All scale bars = 50 μm.

physiological condition, given the higher lipid and energy contents of their control group.

Chemical contaminants have been shown to induce physiological stress in teleosts. Beyers et al. (1999) reported that largemouth bass (*Micropterus salmoides*) utilize energy relocation to compensate for the additional energetic costs associated with toxic exposures. As described in Selye's general adaption syndrome (Selye, 1955), the authors observed a two stage energy relocation in the largemouth bass: first, an allocation of resources from somatic and reproductive growth, which have little effect on the overall energy status of the animal; and second, the allocation of body reserves such as somatic lipid and protein, which can put the animal in an energy-deficient state. Furthermore, when the stressor persists for sufficient length of time and magnitude, the animal would inevitably enter exhaustion, the third and final stage of stress adaption (Selye, 1955).

At the two highest dietary SeMet levels, physiological assessments indicated that green sturgeon were in the exhaustion stage. Characteristics such as glycogen depletion of hepatocytes, increased histopathology in the liver and kidneys, depressed

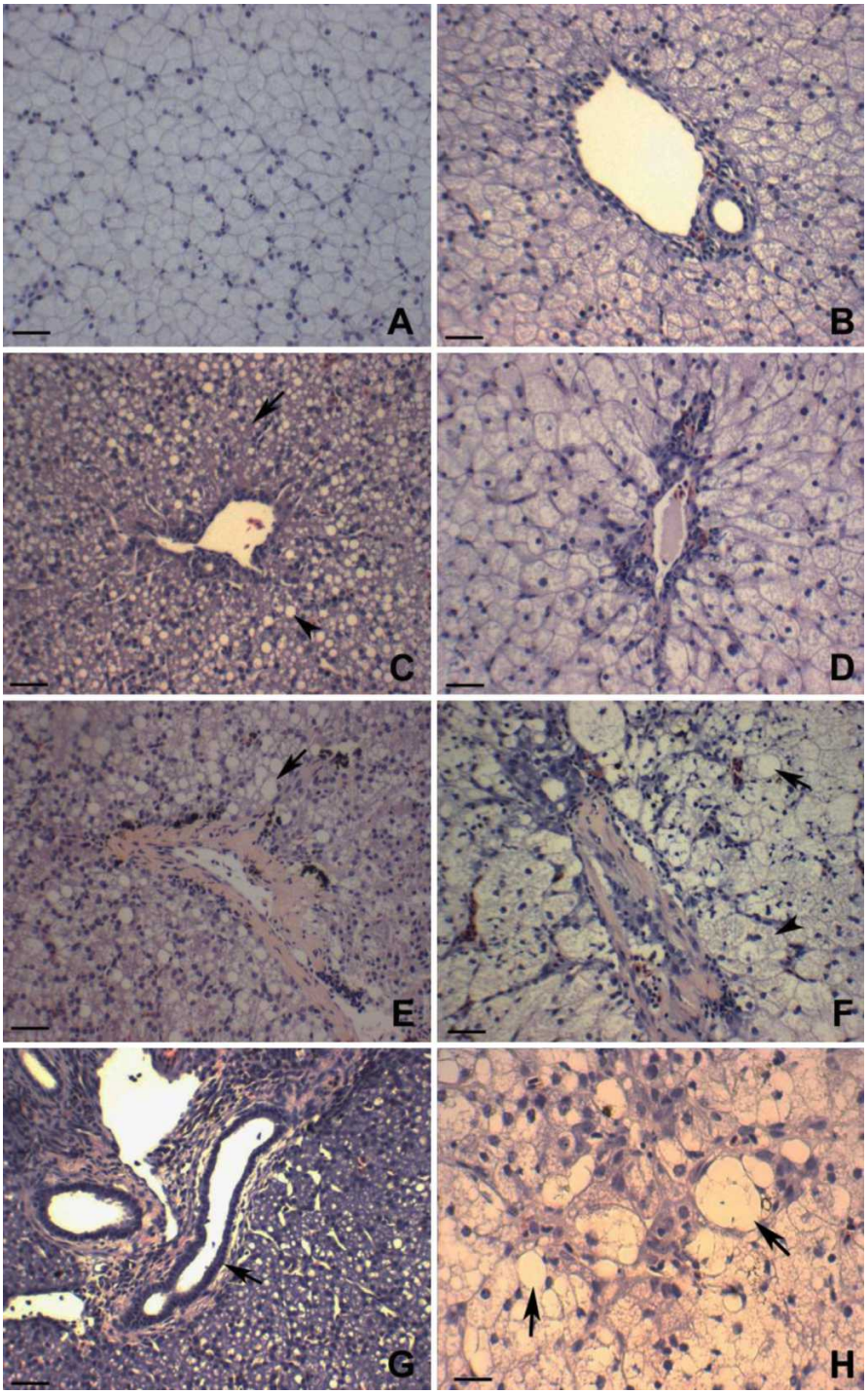

Fig. 2. The liver of *Acipenser medirostris* (left) and *A. transmontanus* (right) stained with hematoxylin/eosin: (A) and (B): Livers of individuals from control groups. (C) Liver of *A. medirostris* exposed to 50 mg SeMet/kg diet for 8 weeks showing moderate glycogen depletion (GD) (arrow) and vacuolar degeneration (VD) (arrow head). (D) Liver of *A. transmontanus* exposed to 50 mg SeMet/kg diet for 8 weeks showing slightly enlarged hepatocytes with unclear cell membranes. (E) Liver of *A. medirostris* exposed to 100 mg SeMet/kg diet for 8 weeks showing severe VD (arrow). (F) Liver of *A. transmontanus* exposed to 100 mg SeMet/kg diet for 8 weeks showing VD (arrow) and necrotic cells (arrow head). (G) Liver of *A. medirostris* exposed to 200 mg SeMet/kg diet for 8 weeks showing severe GD,VD, and dilation of bile duct (arrow). (H) Liver of *A. transmontanus* exposed to 200 mg SeMet/kg diet for 8 weeks showing VD (arrows). All scale bars = 50 μm, except the scale bar at (*H*) = 25 μm.

growth rates, and increased mortality were observed in these animals. By week 4, the animals have entered the second stage of energy mobilization, as seen in the largemouth bass (Beyers et al., 1999), in which more body constituents, such as lipid and protein, were utilized to meet the additional energy cost associated with Se toxicity. In comparison, the white sturgeon seemed to remain in the resistance state, given that their protein levels remained unaffected by SeMet. Furthermore, their body lipid contents were also significantly higher. The species difference, again, may be due to the rapid initial growth phase of juvenile green sturgeon, in which the associated high metabolic cost led to a comparatively more energetically vulnerable status. The exact cause of the observed reduction in body lipid is unknown, however, as multiple factors such as reduced food intake due to unpalatability of SeMet enriched feed and increased energy demand for Se detoxification may be involved.

4.3. Se burden

In general, whole body Se burden increased with dietary Se level and exposure duration; however, by week 4, the extent of Se bioaccumulation have slowed down in green sturgeon (Table 2). Avoidance to Se-contaminated food has been reported in waterfowl (Heinz and Sanderson, 1990) and teleost species (Hilton et al., 1980). Unpalatability of foods containing high concentrations of Se was suggested as a factor leading to food avoidances observed in birds and fish species (Ogle and Knight, 1989). In the current study, decreased feeding was noted in green sturgeon, from week 4 onwards, in the two highest SeMet groups. However, similar observation was not made during the first 4 weeks of exposure. Other Se toxicity mechanisms, such as musculature dysfunction may have also contributed to decreased food consumption in this study. Substitution of methionine (Met) by SeMet, in the disulfide bond of muscle actin filament, can generate radical oxygen species (ROS) leading to mechanical malfunction of the organ (Dalle-Donne et al., 2001; Palace et al., 2004). Histological changes observed in the white muscle of both sturgeon species (results not shown) in this study support possible musculature malfunctioning. Similarly, SeMet substitution may have also occurred in the heart muscle, as indicated by mild histological changes in the heart tissues (results not shown), and may have compromised the cardiovascular function of these animals. Thus, it is more likely that the decrease in feeding observed in the latter 4 weeks, the starvation effect, was a secondary effect of Se toxicity, such as locomotor dysfunction, rather than unpalability relating to the high SeMet content.

The highest Se burden was observed in the green sturgeon livers, at 6 weeks. However, the high liver [Se] may be a combined effect of decreased HSI (half the size of that of the controls), negative growth rates (%BWI/d), and decreased food consumption. Lee et al. (2011) reported similar findings in juvenile green sturgeon fed various levels of dietary MeHg for 8 weeks. Regardless of the mechanisms leading to the high organ Se accumulation, extensive liver damages were observed and likely were important factors contributing to the significant growth rate decline observed in green sturgeon and their subsequent high mortality.

Urine is the primary excretion route for Se. Although mammals can also excrete excess Se via feces and exhalation, the urine plays a quantitatively greater role in whole body Se homeostasis (Ellis et al., 1997; Ivancic and Weiss, 2001). Similarly, urine is also the primary Se excretory pathway in white sturgeon (Huang et al., 2012). In the current study, the significantly higher Se burden observed in white sturgeon kidneys suggests a more active depuration of Se (compounds) relatively to that of green sturgeon. However, study on both species using oral intubation and intravenous injection methods demonstrated similar SeMet assimilation and metabolism among the sturgeon (Silas S.O. Hung, University of California at Davis, unpublished date). Thus, the Se concentration plateau observed in the green sturgeon kidneys at post week 4 was likely due to decreased feed consumption rather than decreased urinary Se.

4.4. The trunk kidney

Histological changes in the kidneys in fish have been previously studied and are reliable and sensitive biomarkers for a wide variety of chemical exposures, including SeMet (Sorensen et al., 1984; Handy and Penrice, 1993; Thophon et al., 2003). In this study, the kidneys of sturgeon exposed to SeMet showed marked abnormalities, including TED, CD, and ITD. Collapsed glomerular capillaries, mesangial cell hypertrophy, abnormally abundant matrixes, thickened Bowman's capsule layers, and collapsed or enlarged Bowman's space were also observed in the renal corpuscles of SeMet exposed sturgeon. Similar damages were reported in green sunfish (*Lepomis cyanellus*) from Se-contaminated lakes (Sorensen et al., 1982, 1984) and in striped bass (*Morone saxatilis*) fed Se-contaminated live feed (Coughlan and Velte, 1989).

The extensive kidney lesions seen in both sturgeon species can be attributed to the primary excretory role of Se compounds (Suzuki, 2005) of the organ. The significant increase in green sturgeon whole body moisture content may be indicative of a compromised osmoregulation, given the extensive damages seen in the tubular epithelium. Other factors such as deprivation of energy and higher damages in the livers may also have contributed to the severe kidney lesions observed in green sturgeon, despite having a comparatively lower kidney Se burden compared to the white sturgeon.

4.5. Liver

The livers of both sturgeon species exposed to SeMet treatments exhibited adverse histological changes such as GD and VD, and are consistent with the histopathological lesions reported by Tashjian et al. (2006). Swollen hepatocytes and vacuolation were also reported in livers of green sunfish exposed to Se-elevated water (Sorensen et al., 1982, 1984). Reproductive failure was noted in the study and marked population decline followed suit. In the current study, the extent of the liver lesions may have also affected organ function, as seen in the decreased hepatocyte glycogen storage. Such will have an effect on glycogenesis and glycolysis, leading to an interruption of energy metabolism, as supported by the decrease in whole body energy content, growth, and the higher mortality in green sturgeon.

In addition, GD and single cell necrosis were also reported in Sacramento splittail (*Pogonichthys macrolepidotus*) fed SeMet-supplemented diets (Teh et al., 2004). Significant glycogen depletion was suggested as a result of increased liver glycogenolysis due to the excessive energy demand for repairing SeMet-induced damage and/or reduced food intake (Teh et al., 2004). Significant GD seen in the current study is thought to be an adaptation by the sturgeon to meet the high energy demand when exposed to high levels of dietary SeMet.

Laboratory studies reported hepatic oxidative stress in mallard ducks (*Anas platyrhynchos*) exposed to dietary SeMet (Hoffman, 2002). Increased dietary Se elevated plasma and hepatic GSH peroxidase activities, followed by an increased ratio of oxidized to reduced glutathione (GSSG:GSH) and hepatic lipid peroxidation. The oxidative effects were associated with teratogenesis, reduced growth, diminished immune function, and histopathological lesions. Similarly, oxidative stress is believed to have induced the histological changes observed in the current study. Deposition of dark pigments, which is thought as indicators of oxidative stress in northern pike (*Esox Lucius*; Drevnick et al., 2008), were also observed in the livers of sturgeon in the highest SeMet treatment groups and were found to be especially numerous in green sturgeon. Thus, liver damage, likely a result of Se-induced oxidative stress, may be a major factor contributing the higher susceptibility to Se toxicity by the green sturgeon in this study.

It is possible that the comparatively faster initial growth rates of juvenile green sturgeon have resulted in their energetically vulnerable states. As growth requires an increase in protein synthesis, green sturgeon may have experienced a higher frequency of Met substitution by SeMet in their functional proteins. Consequently, normal physiological functions may have been compromised by an increase in non-functional proteins, as well as the associated oxidative stress. The high energetic demands of their initial growth phase may have also compromised the species' ability to repair damages induced by Se Toxicity, leading to the stunted growth and higher mortality observed during the latter part of exposure trial.

5. Summary

The objective of this study was to compare the effects of high Se diets in the juvenile stage of two sturgeon species native to SFBD. Effects on growth parameters and histopathological alterations clearly indicated that green sturgeon is more sensitive to Se-laden diets compared with white sturgeon. Furthermore, the low SeMet diet (19.7 ± 0.6 mg Se/kg dw), which caused severe adverse effects in green sturgeon, is similarly to that of the levels found in SFBD benthic macro-invertebrates, which are a major dietary component of young sturgeon. As such, our results suggest that juvenile green sturgeon is more sensitive to Se toxicity and should be monitored and managed separately from white sturgeon when developing conservation measures to protect this threatened SFBD population segment from Se exposure.

Acknowledgment

Funding for this study was provided by CalFed Project number SP2006-035 and partially by P.O.R. Sardegna F.S.E. 2007-2013—Axis IV—L.a. l.3.1. and Fondazione Banco di Sardegna, Italy. We are also grateful for the technical assistance provided by Paul Lutes and Eric Hallen of the Center of Aquatic Biology and Aquaculture at the University of California, Davis. The infrastructure support of the Department of Animal Science and the College of Agriculture and Environmental Sciences of the University of California, Davis was also acknowledged.

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Total Maximum Daily Load
Selenium in North San Francisco Bay

Preliminary Project Report

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California Regional Water Quality Control Board
San Francisco Bay Region

January 2011

For details of technical analysis, data interpretations and model development in support of the TMDL see Technical Memoranda prepared by Tetra Tech Inc.:

Technical Memorandum 2: North San Francisco Bay Selenium Data Summary and Source Analysis. July 2008.

Technical Memorandum 3: North San Francisco Bay Selenium Toxicological Assessment. April 2008.

Technical Memorandum 4: Conceptual Model of Selenium in North San Francisco Bay. August 2008.

Technical Memorandum 5: Recommendations for Numerical Model Development. August 2008.

Technical Memorandum 6: Application of ECoS3 Model for Simulation of Selenium Fate and Transport in North San Francisco Bay. February 2010.

These Reports are available on San Francisco Bay Water Board website:
http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/seleniumtmdl.shtml

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1 INTRODUCTION

This Preliminary Project Report summarizes the data and supporting information acquired over the past three years, and provides interpretation of technical analyses conducted to support development of a Total Maximum Daily Load (TMDL) to address and reduce selenium impairment in the North San Francisco Bay (North Bay).

The report presents a scientific basis for the proposed numeric target for the TMDL, protective of human health, wildlife and aquatic life, and contains the results of impairment assessments, sources and loadings analysis, and linkage analysis. A modeling framework for simulation of selenium transformations and biological uptake processes in the North Bay comprising a numerical estuary model and a bioaccumulation DYMBAM model is also discussed. In the following sections the available data and information on the key processes and conditions leading to the impairment are presented together with the information gaps and uncertainties identified while conducting the technical analyses.

Additional data collection and interpretation of information that will likely become available over the next two years are recommended before the final decision could be made about how to proceed with this TMDL.

2 PROBLEM STATEMENT

San Francisco Bay is listed under section 303(d) of the Clean Water Act as impaired for selenium because bioaccumulation of this element has led to recurring health advisories for local hunters against consumption of diving ducks. Moreover, elevated selenium concentrations found in biota often exceed levels that can cause potential reproductive impacts in white sturgeon and are often higher than levels considered safe for fish and other wildlife species in the estuary.

The problem has been somewhat exacerbated by the introduction of the Asian clam (*Corbula amurensis*) into the Bay in 1986. This non-native clam is a prodigious filter-feeder, and by consuming large quantities of selenium-laden particles this exotic species provides a pathway for biotransformation of a considerable mass of selenium into the benthic food web and thus to diving ducks and large fishes such as sturgeon. The estimated whole body selenium concentrations found in sturgeon often exceed the proposed draft United States Environmental Protection Agency (USEPA) limit of 7.91 µg/g (USEPA 2004) and are above the level of concern (4-12 µg/g) indicated by the US Fish and Wildlife Service recommended ecological risk guidelines (Presser *et al.* 2004). Increased levels of selenium in the Bay-Delta

have been recognized as a possible contributing factor to the observed decline of some key species, e.g. white sturgeon, Sacramento splittail, starry flounder and surf scoter.

Sources and pathways leading to the possible impairment in northern and southern segments of the Bay differ significantly and therefore a separate approach to addressing the problem is warranted. The widespread selenium food web enrichment is most pronounced in northern segments of the Bay extending from the Delta to the Central Bay, while Lower and South Bay segments indicate only a localized enrichment. The northern segments of the Bay are dominated by the freshwater inflows from Sacramento and San Joaquin Rivers that contribute substantial amounts of selenium enriched sediment and irrigation runoff from Central Valley. The Lower and South Bay segments receive much lower freshwater inflows and the observed selenium levels appear to be dominated by groundwater discharges and dewatering operations.

Thus, this TMDL is being developed for the North San Francisco Bay segments (North Bay) only, which for the purpose of this project include a portion Sacrament/San Joaquin Delta, Suisun Bay, Carquinez Strait, San Pablo Bay and Central Bay (Figure 1). It aims at identifying sources and prioritizing management practices that could lessen possible detrimental effects of selenium on wildlife and, subsequently, will lead to reducing selenium concentrations in fish tissue to the levels that are, to best of our knowledge, safe and protective of beneficial uses. When completed the TMDL will include the fish tissue-based numeric target and associated total daily maximum loads, allocations, and implementation actions.

2.1 Basis for 303(d) Impairment Listing

In 1987, the California Department of Health Services issued a human health advisory against consumption of two species of ducks (Greater scaups and Surf scoters) from the Bay-Delta area due to elevated concentrations of selenium in tissue of the waterfowl. This advisory reflected the impairment of San Francisco Bay beneficial uses and provided a means for placing the Bay on the 303(d) list of impaired water bodies. The health advisory was based on the initial results reported by the Selenium Verification Study that begun in 1985 (SWRCB 1991).

The purpose of the Verification Study was to provide a comprehensive assessment of selenium and trace elements in a wide array of aquatic and terrestrial organisms from previously identified areas of concern. The selenium contamination was measured in 26

locations throughout the state including the areas in the San Francisco Bay and the Delta. The results of the study showed very high concentrations of selenium in scoters (more than 30 µg/g wet weight in liver) as well as elevated levels of selenium in muscle tissue of white sturgeon (average of 4.1 µg/g wet weight). The levels of selenium in scoters were three times higher than those determined by the US Fish and Wildlife Services (USFWS) to cause selenium toxicosis and reproductive impairment.

Figure 1: Segments of San Francisco Bay

The study also found high concentrations of selenium in clams and other animals that are a source of food for these migratory waterfowl and certain larger fishes. On average selenium concentrations in the muscle of white sturgeon, which feeds primarily on benthic organisms were five times higher than, for example, in striped bass, which are primarily piscivorous. The study concluded that food habits played a role in selenium accumulation, and that the species with elevated levels of selenium in their tissue were either bottom-dwellers or species with diets comprising of benthic organisms.

As a result of the elevated selenium levels in wildlife and the issuance of the health consumption advisory, the 1998 303(d) list identified San Francisco Bay as impaired by selenium. The current 303(d) list (2010) continued the listing of most segments of the Bay (see Table 1). Despite the fact that the Bay was listed as impaired prior to adoption of the Water Quality Control Policy for developing California's Clean Water Act Section 303(d) List (2004) the listings are consistent with the current policy. The listing factors, among others, include a health advisory against the consumption of edible resident organisms and bioaccumulation of pollutants in aquatic life tissue.

Table 1: The San Francisco Bay segments listed as impaired by selenium

San Francisco Bay segment or Water Body		2010 303(d) List	Indicator of Impairment
North Bay	Sacramento-San Joaquin Delta	X	Hatchability in nesting diving birds Health consumption advisory in effect for scaup and scoter (diving ducks)
	Suisun Bay	X	
	Carquinez Strait	X	
	San Pablo Bay	X	
	Central San Francisco Bay	X	
Lower & South Bay	Central Basin (Part of Lower Bay)	X	Health consumption advisory in effect for benthic-feeding ducks
	South San Francisco Bay	X	
	Oakland Inner Harbor – Pacific Dry Dock	X	
	San Leandro Bay	X	

While selenium concentrations in the North Bay do not exceed the National Toxics Rule chronic saltwater criterion (5 µg/L) for protection of aquatic life, the observed bioaccumulation of selenium in fish is the basis of impairment of the estuarine habitat (EST) and poses a threat to other estuarine organisms including waterfowl and shorebirds. Other designated uses of the Bay such as preservation of rare and endangered species (RARE) as well as

commercial and sport fishing (COMM) are also affected by selenium. These beneficial uses are described in Table 2.

Table 2: Beneficial uses of the North Bay potentially impaired by selenium

Designated Beneficial	Description
Estuarine Habitat (EST)	Uses of water that support estuarine ecosystems, including, but not limited to, preservation or enhancement of estuarine habitats, vegetation, fish, shellfish, or wildlife (e.g., estuarine mammals, waterfowl, shorebirds), and the propagation, sustenance, and migration of estuarine organisms.
Preservation of Rare and Endangered Species	Uses of waters that support habitats necessary for the survival and successful maintenance of plant or animal species established under state and/or federal law as rare, threatened, or endangered.
Ocean, Commercial and Sport Fishing (COMM)	Uses of water for commercial or recreational collection of fish, shellfish, or other organisms in oceans, bays, and estuaries, including, but not limited to, uses involving organisms intended for human consumption or bait purposes.

2.2 Project Objectives

The proposed project is intended to evaluate the contributions of existing and future selenium discharges to the impairment of beneficial uses in the North San Francisco Bay associated with controllable water quality factors i.e. resulting from human activities that can influence water quality and be reasonably controlled through prevention, mitigation, or restoration actions. The specific goals are to:

- Reduce selenium impairment and attain water quality objectives established for the North Bay
- Protect and enhance the overall aquatic health and wildlife habitat including rare and endangered species habitat
- Protect beneficial uses of the North Bay and enhance its aesthetic and recreational values

3 BACKGROUND AND ENVIRONMENTAL CONDITIONS

3.1 Environmental Setting

San Francisco Bay, with an area of approximately 1,600 square miles, is the largest estuary on the West Coast. The region is recognized as having utmost ecological and economical importance. It supports a variety of natural habitats and a diverse wildlife population as well as provides drinking water for more than 70 percent of Californians and irrigation water for 4.5 million acres of farmland. The North Bay, in particular, supports a diverse fish biota. The fish supported include both sportfish and threatened and endangered fish species. The five most common sport fish in the North Bay are: (SFEI 2000; listed in order of catch frequency):

- Striped bass (*Morone saxatilis*)
- Halibut (*Paralichthys californicus*)
- Jacksmelt (*Atherinopsis californiensis*)
- White sturgeon (*Acipenser transmontanus*)
- White croaker (*Genyonemus lineatus*)

In addition to the sport fish listed above, the North Bay supports the following threatened and endangered fishes (Beckon and Maurer 2008):

- Chinook salmon (*Oncorhynchus tshawytscha*)
- Delta smelt (*Hypomesus transpacificus*)
- Green sturgeon (*Acipenser medirostris*)
- Longfin smelt (*Spirinchus thaleichthys*)
- Sacramento perch (*Archoplites interruptus*)
- Sacramento splittail (*Pogonichthys macrolepidotus*)
- Steelhead trout (*Oncorhynchus mykiss*)
- Tidewater goby (*Eucyclogobius newberryi*)

The Bay is commonly divided into segments including Sacramento/San Joaquin Delta, Suisun Bay, Carquinez Strait, San Pablo Bay, Central Bay, and Lower and South Bay (Figure 1). Each segment has a distinct ecological structure defined by the local tidal datum, amount of fresh water influx, sediment input, and the underlying hydrology. The North Bay extending from the Sacramento/San Joaquin Delta through Central Bay differs significantly from the South Bay as it receives almost 90% of the entire fresh water and sediment inflow into the Bay (SFEP 1992).

The northward-flowing San Joaquin and southward-flowing Sacramento Rivers discharge into the northern reach of the Bay and carry about 60 percent of the state runoff draining approximately 152,500 square kilometers or 40 percent of California's surface area (Conomos *et al*. 1985). The Sacramento River typically accounts for 80 percent of the fresh water inflow coming through the Delta into the Bay and the San Joaquin River for 15 percent. The presence of freshwater inflow into the North Bay causes stratification of Bay waters and generates horizontal salinity gradients. Salinity gradually increases from one part of salt per thousand (ppt) in the Delta to approximately 30 ppt near the mouth of the Bay (Cohen 2000). Tidal action, river flow and stratification that occur in the North Bay result in the average residence time being three to six times shorter than in the southern portion of the Bay.

Sacramento and San Joaquin Rivers are fundamental to the health and continuation of the shallow water habitats in the North Bay area; however, they also provide a conduit for selenium rich drainage and agricultural runoff. Freshwater inflows from the Central Valley watershed are the major source of new sediment input into the Bay. Most new sediment (approximately 80 percent) originates in the Sacramento - San Joaquin River drainage and enters primarily as suspended load during the high winter flows. Much of the winter sediment load initially settles out in San Pablo Bay. During the low flow summer months, wind-generated waves and tidal currents re-suspend the previously deposited sediment and redistribute it over a wider area. Selenium affiliated with sediments is effectively mobilized and could enter into food webs contributing to long-term dietary exposure of fish and wildlife (Lemly 1999). Therefore sediment dynamics exerts an important control on the distribution, transport and speciation of selenium in the Bay.

3.2 Selenium Characteristics, Speciation and Environmental Fate

Selenium is a naturally occurring trace element that is widely distributed but dispersed in the environment. It is commonly found in marine sedimentary rock formations and soils developed from parent seleniferous material.

At trace concentrations selenium is an essential nutrient for plants and animals and it is important to human health. As a vital constituent of selenoproteins, selenium plays a significant role in production of thyroid hormones, in the functioning of immune system and in prevention of oxidative stress or inflammation (Rayman 2000). However, the margin between essential concentrations of selenium in diet of plants, animals or humans and the concentrations that can cause toxicity or poisoning is the smallest among all known micronutrients.

Selenium Properties and Distribution in the Environment

Selenium has an atomic number of 34, melting point of 217^{o}C, boiling point of 685^{o}C, and an atomic weight of 78.96. In the periodic table it is located between non-metallic sulfur and metallic tellurium. In nature, selenium is strongly associated with sulfur. Because the radius of Se^{2-} is only slightly larger than of the S^{2-} anion selenium substitutes readily for sulfur in the structures of sulfide minerals (USGS 2004). Thus, selenium usually occurs combined with other compounds, such as in sulfide ores of other metals such as silver, copper, lead and nickel.

Average concentrations of selenium found in sediments and soils usually range from 0.01 to 0.02 mg/kg with most seleniferous soils containing less than 2 mg/kg (USDHHS 2003b, Chapter 6). However, Cretaceous and Tertiary marine and sedimentary deposits underlying and surrounding basins such as San Joaquin Valley, and those found in western states are enriched in selenium. Presser (1994) identified seleniferous deposits in the Coast Ranges of California and Central Valley with concentrations of Se reaching 45 mg/kg and median values exceeding 6.5 mg/kg.

Enrichment of selenium in soils and groundwater commonly occurs in arid and semi-arid irrigated areas where application of irrigation water accelerates weathering processes and mobilizes already elevated levels of selenium in the soil profile. To reduce effects of salinization of agricultural lands in these areas, such as the southern Central Valley, large volumes of water have to be used to flush the excess salt and selenium that accumulates in the root zone (Seiler *et al*. 2003). Drainage of irrigation excess water through the system of drains and canals is then necessary to prevent waterlogging of the soils. These drains, however, provide a conduit to carry seleniferous groundwater to surface waterbodies and wildlife areas as it was well documented in the case of disposal of agricultural drainage water into the Kesterson Wildlife Refuge. Reported selenium concentrations detected in irrigation drainage are very high and vary between 75 and 1400 µg/L (Amweg *et al.* 2003). Arid climate amplifies further evaporation related enrichment that takes place in enclosed surface waterbodies and wetlands resulting in selenium concentrations potentially reaching toxic levels.

Selenium exists in a number of chemical forms and exhibits a complex biochemistry. Most common selenium species include: elemental selenium (Se^{0}) selenide (Se^{2-}), selenite Se^{4+} (SeO_3^{2-}) and selenate Se^{6+} (SeO_4^{2-}). Oxidation state is the key factor determining the fate of selenium in the environment. The concentration, speciation and partitioning of selenium in a

given environment are mostly govern by complex interactions between pH and redox conditions, presence of metal oxides and biological interactions (USDHHS 2003b Chapter 6). As described by Lemly (1997) the aquatic cycling of selenium includes four major pathways: 1) it can be absorbed or ingested by organisms, 2) it can bind or complex with particulate matter, 3) it can remain free in solution, and 4) it can be released to the atmosphere through volatilization.

In natural freshwater and estuarine ecosystems selenium concentrations are typically low ranging from 0.1 to 0.4 µg/L with background concentrations below 1 µg/L (Lemly 1997, Eisler 1985). Selenium concentrations in present-day seawater average approximately 0.09 µg/L (Hem 1985). Selenate and selenite are the most soluble and the most mobile forms of selenium that predominate in well-oxygenated, aerobic surface waters. Out of these two common selenium species, selenite is more readily taken up by bacteria, which, in turn, serves as a path for rapid biotransformation into organoselenides. This biologically reduced selenium, often referred to as particulate selenium, is then directly available to rooted plants, bottom-dwelling invertebrates and detrital-feeding fish and wildlife (Abu-Saba and Ogle 2005, Amweg *et al*. 2003).

Anthropogenic Sources and Uses

Despite wide distribution of selenium in the environment, deposits of selenium are not sufficiently concentrated to justify mining. Instead nearly all selenium is produced as a byproduct of the electrolytic refining of copper (SWRCB 1989). The main anthropogenic activities that may release selenium compounds to the environment include glass manufacturing, chemical and pigment manufacturing, electronics, agriculture and, pharmaceutical and nutrition industries (Table 3). The most significant emissions of atmospheric selenium result from combustion of coal and petroleum fuels (USDHHS 2003a, b). Incineration of rubber tires, paper, and municipal waste is thought to be the second largest source of atmospheric selenium.

USGS (2004) estimated that approximately 90 percent of selenium used in pigments, fertilizers, animal feeds, chemicals and pharmaceuticals dissipate into the environment. Furthermore, the content of selenium in glass and free-machining alloys is not accounted for during recycling of those materials as selenium is likely to volatilize during melting operations.

Table 3: Description of selenium sources and uses

Type of Use	Description	Estimated Se Use (%)
Glass Manufacturing	Used together with other chemical compounds to produce color glasses (black and bronze-colored architectural glass; pink, purple and yellow glass; as well as ruby glass used for lenses in traffic signal and navigation lights) Used as a decolorizer for the natural gray heat absorbent flat glass for automobile and modern office building windows Used in powdered and granulated glass applied onto the surfaces of ceramic products to seal and color them	25
Chemicals & Pigments	Catalysts and oxidizing agents in organic chemical processes Pigments used in the coloring of plastics processed and used at high temperatures, paints, enamels and rubber (e.g. for cable and steam line coverings)	22
Electronics	Photographic exposure meters and rectifiers for home entertainment equipment Plain paper xerographic copiers (selenium is used to coat metal cylinders from which a photographic image is transferred). Selenium is gradually being replaced in copiers by silicon and other materials Solar photocells	10
Metal Manufacturing	An additive to improve machinability of copper, lead and steel alloys	24
Other	Catalyst in preparation of various pharmaceuticals Feed additive for poultry and stock Dietary supplement Cosmetics (Antidandruff shampoos)	19

Compiled from USGS (2004)

3.3 Ambient Selenium Levels in the North Bay

Concentrations of selenium in the North Bay water column and bottom sediments have been monitored since the 1980s. Early on the monitoring effort focused on the northern segments of the Bay because sub-surface drainage of agricultural areas in the San Joaquin Valley and waste streams from oil refineries in the Suisun Bay and Carquinez Strait conveyed large amounts of selenium to the Bay. Regional Monitoring Program (RMP) and the data collected by Dr. Greg Cutter's research group at Old Dominion University[1] are the two most comprehensive sources of selenium data in the North Bay. Sampling design, frequency and

[1] Funded by the U.S. Bureau of Reclamation, CALFED (Grant 01WRPA0077), California DWR, and National Science Foundation, Environmental Geochemistry and Biogeochemistry Initiative (Grant: OCE-9707946).

quality assurance procedures are described in detail in SFEI (2006), Cutter and Cutter (2004) and Doblin *et al*. (2006). General sampling locations are shown in Figure 2. Technical Memorandum No. 2 prepared by Tetra Tech (2008a) provides a summary of all the available data and describes spatial and temporal changes in water and sediment quality.

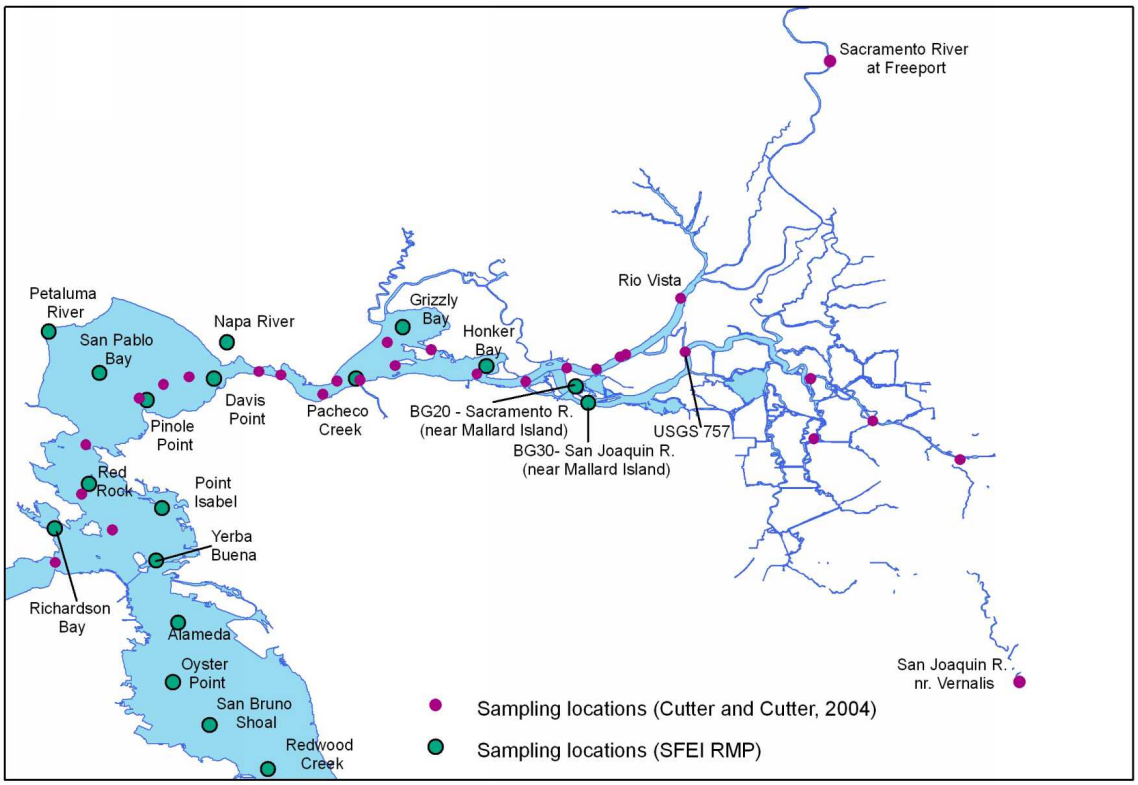


Figure 2: Locations of RMP long-term monitoring sites and sampling by Cutter and Cutter (2004) during November 1999 *(Tetra Tech 2008a)*

The ambient total selenium levels in the North Bay measured between 1993 and 2005 are consistently low and do not exceed 0.5 µg/L. The mean dissolved and total selenium concentrations at each monitoring location range from 0.12 to 0.18 µg/L and 0.13 to 0.24 µg/L respectively. Dissolved selenium is the predominant form present in the water column. Particulate selenium, calculated as a difference between total and dissolved selenium, accounts for approximately 10% of the total selenium. The most recent data collected during 1999-2005, i.e., following the improved wastewater control measures implemented by the oil refineries in 1999, indicate a slight decrease in concentrations of dissolved and total selenium at 0.10 µg/L (n = 105) and 0.13 µg/L (n = 100). In comparison, mean dissolved and total

selenium concentrations for the period of 1993-1999 at the same monitoring locations were 0.17 µg/L (n = 258) and 0.20 µg/L (n = 230).

Spatially, total selenium concentrations are marginally higher in the mid-estuarine regions of Suisun and San Pablo Bays when compared to the freshwater and marine portions of the estuary (Figure 3). Total selenium concentrations in the Central Bay are lower, most likely due to ocean exchange and dilution. A few locations near the confluence of local tributaries (e.g., Petaluma and Napa River) show higher total selenium than the rest of the Bay.

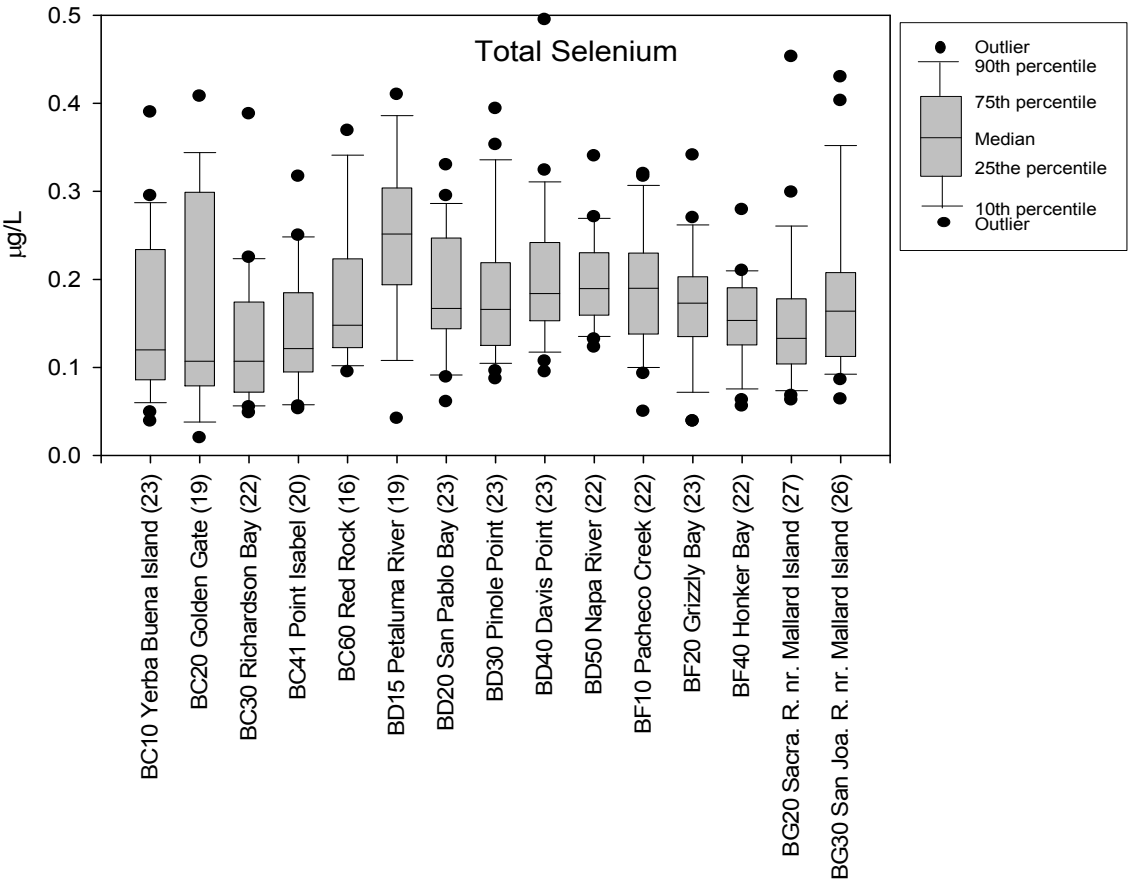


Figure 3: Total selenium concentrations at long-term RMP monitoring sites for the period of 1993-2005 (*Tetra Tech 2008a*)
Values in parentheses are numbers of samples *(Data: RMP)*.

Figure 4 shows selenium speciation in the North Bay and at the downstream reaches of the Sacramento and San Joaquin Rivers where they enter the Delta. The composition of selenium species in the North Bay is markedly different to that observed in the Delta. In the Bay water column selenate is the dominant form and averages above 50% of total selenium. However, a relatively high proportion of organic selenide and selenite is still present,

accounting for approximately 20% each. In the freshwater flows from Sacramento and San Joaquin Rivers selenate concentrations account for more than 70% of total selenium with the remainder equally distributed between selenite and organic selenide.

The changes in selenium composition resulting from the improvements in the wastewater treatment at the refineries are clearly visible during low flow conditions surveyed in 1986 and 1999. In 1986, the selenite fraction of total selenium exceeded 35% and almost matched selenate. Since then, selenite concentration decreased significantly and it now accounts for approximately 15% of total dissolved selenium during low flow.

Over the long-term, dissolved and total selenium concentrations show temporal variations, both inter-annual and seasonal but the overall selenium levels remain low in the North Bay. The temporal patterns in dissolved selenium closely resemble those in the total selenium. Data from the RMP random sampling period of 2002-2008, indicated that dissolved and total selenium concentrations were usually below 0.15 µg/L, with an average for the entire North Bay of 0.10 µg/L. Total selenium concentrations are higher in the upper estuary (Suisun Bay) than in the San Pablo and Central Bays.

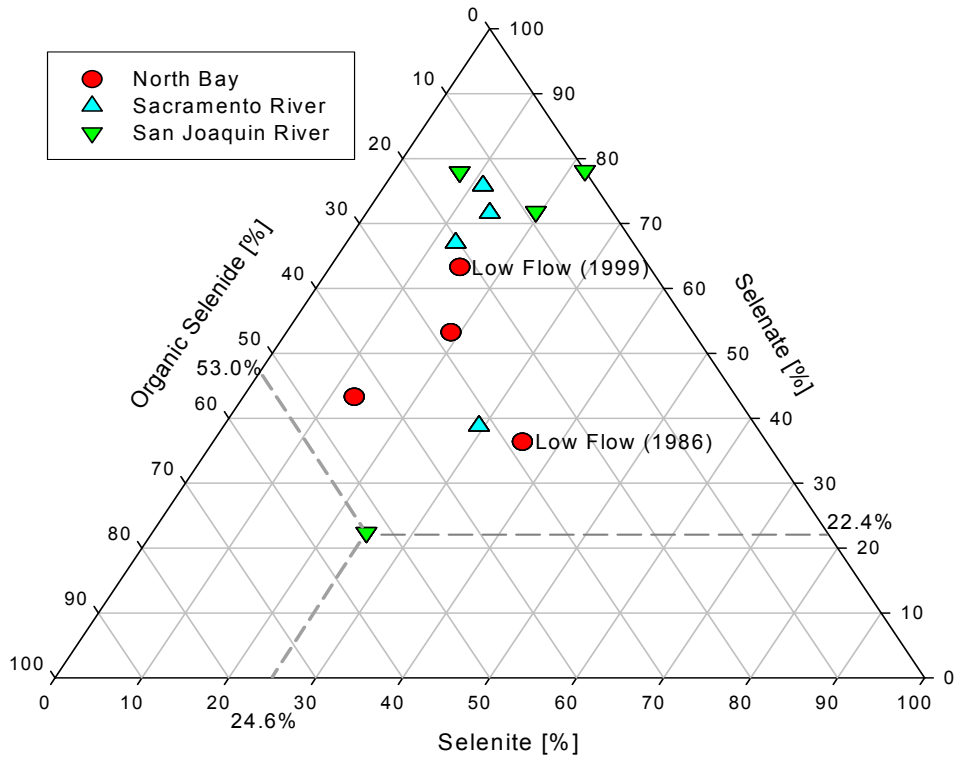


Figure 4: Speciation of dissolved selenium in North Bay and main tributaries
(Data: Cutter and Cutter 2004)

Although most selenium in the water column at any given time is in one of the dissolved forms, the suspended particulate material still comprises 2 to18.5% of the total selenium. This particulate selenium is also more readily available to bivalves and zooplankton. Suspended materials in the North Bay waters include mineral particles, particulate organic matter (non-living), and living organic matters, primarily algae and bacteria. These suspended particles may originate from the various non-point sources discharging to the Bay, may be generated in situ, or may be eroding from the sediment bed. Studies indicate that particulate selenium is a function of phytoplankton productivity and riverine inputs of sediment to the Bay (Abu-Saba and Ogle 2005). In general, particulate elemental selenium is associated with bed sediments while particulate organic selenium is associated with algal/bacterial uptake, and selenite and selenate are sorbed to mineral particles and/or particulate organic matter.

Doblin *et al*. (2006) reported concentrations of total suspended particulate material (TSM) and selenium on particles in San Francisco Bay for the time period from 1997-1999. Particulate selenium concentrations, including elemental selenium and particulate selenate and selenite, generally track the pattern in total suspended material and decrease along the salinity gradient especially during high flow conditions (Figure 5), and are usually lower during high flow than low flow. However, the levels of organic selenium remain similar during low and high flow periods and even increase with travel distance in the estuary, indicating that biotransformation of selenium may occur in the estuary where more oxidized forms of selenium (likely selenite) are incorporated into a wide variety of organic compounds.

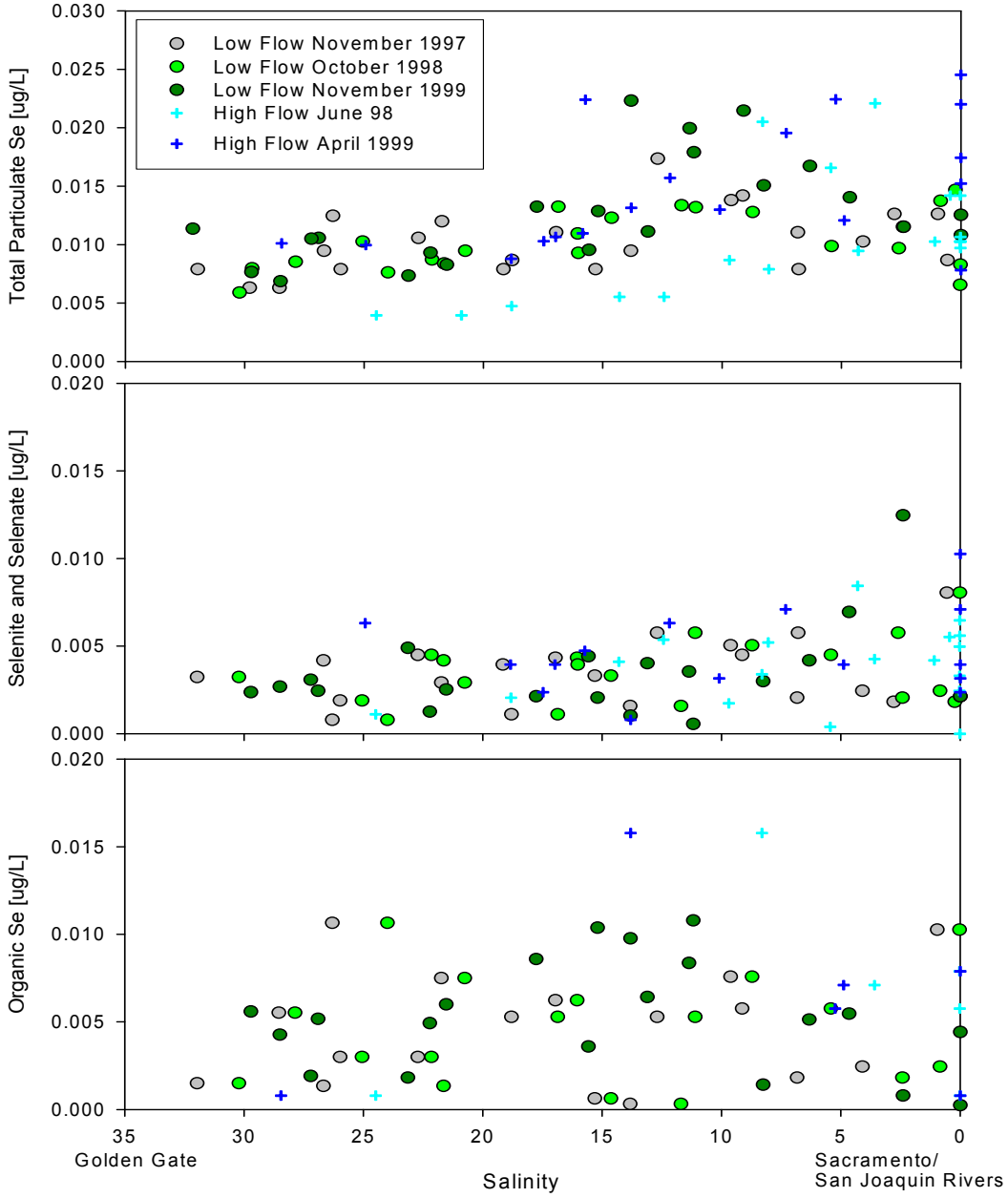


Figure 5: Distribution of particulate selenium along the salinity gradient during different flow conditions *(Data: Doblin et al. 2006)*

4 NUMERIC TARGETS

Numeric targets identify specific water column, sediment and/or tissue indicators that express the desired conditions of the water body and ensure attainment of the water quality standards including water quality objectives and beneficial uses. TMDL targets are often set to applicable numeric water quality objectives. However, the existing water column based criteria may not ensure adequate protection of aquatic organisms in the North Bay. Despite very low ambient selenium levels in the water column, concentrations in some fish tissue samples exceed ecological risk guidelines (Presser *et al*. 2004), which form the basis of the impairment in the Bay. Therefore, we propose a sturgeon-based fish tissue numeric target as the most direct way to address selenium impairment and assess protection of beneficial uses (Table 4).

Table 4: Proposed numeric target for selenium in the North San Francisco Bay

TMDL- North Bay	Fish Tissue µg/g – dw
Numeric target	6 - 8.1

The proposed target aims at protection of white sturgeon, the fish that is particularly vulnerable to selenium exposure in the North Bay. Sturgeons are long-lived fish found year-round in the Bay with a high propensity to bioaccumulate selenium because of their feeding preferences and reproductive biology. They feed predominantly on benthic organisms including the invasive clam, *Corbula amurensis,* which is very efficient in accumulating and retaining selenium. Sturgeon exposure is further exacerbated by its long reproductive cycle during which selenium is transferred and stored in developing eggs, forming a stable selenium reservoir in reproductive females.

The selected target is set to the range of values that the USEPA is considering as wildlife criterion for San Francisco Bay/California (D. Fleck, USEPA, *pers. comm*.) and is based on an estimate of the concentrations at which an effect is observed in 5% (EC5) to 10% (EC10) of the population. The tissue concentration within this range is deemed to be sufficiently protective of the most sensitive fish species that reside in San Francisco Bay. The USEPA has not yet offered the scientific rationale for recommending a specific value. Therefore, in this Chapter, we provide a scientific context for establishing a numeric target for the TMDL, an overview of the selenium toxicity relevant to fish and birds in the North Bay, and the

applicable existing objectives and health and risk criteria, which equate to attainment of water quality standards.

4.1 Selenium Bioaccumulation and Impact on Aquatic Life

Evidence of fish and wildlife contamination, leading to reduced survival and deformities due to selenium in aquatic and terrestrial food webs, has been documented extensively (Hamilton 2004, Fan *et al*. 2002, Skorupa 1998). These studies confirmed that once selenium enters the aquatic environment it has a high potential to bioaccumulate in zooplankton and benthic invertebrates, and, subsequently, to biomagnify as it reaches top level predators such as fish, birds and mammals.

Bioaccumulation describes selenium's tendency to be taken up from the environment and stored at increased concentrations by organisms. The rate of bioaccumulation is often site-specific and highly dependent on the selenium forms present, the environmental conditions and the type of the organism. In San Francisco Bay, selenium uptake and bioaccumulation effects are particularly evident in the dominant estuarine clam *Corbula amurensis* (Schlekat *et al*. 2004, Linville *et al*. 2002). The studies found that this clam displayed a 10-fold slower rate constant for selenium loss compared to common crustaceans, such as copepods and mysids, leading to increased bioaccumulation of selenium. In 1995-1997 Se concentrations in *C. amurensis* found in the North Bay varied seasonally from 5 to 20 µg/g dry weight (dw). These concentrations are within the range of values that are linked to a high frequency of developmental toxicity in wildfowl based on diets of more than 8 µg/g dw and teratogenic effects observed in fish at dietary selenium concentrations above 5 µg/g dw (Schlekat *et al.* 2004). In addition, stable isotope analyses used by Stewart *at al*. (2004) revealed that bottom feeding fish (e.g. white sturgeon and splittail) exhibited isotope signatures indicative of diets that included bivalves and therefore could be under greater risk from selenium.

Biomagnification occurs where there is a progressive buildup of selenium in organism at higher trophic levels. Figure 6 depicts conceptually how selenium biomagnifies in the tissues of organisms present in San Francisco Bay. Lemly (1997) reported that biomagnification might lead to a two to six-fold increase in selenium concentrations between primary producers and forage fish. This, in turn, may have detrimental effects on fish and waterfowl even when selenium in the water column is present at low concentrations.

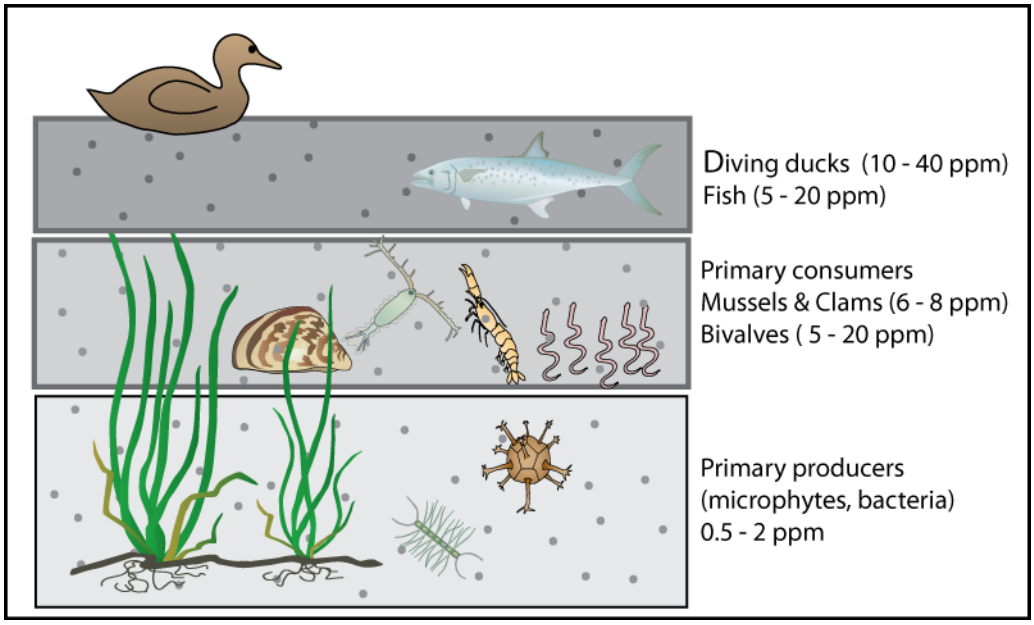


Figure 6: Conceptual representation of selenium biomagnification in the North Bay
(Concentrations illustrate the range of selenium found in the North Bay species. Concentrations are measured as total selenium in tissue and expressed as micrograms per gram (ppm) dry weight)

4.2 Toxicity and Selenium Related Risks

Aquatic and terrestrial organisms are highly sensitive to selenium contamination. They require 0.5 µg/g dw of selenium in their diet to sustain metabolic processes, however, concentrations that are only an order of magnitude greater than the required level have been shown to be toxic to fish (USEPA 2004). The main toxicological effects in fish and aquatic birds involve reproductive abnormalities, teratogenic deformities, selective bioaccumulation, and growth retardation (Eisler 1985).

Toxicity of selenium to wildlife has been researched for many years and numerous studies have documented that, in contrast to many other microelements, chronic toxicity resulting from dietary and food chain exposure causes a much greater problem than toxicity associated with water exposure (for example see: Lemly 1997, Canton and Van Derveer 1997, Hamilton 2002). Reproductive effects in fish and aquatic birds have been identified as the most sensitive biological indicators of aquatic ecosystem-level impacts of selenium.

This section summarizes the available information on the toxicity of selenium to fish and birds and reviews concentrations associated with toxic effects to help establish the numeric target. The discussion of selenium toxicity takes into account the studies and methods described in

the *North San Francisco Bay Toxicological Assessment* (2008b) prepared by Tetra Tech Inc. and refers to the review of existing selenium dietary exposure benchmarks by Beckon and Maurer (2008). The toxicity-based screening values have been derived from the available scientific literature that considered either dietary or dietary and waterborne selenium exposures.

Evaluation Methods

Eighty fish toxicity studies reported from 1987 to 2007 were identified and evaluated using a set of predefined exclusion and acceptability criteria (Tetra Tech 2008b). The reported effects from each study that met the initial criteria were grouped into one of two categories: major and minor effects. Major effects are those that have the potential to impact fish or birds at the organism and/or population level (e.g., increased mortality, reduced fecundity, reduced growth). The lowest observed adverse effect levels (LOAELs), effect thresholds, species mean chronic values (SMCV), effect concentration (EC01 or EC10) and species sensitivity distributions (e.g. Hamilton 2003, 2004) were then used in the derivation of proposed screening values.

When there is a large body of literature, with many reported LOAELs, the lowest observed adverse effect level is likely to be indicative of the concentration at which effects first appear. However, when there are only a few studies, which is often the case in this assessment, it is likely that effects begin at a level below the lowest LOAEL reported. Effect thresholds are calculated as the geometric mean of the no observed adverse effect level (NOAEL) and LOAEL reported for the same effect in an individual study. Since toxicity tests do not generally test many different concentrations, and effects may occur at concentrations below the LOAEL, calculating the geometric mean of the NOAEL and the LOAEL is a way to add a margin of safety to the LOAEL. A similar approach is recommended for establishing risk-based ecological soil screening levels (USEPA 2005) and for developing water quality criteria (USEPA 1985).

To provide a better comparison between toxicity effects reported by different studies, tissue concentrations expressed as wet-weight values were converted to dry-weight values and, similarly, if not reported, the whole-body concentrations were calculated using the USEPA methods (USEPA 2004). The USEPA recommends the whole-body tissue based medium as the best means of expressing the chronic criterion value because of the general availability of the data and practicality of performing the tests.

After applying the screening criteria, 19 studies with usable toxicity data were identified as suitable for derivation and comparison of the screening levels for fish and 23 studies for birds. The studies reported toxic effects associated with dietary or dietary and waterborne exposure for six species of fish: bluegill, fathead minnow, rainbow trout, Chinook salmon, Sacramento splittail and white sturgeon. All experiments, with the exception of one involving Chinook salmon, were conducted in freshwater.

Selenium Toxicity Thresholds in Fish

The available selenium toxicity data showed a broad range of sensitivity among tested fish and included observed threshold effects at very low concentration levels suggesting that the dataset provides a good approximation of the expected effects that are applicable to most fish species (Figure 7). The larvae of rainbow trout exhibited the most sensitivity to Se toxicity with the whole-body LOAEL concentration of 2.3 µg/g-dw for the growth endpoints. The lowest species mean chronic value (SMCV) of 3.0 µg/g-dw was estimated for channel catfish followed by the bluegill and fathead minnow with SMCVs of 5.6 and 6.0 µg/g-dw. However, the North San Francisco Bay does not support these freshwater fish species nor were they considered at risk specifically for selenium toxic effects in the Bay/Delta estuary in the Beckon and Maurer (2008) review.

Sacramento splittail and sturgeon

The effect thresholds and LOAELs for juvenile Sacramento splittail and white sturgeon, the two important species of concern in the North Bay, are above 6 and 10 µg/g-dw respectively (Figure 7). These estimated screening levels correspond well with thresholds for reproductive toxicity in fish (Beckon and Maurer 2008).

Both, the Sacramento splittail and white sturgeon, feed primarily on benthic organisms including introduced bivalves that have been proven to be very proficient selenium bioaccumulators. This in turn may lead to a greater potential for selenium toxicity for these fish. Clams and other mollusks were found to predominate the stomach contents of white sturgeon caught by anglers in Suisun Bay (1965-1967), reaching up to 77% of stomach volume. The diet of the splittail collected in Suisun Marsh was dominated by detritus with the proportion of bivalves increasing markedly after the decline of Mysid shrimp in the San Francisco Estuary (Feyrer *et al.* 2003).

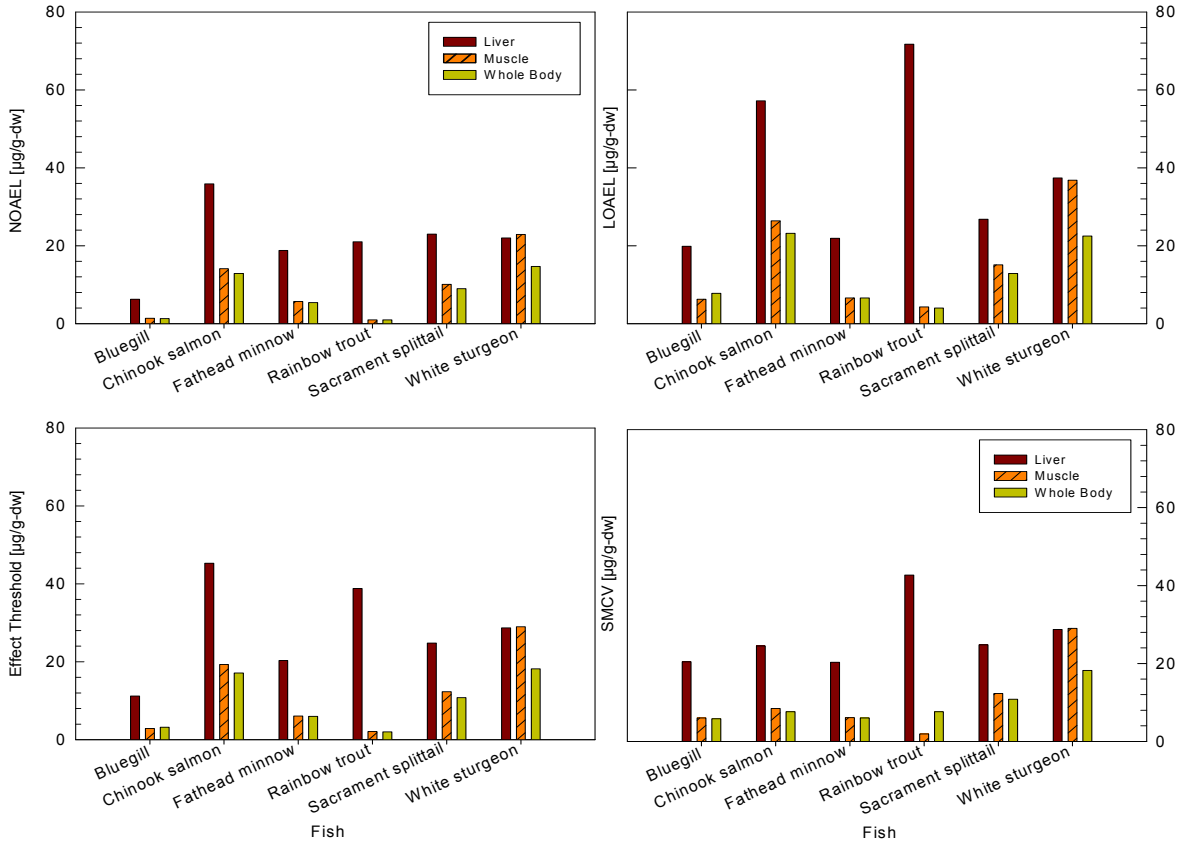


Figure 7: Selenium concentrations in selected fish at which adverse effects may occur

(Figure compiled from the data presented in Table 3-3 (Tetra Tech 2008b) showing the most stringent toxicity levels from studies of juvenile fish)

Despite the diet comprising primarily bivalves, splittail tissue collected in 2000 from Suisun Slough (USGS, unpublished data) did not show elevated levels of selenium. In fact, the observed muscle concentrations in juvenile fish varied from 1.5 to 3.5 µg/g-dw, and in adult fish from 1.5 to 4.1 µg/g-dw, and were well below known toxicity thresholds. These concentrations are also indicative of background level diets not exceeding 1 µg Se /g. Deng and others (2007) observed relatively slow selenium depletion in the muscle of splittail fed a 12.6 µg/g diet for 9 months that was then followed by 21 weeks of a control diet of 0.4 µg/g. At the end of the experiment the measured concentrations ranged from 11 to 13 µg/g in fish exposed to higher dietary selenium and remained constant at approximately 3 µg/g in fish fed the control diet for the entire experiment. Furthermore, faster elimination rates were detected at the end of a 21-week depuration in fish previously exposed to very high dietary selenium (26.0 and 57.6 µg/g) that might indicate the ability of splittail to cope with the short-term

exposure without adverse effects. The authors concluded that based on the observed growth, tissue accumulation and histopathology, splittail that survived the 9-month exposure to 12.6 µg/g or less could thrive under normal dietary exposure.

One explanation for low tissue concentrations in the North Bay could be related to the fact that splittail may not be consuming Asian clam for several months each year. This fish is known to spawn in inundated terrestrial vegetation in the upper Estuary and their recruitment is strongly associated with the magnitude and duration of floodplain inundation during wet season winter months when the clam population usually experiences a notable decline (Deng *et al.* 2007, Parchaso and Thompson 2002). During laboratory experiments Teh and others (2004) determined that at least 9 months of chronic exposure to a diet of 6.6 µg/g was necessary to induce possible deleterious health effects and these conditions are unlikely to occur in the part of the estuary frequented by splittail.

The relatively high selenium concentrations exceeding 10 µg/g-dw found in the muscle of white sturgeon collected by the RMP from San Pablo Bay between 1997 and 2006, might be linked to a diet composed of bivalves and in particular the Asian clam. Even higher concentrations exceeding 30 µg/g-dw were measured in adult sturgeon caught near Pittsburg in 2000-2001 (USGS data). However, Linares and others (2004) reported selenium in 39 sub-adult sturgeon caught between 2002 and 2004 at levels below 11.9 µg/g-dw with an overall mean concentration of 6.59 ± 0.45 µg/g-dw.

Linville (2006) observed similarly high but greatly variable selenium concentrations in the experimental study with white sturgeon fed with mostly seleno-methionine diets of 15 to 45 µg/g and concluded that the laboratory results were consistent with the conditions in San Francisco Bay-Delta where the Asian clam was also a common food source for white sturgeon. Despite the high variability in observed selenium bioaccumulation rates Tashijan *et al*. (2006) suggested that juvenile white sturgeon are relatively less sensitive to selenium toxicity than other fish species and even the dietary concentrations exceeding 190 µg/g-dw did not affect the survival of sturgeon (the mean survival rate was 99±0.43%). This study also determined on the basis of frequency of kidney lesions, that the adverse effects occurred when white sturgeon were fed 20.5 µg Se /g in the diet. When all sensitive endpoints were considered, no effects were observed with a diet of 9.6 µg Se /g. The corresponding whole-body tissue concentrations with sturgeon fed these diets were 14.7 µg/g-dw (LOAEL) and 11.8 µg/g-dw (NOAEL) respectively.

However, certain developmental defects such as edema and skeletal deformities could occur at lower tissue concentrations (B. Beckon, US FWS, *pers. comm*.). The experimental results reported in the above two studies indicate that these effects begin to get significant when the EC10 exceeds 8.13 µg/g dw (Figure 8).

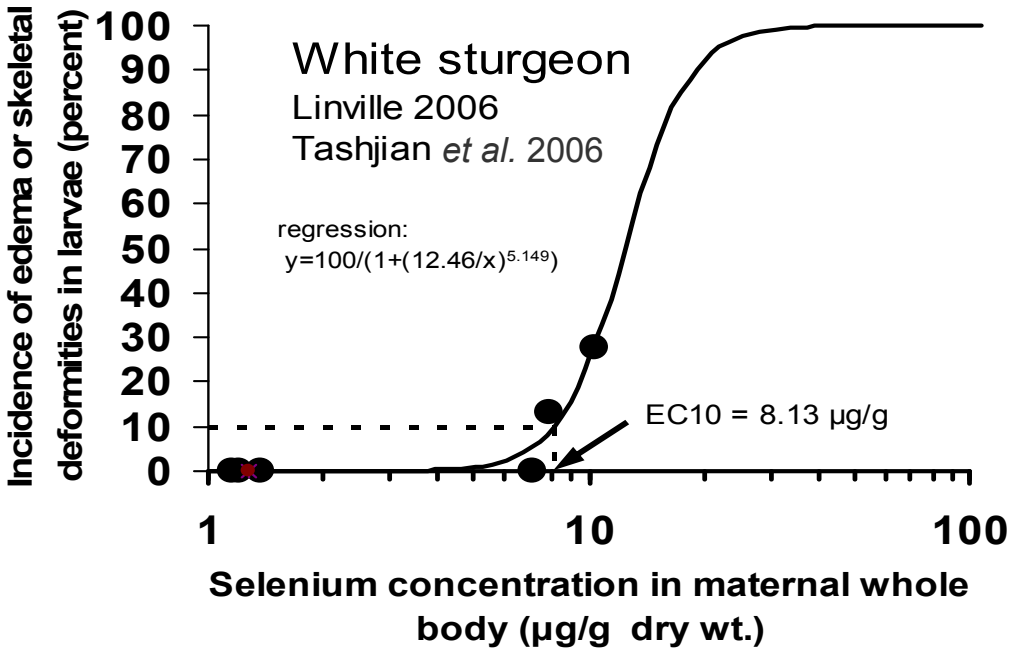


Figure 8: Relationship between selenium in the whole bodies of adult female white sturgeon and the occurrence of edema and/or skeletal deformities in the larvae that hatch from their eggs.

(data from Linville 2006 and Tashjian *et al.* 2006, converted from muscle Se concentrations to whole body concentrations, after Beckon, *pers. comm*.)

Compared to white sturgeon, very little direct information is available for the threatened green sturgeon. In one study that tested the green and white sturgeon response to changed environmental conditions, Kaufman *et al*. (2008) concluded that green sturgeon exhibited much greater sensitivity to selenium. The noticeable declines in predator avoidance and reduced swimming performance in green sturgeon were detected at the dietary dose of 20 µg SeMet/g. However, selenium concentrations and dose spacing used in the experiment were too high to be applicable to the conditions in the North Bay and to accurately determine the toxicologically significant thresholds. In general, white sturgeon is considered to be a representative surrogate species for the green sturgeon (Beckon and Maurer 2008, D. Fleck USEPA *pers. comm*., April 28, 2010).

The protection of the green sturgeon using a numeric target developed based on the white sturgeon data is supported by the habitat and life history of the two species. Green sturgeon are the most anadromous of the sturgeon species and adults and sub-adults spend a large portion of their lives in coastal marine waters outside of the estuary. Typically green sturgeon use the San Francisco Bay during their infrequent (every 2 to 4 years) spawning migrations up to 240 miles upstream the Sacramento River. Juveniles may rear in freshwater and then estuarine waters for 1 to 4 years before dispersing into salt water (73 Federal Register 52084 52110, Sept 8, 2008). Data for white sturgeon indicate that young fish appear to have low selenium levels in spite of spending prolonged periods of time in the estuary (Linares *et al.* 2004).

Chinook salmon

In contrast to sturgeon and splittail, the diet of young Chinook salmon in the Delta consists primarily of insects and crustacean potentially resulting in lesser exposure to selenium. Hamilton *et al*. (1990) conducted a growth and survival study with Chinook salmon in standardized freshwater and brackish water during which swim-up larvae were fed one of two different diets. The survival rate of 94.1 to 95% was observed in larvae exposed for 60 days to seleno-methionine diet at concentrations of 9.6 and 5.3 µg/g-dw, respectively. At the higher (95%) survival rate the selenium concentration in tissue of the tested fish was 3.1 µg/g-dw with the mean larval weight just marginally less than the weight of fish with tissue concentration of 0.9 µg/g-dw and selenium diet of 1 µg/g-dw. The residence time of Chinook salmon juveniles in the estuary was also estimated to range from a maximum of 64 (Beckon and Maurer 2008) to less than 40 days (MacFarlane and Norton 2002), which corresponds to the exposure time used in the experiments that did not result in any significant adverse effects.

The calculated whole body effect thresholds based on the results from the study by Hamilton *et al*. (1990) are 7.6 µg/g-dw for freshwater and 17.1 µg/g-dw for brackish water. These calculations exclude the results of the experiments in which larvae were fed field-collected mosquitofish, from San Luis Drain thought to be potentially contaminated by pesticides and heavy metals. These effect thresholds were higher than those established for bluegill and catfish. This is contrary to the findings reported by Beckon (2007), who employed a biphasic model to all the data from the study by Hamilton *et al*. (1990), and estimated that 20% mortality may occur in Chinook salmon with tissue concentration in excess of 2.5 µg/g-dw. The optimum selenium concentration in that interpretation was assumed to be approximately 1 µg/g whole body-dw. This concentration is lower than the natural background

concentrations found in fish from areas where selenium is attributed to natural geologic sources (Eisler 1985).

The results of a stochastic population model simulating the chronic level exposure in cutthroat trout which have similar early life-stage characteristics to those of rainbow trout or Chinook salmon also confirm that adverse effects from selenium occur at somewhat higher concentrations. Van Kirk and Hill (2007) simulated the conditions in the upper Snake River basin and showed that resident cutthroat trout populations were more sensitive to selenium contamination than migratory populations. Based on the modeling results the authors recommended 7 µg/g-dw as the maximum allowable concentration in whole-body fish tissue to protect cutthroat trout.

Salmonids in the North Bay are potentially among the most sensitive species of fish; however, their migratory nature, the length of time they spend in the estuary and their predominant diet of insects and crustacean imply that these fishes are at lesser risk from selenium than sturgeon or Sacramento splittail.

Toxicity Mitigating Conditions

Environmental factors and water quality parameters have been used in developing the aquatic life criteria for toxic pollutants in recognition of their mitigating effects, and to account for the site-specific conditions in a particular water body. Sulfate content and salinity are among the factors that have been shown to potentially alleviate selenium related toxicity to aquatic organisms. Antagonistic effects from sulfate content on either uptake or acute toxicity of selenate have been reported for algae, aquatic invertebrates, Chinook salmon and fathead minnows (USEPA 2004).

Hansen *et al*. (1993) demonstrated that sulfate concentrations significantly reduced the accumulation of selenium in two aquatic invertebrates: *Chironomus decorus* and *Daphnia magna*. Based on the results of the laboratory experiments the study concluded that although increased levels of sulfate could not totally prevent selenate absorption, over 40% reduction in tissue selenium concentrations was observed in both invertebrates for the Se to S ratios between 1:0 to 1:480. Similarly, juvenile rainbow trout acclimated in high salinity water (16.8 dS/m) prior to dietary exposure were more resistant to 180 µg/g dietary seleno-methionine treatment and experienced limited mortality (33 and 0%) compared to tests in freshwater where 100% mortality occurred (Schlenk *et al.* 2003). This reduction in selenium uptake has been attributed to salinity and the presence of sulfate ions that may prevent the interaction of seleno-methionine with proteins on subcellular level.

Hamilton and Buhl (1990) conducted 24-hr and 96-hr acute toxicity tests with advanced fry of Chinook salmon and coho salmon in fresh and brackish waters simulating the conditions in the San Louis Drain. Although the study focused on examining the impact of multiple contaminants and the sensitivity of various life stages of fish, the reported acute toxicity to selenate and selenite expressed as LC50s were consistently higher in the standardized brackish water compared to tests in freshwater. In addition, the authors estimated the margin of safety from the pooled LC50 data for Chinook salmon expressed as a difference between selenium levels resulting in no effects and toxic effects. The margin of safety for both selenate and selenite was significantly higher in brackish water with the value for more toxic selenite estimated at 276 in freshwater and 468 in brackish water. Similarly, in a chronic toxicity study with fingerlings size Chinook salmon exposed to dietary selenium for 120 days, the fish survival was significantly reduced in freshwater but not affected in brackish water (Hamilton *et al.* 1990). In a 10-day seawater challenge test that followed the dietary exposure, the fish survival was significantly reduced but only in fish fed in excess of 35 µg Se/g. Evidence of no effects on growth or survival in fish fed 26 µg Se/g prior to a 3-month seawater challenge was also provided.

Even though the data are limited, fish seems to exhibit much higher resilience to selenium toxicity in saltwater with higher sulfate content than freshwater. The results of these studies suggest that levels of sulfate occurring in the North Bay are likely to provide added level of protection against selenium toxicity and at the same time account for an implicit margin of safety in our review of the screening values for fish.

Selenium Toxicity Thresholds in Birds

Selenium toxicity in birds has been recognized as an issue of concern since the 1980s (Ohlendorf and Fleming 1988, Skorupa 1998).This evaluation of selenium toxicity focuses on six bird species that have been identified by Beckon and Maurer (2008) to be the most at risk from selenium and are common in the San Francisco Bay/Delta area. These species include black scoter, California clapper rail, greater and lesser scaup, surf scoter and white-winged scoter and are considered to be exposed to selenium because of their main feeding habits and/or wintering locations. Although San Francisco Bay is described as an important habitat and wintering area for waterfowl, no direct toxicity information is available for any of the birds species listed above. Instead, this section of the report summarizes the available information on avian toxicity in general and examines toxic concentrations in the diet and eggs of typical laboratory test species.

The dietary screening levels reflecting potential adverse effects for bird species in the North Bay were determined based on a review of more than 40 selenium toxicity studies. Chickens and mallards were the bird species for which most information was available. The dietary toxicity data showed a similar broad range of sensitivities and variability as presented for fish (Figure 9).

The evaluation of toxicity studies confirmed that reproductive success, such as egg hatchability, egg fertility and chick survival was the most sensitive endpoint in the tested birds, especially in mallards. In addition, the results for chickens indicated the growth/survival was also one of the sensitive endpoints. A large variability in the effect threshold ranging from 1.5 to 17.3 may suggest that these birds have potentially greater resilience to selenium toxicity. Similarly, immature mallards seem to be able to tolerate relatively high selenium concentrations reaching 17 µg/g-dw without experiencing adverse effects (Heinz *et al.* 1990).

Since no toxicity data on bird species of concern in the North Bay are available, data from the available bird studies were used and allometric scaling applied to better estimate the pertinent risk levels (Tetra Tech 2008b). In ecological risk assessment, allometric scaling is often used to extrapolate toxic responses observed in avian test species to the wildlife endpoint species of interest (Sample and Arenal 1999). The allometrically adjusted toxicity values account for differences in body weight, metabolism, pharmacokinetics and sensitivity to allow for the best available estimate of species-specific toxicity when data are lacking.

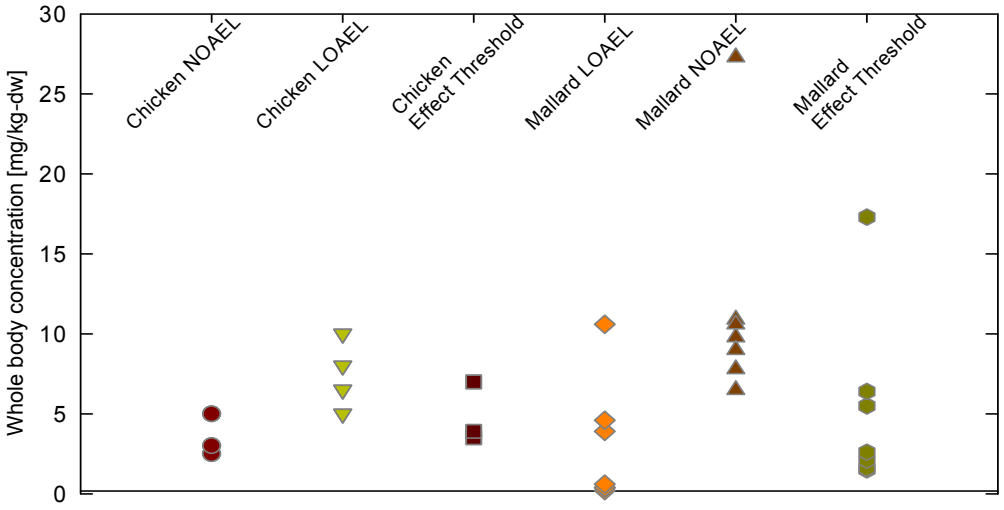


Figure 9: Observed range of dietary selenium concentrations at which adverse effects in birds may occur

In an effort to relate the known toxicity levels observed in chickens and mallards to the species of concern in the North Bay the allometrically adjusted toxicity values were calculated using the following equation:

$$TRV_a = TRV_t \left(\frac{BW_t}{BW_a} \right)^{(1-b)}$$

where:

TRV_a - allometrically adjusted toxicity value

TRV_t - toxicity reference for a test species

BW_t *and* BW_a - body weights (in kg) for the test and wildlife species, respectively, and

b - allometric scaling factor (this factor is not specific to Se but is a mean value for other contaminants)

The available dietary toxicity values considered as the most indicative of reproductive success were used in the calculation of allometrically adjusted screening values for birds in the North Bay. The calculated results in Table 5 show large variations depending on the type of the original test species and the toxicity thresholds used. The adjustment based on the studies for mallard ducks that share many common characteristics with most birds of concern in the North Bay indicates that clapper rail could be sensitive to dietary Se concentration of 2.2 µg/g-dw and that diving ducks (scaups and scoters) show fairly consistent sensitivity threshold within a range of 3.2 to 5.6 µg/g-dw (mean 4.1).

Table 5: Allometrically adjusted dietary selenium screening values for birds in the North Bay

Bird Species	Dietary Screening Value [µg/g-dw]	
	Mallard [a]	**Chicken** [b]
California clapper rail	2.2	0.9
Greater scaup	3.9	1.6
Lesser scaup	3.2	1.3
White-winged scoter	5.6	2.3
Surf scoter	4.1	1.7
Black scoter	3.9	1.6

a – EC10 for reduced hatching success from Adams *et al*. (2003) and Ohlendorf (2007) of 4.4 µg/g-dw

b – effect threshold for reduced hatching success of 3.9 µg/g-dw from Ort and Latshaw (1978)

Clapper rail

Although clapper rail depends on a diet that includes benthic invertebrates, these birds feed predominantly on plaited horse mussels (>50%), and not on Asian clams. Therefore their dietary selenium intake is likely to remain low. According to Beckon and Maurer (2008) only a relatively small proportion of clapper rail diet comprises Macoma clams (>7%), yellow shore crabs and snails account for less than 5% of the diet, and spiders and plant material account for 15% each. The preferred clapper rail diet, together with the fact that their principal habitats include low portions of coastal wetlands and tidal sloughs where the Asian clam is less common, are likely to limit the exposure of clapper rail to dietary selenium.

The recently published results of a study that investigated the reproductive success of clapper rail in six bay area marshes (including two marshes in the North Bay area: Corte Madera and Wildcat) during four breeding seasons from 1991 through 1999 (Schwarzbach *et al*. 2006) revealed that mean egg tissue selenium concentrations ranged between 1.89 and 2.22 µg/g-dw and were within the normal range for avian eggs (1 to 3 µg/g-dw: Skorupa and Ohlendorf 1991) signifying no effect on reproduction. Furthermore, the egg selenium concentrations declined significantly since the 1980s and were at half of the concentrations found in 1986-87 (mean: 4 µg/g-dw; range 1.6 – 7.4 µg/g-dw). As concentrations in eggs are the most direct way to determine avian embryonic exposure and effects we conclude that under current conditions the endangered clapper rail are not at risk from selenium exposure.

Surf scoter and Greater/Lesser scaup

Among the North Bay birds, only scoters and scaups are likely to be exposed to selenium concentrations in their diet that may exceed the screening levels, with the greater and lesser scaup and surf scoter being most at risk because of their feeding habits. These diving ducks are common in the North Bay and they feed primarily on benthic mollusks, especially clams and mussels, crustaceans and insects. The results from the 2002 bird study involving tissue and gut content analysis of surf scoters showed that the entire gut content of scoters caught in Suisun Bay was comprised of the invasive clam *C. amurensis,* while in scoters caught in San Pablo Bay the gut content consisted of 25% of *C. amurensis* and 75% of the soft shelled clam, Mya arenia (J. Hunt,SFEI, *pers. comm*). Average selenium tissue concentrations in scoters measured in Suisun Bay and San Pablo Bay were below 4 µg/g-ww indicating a 50% reduction compared to the levels observed in 1989 that exceeded 11 µg/g-ww (Figure 10).

The concentrations of selenium in greater scaups in 2002 and 2005 on average did not exceed 5 µg/g-ww; the levels in San Pablo Bay and Suisun Bay were slightly higher in the

most recent samples than in 1986-1987. Nevertheless, the results show that typically, for both species, selenium concentrations in 2002-2005 were lower in most regions of the Estuary than in the peak concentration years of the late 1980s.

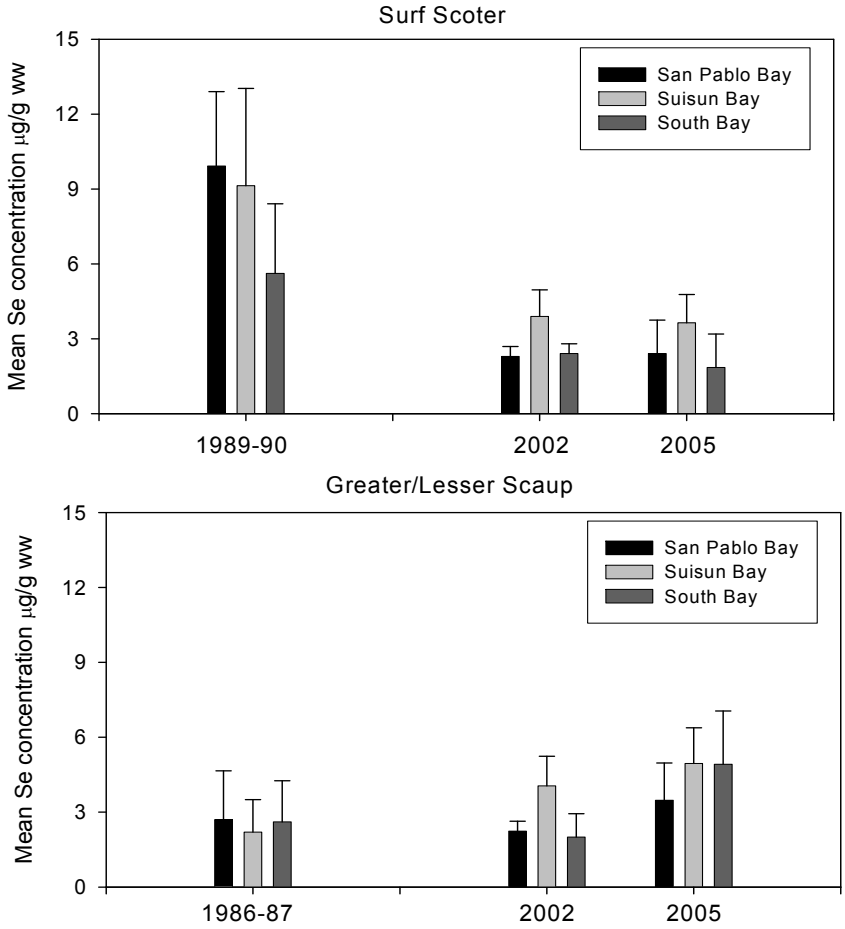


Figure 10: Selenium tissue concentration in diving ducks from San Francisco Bay
(columns represent average concentrations and bars show standard deviation)
Data: DFG 1987, 1988, 1991; SFEI- J. Hunt *pers. comm*.

A similar reduction in selenium concentrations in aquatic birds from Central Valley has been detected based on the data collected from 1986 to 2005 in the Grasslands area. Paveglio and Kilbride (2007) reported that selenium concentrations in the livers of mallards, pintails, coots and stilts from the North Grasslands declined by 38 to 68 percent throughout the 20-year period. For birds collected in North Grasslands in 2005 the average concentrations of selenium in livers varied from 5 to 8.5 µg/g-dw. The 95% confidence intervals (7.1 - 11 µg/g-dw) were highest in black-necked stilts. The authors affirmed that all 95% confidence

intervals for the 2005 data from North Grasslands were below the potential reproductive impairment range of 20 to 30 µg/g-dw derived from the US FWS data.

The data from the National Irrigation Water Quality Program have shown that ducks exhibit greater sensitivity to embryonic selenium exposure than other species studied and the response functions developed for ducks represent a generic surrogate for other sensitive birds (Seiler *et al.* 2003). Yet predictions of the teratogenic effects based on the selenium-response functions showed that selenium concentrations of 15 µg/g-dw in eggs would have a minimal adverse impact (~EC01) and the duck eggs' exposure to 20 µg/g Se dw would cause incidence of teratogenesis to increase to 5 percent (EC05).

Moreover, studies indicate that both, selenium accumulation and depuration rates in birds, are rapid. It would take just over 70 days for waterfowl to return to background selenium levels once they leave the selenium rich source, and only within 8 to 10 days selenium concentrations are likely to fall below the known effect thresholds (Heinz *et al.* 1990, Wilson *et al.* 1997). The rapid depuration of selenium by diving ducks during their more than 50-day spring migration from San Francisco Bay to breeding grounds in Alaska and Northern Canada might be responsible for lack of detrimental physiological effects reported and for minimal amounts of selenium deposited in developing eggs. This way the potential for adverse effects in transient and migratory species that are most at risk from selenium in the North Bay is greatly reduced.

DeVink *et al*. (2008) simulated late spring migration exposure to environmentally relevant doses of dietary selenium in an experimental study with captive scaups. The authors found no treatment effect on body mass, breeding probability, or clutch initiation dates after a 30-day exposure to 15 µg/g and 7.5 µg/g of Se as selenomethionine, after which excess selenium was removed from the diets prior to laying. Moreover, the results showed that egg selenium concentrations decreased rapidly after selenium-supplemented diets were removed and within 12 and 8 days post treatment were below the teratogenicity threshold of 9 µg/g-dw. The overall conclusions indicated that these dietary exposures were not sufficient to adversely affect body mass or reproduction in scaup that subsequently migrated to uncontaminated breeding areas.

The selenium diets used in the study reflected the maximum reported concentrations (7.4 µg/g) in zebra mussels from sites along the St. Lawrence River and an environmentally elevated dose (15 µg/g) greater than the maximum reported concentration (11.5 µg/g) in zebra mussels from the Great Lakes. Areas surrounding Lake Erie have recently experienced

significant increases in diving duck populations that are attributed to the invasion of the zebra mussel. Selenium concentrations in *C. amurensis* in the North Bay are very similar to those found in zebra mussels and used in the study. The levels in *C. amurensis* measured in 1999 ranged from 7.2 to 16.7 µg/g (mean 11.0 µg/g) and in 2008 the mean was 9.5 µg/g. One of the most compelling signs so far that the conditions in the Bay may have lesser than expected impact on diving ducks comes from the recent analysis of selenium in eggs of scoters. In 2005-2006 twenty three female scoters from the Bay area were marked with satellite transmitters and their migration was tracked to the breeding areas (Wainwright-De La Cruz, USGS, *pers. comm*.). Eleven fresh eggs were collected from three nests of the marked birds. The concentrations of selenium in these eggs were 1.71 +/- 0.12 µg/g-dw, well below those thought to be of concern for other sensitive bird species and within the normal range of concentrations:1 to 3 µg/g-dw; (Skorupa and Ohlendorf 1991).

Existing Screening Levels for Fish and Birds

Screening values reflective of safe selenium concentrations in water, sediment, food and tissue of aquatic organisms were reviewed and proposed in the past (Presser and Louma 2006, Hamilton 2002, Lemly 1998, Skorupa 1998). In establishing these threshold levels researchers considered numerous factors including the most sensitive endpoints, different life stages, type of exposure, dietary determinations and other conditions. To ensure protective conditions for all types of wildlife and habitats the suggested threshold levels tend to be set to the lowest value established from a limited number of experimental studies and field measurements, even though, a wide range of sensitivities to selenium might have been observed. This approach may lead to recommending screening values that are lower than background concentrations in areas naturally enriched in selenium. For example, minimum selenium concentrations in Yellowstone cutthroat trout in proximity to phosphate deposits in Idaho but not affected by the mine operations were reported to range from 0.1 to 4.7 µg/g-dw (Golder Associates 2006) while in other areas concentrations within the range of 1 to 2 µg/g-dw would be representative of background levels. In Central Valley, natural enrichment in selenium in soils contributes to elevated ambient selenium levels San Joaquin River.

Table 6 shows the screening level concentrations most commonly referred to in the scientific literature that encompass the variety of concerns. The concentrations exceeding the upper limits are likely to have adverse effects. The recommended ecological risk guidelines that were used to evaluate the success and effectiveness of the measures implemented to

mitigate selenium contamination at the Grassland Bypass Project in Central Valley are shown in Table 7.

Table 6: Threshold selenium concentrations in fish and aquatic birds

Fish/Birds	Presser and Luoma (2006) [a]		Lemly (1998) [b]	
	Diet	**Tissue**	**Tissue**	**Measured Concentrations**
	µg/g – dry weight		µg/g – dry weight	
Fish Thresholds				
General	2 – 8	4 – 12	Whole body	6 – 9 no effect (<3) [c]
Sensitive Species	2 – 5	1.5 – 6		
Eggs	> 3	5 – 10	Eggs	6 – 17 teratogenic (<3)
Liver		12 – 15	Liver	4 – 7 no effect (<8)
Bird Thresholds				
General	3 – 7	3 – 10 (egg)	Muscle	7 – 19 no effect (<3)
Sensitive Species	2 – 5	6 – 7 (egg)		
	-	-	Eggs	4 – 9 no effect (<3)
Liver		20 – 30	Liver	23 – 32 teratogenic (<10)

a – Compiled from Presser and Luoma (2006) (Tables 13, 14 and 15)

b – Lemly (1998) (Table 1), values represent measured concentrations showing whether adverse effects are likely to occur

c – Values in parenthesis indicate concentrations typical for uncontaminated aquatic systems

Table 7: Ecological risk guidelines for selenium concentrations
(from Beckon *et al*., 2001)

Medium	Effects on	No Effect	Concern	Toxicity
		µg/g – dry weight		
Warm water fish (whole-body)	Fish growth/condition/survival	<4	4–9	>9
Vegetation (as diet)	Bird reproduction	<3	3–7	>7
Invertebrates (as diet)	Bird reproduction	<3	3–7	>7
Sediment	Fish and bird reproduction	<2	2–4	>4
Avian egg	Egg hatchability	<6	6–10	>10
		µg/L		
Water (total recoverable Se)	Fish and bird reproduction (via foodchain)	<2	2–5	>5

Selenium Guidelines for Great Salt Lake (State of Utah)

In 2004 the State of Utah formed a Science Panel to develop a water quality standard for selenium in Great Salt Lake that would prevent impairment of aquatic wildlife. The Science Panel determined reproductive success in birds to be the most sensitive end point and used studies of mallards to recommend the guideline selenium levels in diet and eggs that would be protective of birds commonly nesting on the lake. In recognition of uncertainty the guidelines were initially expressed as a range and included diet selenium concentrations between 3.6 and 5.7 µg/g and egg concentrations between 6.4 and 16 µg/g (Utah DEQ 2008). Finally, Utah recommended the egg tissue-based standard of 12.5 µg/g-dw that is equivalent to 10% effect level concentration (EC10).

These numeric guidelines have been criticized for potentially allowing higher than acceptable levels of exposure in this unique ecosystem with very high environmental and commercial value (J. Skorupa, USFWS, *pers. comm*.). In addition, the availability of food sources rich in selenium and selenium ingestion rates might be extremely variable; hence, measuring selenium concentrations in dietary items may not provide the most sensitive indicator of birds' reproductive success. Subsequently, it was recommended that the concentration of selenium in the eggs be the preferred indicator that determines avian reproductive impairment.

Skorupa (2008, USFWS, *pers. comm*.) suggested that the State of Utah should aim at setting the water quality standard for Great Salt Lake to the value equivalent to no effect concentration (NEC) in avian eggs that could be inferred from the estimates of the EC10. The value of 7.7 µg/g for mallard egg hatchability determined with a generalized biphasic response model (Beckon *et al.* 2008) was considered to be the most technically valid approach for deriving the EC10. This resulted in recommendation of the NEC to be within the range extending from 3 to 7.7 µg/g with the lower boundary representing background means in avian eggs. A geometric mean of the boundary values was used to arrive at the best estimate of NEC for avian eggs that equals to 5 µg/g and this value is not being exceeded in the North Bay.

Given the uncertainties surrounding the magnitude of ecological risks the Science Panel recommended a tiered approach to implement the selenium standard of 12.5 µg/g-dw that requires an increased monitoring and triggers specific regulatory responses when selenium concentrations in eggs increase above 5 µg/g-dw.

Newport Bay Watershed Selenium TMDL

The most recent re-examination of black-neck stilt egg hatchability data by the USFWS staff for the purpose of establishing site-specific objectives for the Newport Bay Watershed TMDL (*report undergoing peer review*) generated two possible NEC for selenium in black-necked stilts: 5.8 µg Se/g dw and 10.2 µg Se/g dw. The value of 8 µg Se/g dw was then recommended as the egg tissue target to be sufficiently protective of the federally listed bird species that reside or forage in the Newport Bay watershed. The fish tissue target of 5 µg/g dw for both fresh and saltwater fish was deemed protective as a dietary target for piscivorous birds.

Site-Specific Thresholds Relevant to the North Bay

In summary, Table 8 shows site-specific concentration data and toxicological effects that are most relevant to the species and conditions in the North Bay.

Table 8: Summary of site specific data and toxicity levels evaluated for this project

Species in North Bay	Mean (standard deviation) [a]	Threshold Concentrations [a] - µg/g–dw		
Fish	µg/g-dw	LOAEL	Effect Threshold	Reproductive toxicity
White Sturgeon				
whole body		12.3 – 22.5	6.2 – 18.2	6 (9) [b] **8.1 (EC10)** [c]
muscle	9.2 (5.5)	12.1 – 36.8	4 – 29.0	
liver	24.1 (10.3)	10.4 – 37.4	3.9 – 28.7	
Sacramento splittail				
whole body		12.9	10.8	
muscle	2.4 (0.9)	15.1	12.3	
liver (Delta only)	11.5 (6.3)	26.8	24.8	
Birds	µg/g-dw	LOAEL	Effect Threshold	Reproductive success
Diving ducks		6.5 –27.3 (diet)	1.5 – 17.3(diet)	7.5 (diet-no effect) [d]
Surf scoter muscle	11.2 (4.4)			
Greater scaup muscle	13.0 (5.2)			
Scoter eggs	1.7 (0.1) [e]			5.0 (eggs-no effect) [f]
Bivalve tissue: local	2.5 (1.8) [g]	-	-	-
Bivalve tissue: invasive	1999: 11.0 (2.5) [h] 2008: 9.5 (2.6) [i]	-	-	-

a – Unless noted threshold concentrations based on the toxicity studies evaluated in Tetra Tech (2008b), Table 3-3 and Table 4-7

b – Toxicity thresholds estimated based on pooled data for coldwater fish (6 µg/g-dw) and warmwater fish (9 µg/g-dw) after Brix *et al.* 2000

c – Estimated by Beckon USFWS (*pers. comm*., see Figure 8)

d – Diet representative of spring-staging with no adverse effects on reproduction (DeVink *et al.* 2008)

e – Concentrations in scoter eggs from San Francisco Bay found in wintering areas (Wainwright-De La Cruz, USGS, *pers. comm*.)

f – Egg tissue-based no effect concentration established for birds in Great Salt Lake (Utah DEQ 2008)

g – US Mussel Watch Program 1986-2005 (O'Connor and Lauenstein 2006)

h – USGS data for *C. amurensis* collected in 1999

i – Tetra Tech data for *C. amurensis* data collected in November 2008

4.3 Existing Water Column Objectives

To ensure protection of aquatic life, numeric water quality objectives for toxic pollutants such as selenium have been established by the USEPA and the California Toxics Rule (CTR).The aquatic life criteria include one-hour average (acute) and four-day average (chronic) concentrations of these pollutants to which aquatic life can be exposed without harmful effect. The criteria for selenium that currently apply are shown in Table 9. Although the USEPA approved the statewide selenium objectives for marine waters in California, they do not apply to San Francisco Bay. The USEPA found substantial scientific evidence that high selenium bioaccumulation was taking place in San Francisco Bay and, under these conditions, concluded that the saltwater criteria did not account for the food chain effects observed in San Francisco Bay. As a result the USEPA promulgated the freshwater National Toxic Rule (NTR) criteria for selenium in the San Francisco Bay/Delta. Water column concentrations in the North Bay do not exceed the NTR criteria.

Table 9: CTR water quality objectives for selenium

Water Quality Objectives	Chronic Objective µg/L (4-day average)	Acute Objective µg/L (1-hr average)
California (saltwater objectives)	71	290
San Francisco Bay and Delta (freshwater objectives)	**5**	**20**

4.4 Human Health Criteria

OEHHA (2006) developed equations to estimate fish contaminant goals (FCG) for selenium using a standard consumption rate of eight ounces per week (32 g/day). The FCGs are designed to estimate contaminant levels that pose no significant health risk to individuals consuming sport fish and could be used to establish fish tissue-based criteria for fish

consumption advisories or pollution mitigation goals. They are similar in nature to the risk-based consumption limits recommended by USEPA (2000), however, the FCG calculations take into account contaminant nutritional requirements. Desired contaminant concentrations for a nutrient with a non-carcinogenic effect, such as selenium, is calculated as follows:

FCG = [(RfD x BW) – BDL]/CR where:

RfD – chemical specific reference dose ($5x10^{-3}$ mg/kg-day)
BW – body weight of consumer in kg (70 kg default)
CR – consumption rate as a daily amount of fish consumed in kg/day (0.032 kg/day)
BDL – background dietary level in mg/day (0.114 mg/day)

The background dietary level was determined based on studies of nutritional requirements and the results of the National Health and Nutrition Examination Survey. The recommended dietary allowance (RDA) for selenium for general adult population is 55 µg/day and the mean selenium intake from diet only, surveyed among all individuals, is estimated at 113.7 µg/day. For those individuals who supplemented their dietary selenium the mean intake was found to be 116 µg/day. OEHHA recommends using the value of 114 µg/day as the background dietary consumption rate for computing FCGs for selenium. Using the above equation and assuming a consumption rate of 8 ounces per week of uncooked fish (32 g/day), which is also a rate used to begin issuing fish consumption advisories, the selenium FCG is 7.4 mg/kg. All known concentrations of selenium in fish in San Francisco Bay are well below 7 mg/kg–ww and therefore do not pose a risk to human consumers.

Similarly, the concentrations measured in the tissue of surf scoter and scaup ranging from 1.34 to 6.4 mg/kg–ww are below the guideline level.

4.5 Tissue-Based Numeric Target

Work is underway to revise the chronic aquatic life criterion for selenium on the national and state level (D. Fleck, USEPA Region 9 *pers. comm.*). However, because of the complex biochemistry of selenium in aquatic ecosystems and its bioaccumulative nature, dependent on resident species characteristics and site-specific conditions, it is unlikely that one criterion, when developed, would be relevant to the conditions in the North San Francisco Bay or other distinct water bodies.

As discussed in the sections above, we have reviewed the scientific literature and guidance documents to develop a numeric target that is applicable to the conditions in the North San Francisco Bay and protective of bird and fish species that are likely at risk from selenium

exposure. Comparison of selenium bioaccumulation via waterborne versus dietary routes shows evidence that water-only toxicity tests could underestimate selenium risk and that selenium biotransformation by algae and zoobenthos adds substantially to the total exposure of higher trophic level organisms. Therefore, we are selecting the numeric target for this TMDL to be expressed as tissue-based concentration.

Even though both fish and birds have the capacity to regulate the levels of selenium in their bodies, the propensity of selenium to bioaccumulate and stay at higher levels is greater in fish than in birds. Despite strong bioaccumulation potential, diving ducks do not show significant adverse impacts. It has been demonstrated that waterfowl that use the area of San Francisco Bay as their wintering grounds depurate selenium quickly after leaving the area where food is enriched with selenium. Their tissue concentrations are likely to return to background levels before the birds reach their breeding grounds and their breeding success is not affected by selenium (Wainwright-De La Cruz, USGS, *pers. comm*., DeVink *et al.* 2008).

Our review of toxicological effects has demonstrated that selenium toxicity in the North Bay is only prominent in benthic-based food webs. Among the benthic-based food webs, the clam-eating bottom feeders such as white sturgeon and Sacramento splittail are most at risk, with white sturgeon being the most susceptible. Thus by establishing a numeric target that is protective of this fish we will ensure that all other species will also be protected.

While selenium toxicity has been studied predominantly in the freshwater environment and research has focused on warm water fish, new information is emerging showing the coldwater fish such as that in the North Bay are more resistant to adverse impact of selenium (Chapman 2007, Schlenk *et al.* 2003). It has been demonstrated that since sulfate levels should be higher in brackish and marine waters than in freshwaters, the numeric target established based on the freshwater toxicity studies is more stringent and, subsequently, offers an added level of conservatism to the target value.

The best available information indicates that the EC10 for white sturgeon should be no higher than 8.13 µg/g-dw (see Figure 8). This estimate takes into consideration gross developmental effects resulting from the transfer of selenium from fish through eggs to developing larvae when fish are most vulnerable. Most recently the USEPA indicated that the EC10 value of 8.1 µg/g-dw is being considered as the fish tissue criterion for San Francisco Bay. Nevertheless, scientific concerns remain whether this threshold offers sufficient protection for fish species like green sturgeon. At this time, due to uncertainties in scientific understanding and lack of

guidelines for the desired level of protection for aquatic life in San Francisco Bay it is difficult to determine a single value as a TMDL target. Instead, we recommend a range of concentrations from **6.0 to 8.1** µg/g-dw as the proposed target. The lower range represents the upper end of the whole body selenium concentration range (4 to 6 µg/g) commonly associated with minimal effects in freshwater fish and is deemed protective of sensitive endpoints in the estuarine environment. The upper range corresponds to the EC10 established for white sturgeon, the fish identified in this TMDL as the species of concern in the North Bay. Overall, this range signifies the desirable level of protection for most sensitive fish species that reside and forage in the Bay. In developing the proposed values we considered various scientific arguments and all relevant data.

5 SOURCE ANALYSIS – SOURCES AND LOADS

Selenium mainly originates from natural sources such as sedimentary rocks, seleniferous soils, and selenium-rich mineral deposits occurring throughout California. Marine shale of Late Cretaceous period formed by sedimentary accumulation and mineralization of marine particulate matter are particularly rich in selenium (SWRCB 1989). Selenium from these sources could be concentrated and redistributed by geological and biological processes, and anthropogenic activities. Agricultural management practices leading to selenium enrichment in irrigation drainage water are often considered as the main cause of surface water contamination in California and the Bay Area. Irrigation remobilizes selenium by leaching it from the soils originating from marine sedimentary deposits. Weathering and erosion of selenium enriched sediments may contribute to the elevated selenium levels in nearby streams and groundwater. Fossil fuels such as coal and crude oil are naturally enriched with selenium. Thus, refining and cracking of crude oils, combustion of fossil fuels and solid waste, microbial activity, and industrial processes also release selenium to the atmosphere and surface waters.

There are several sources contributing selenium into the North San Francisco Bay. The main sources are industrial and municipal discharges including petroleum refineries, urban and non-urban runoff, erosion and sediment transport within the Bay, flow from Central Valley watersheds through the Delta, and atmospheric deposition. Brief descriptions of each source loading contribution, and the uncertainty associated with the load estimates are summarized in Table 10. The magnitude of selenium loads associated with these sources and their temporal variability are discussed in the subsequent sections[2]

During the wet season, riverine sources potentially contribute larger loads than known municipal and industrial facilities discharging to the Bay. While there is usually only limited inflow from the San Joaquin River into the estuary, selenium loads could increase significantly when water from the river reaches the Bay because of typically much higher selenium concentrations. However, it is the dry season that could be critical for selenium bioaccumulation due to its longer residence time in the Bay. Therefore, for source categories with seasonally changing load patterns and available flow information, both dry and wet season loads were calculated and compared.

[2] Selenium load assessment presented in the following sections is based on the Source Characterization Report (2008a) prepared by Tetra Tech, Inc a technical consultant for the project.

Table 10: Characteristics of external and internal sources and loads of selenium in the North Bay

Source		Description	Dominant Se Forms and Species	Load [kg][a]
External	Municipal and industrial wastewater	POTWs and industrial wastewater effluents generally have low concentrations of selenium and they have not changed over the past 20 years. Total selenium concentrations in the effluent are measured and reported on regular basis.	**Predominantly dissolved Se**: selenate (60%), selenite (25%), organic and elemental Se (15%)	**230**
	Petroleum Refineries	Refineries contribute the largest load of selenium among point sources discharging to the Bay. The refinery effluent consists almost exclusively of dissolved forms of selenium with selenate, the less bioavailable form, being the dominant species since 1999.	**Predominantly dissolved Se**: selenate (56 - 64%) organic selenide (~20%) selenite (15 - 22%).	**540**
	Central Valley watersheds via Delta inflow	Delta inflow consists of flow from the San Joaquin and Sacramento Rivers, and forms the major source of selenium to the Bay. The rivers are also the main source of particulate selenium that provides a pathway to bioaccumulation of selenium in benthic organisms. Sacramento River dissolved Se concentrations are considered to represent regional background levels, they have been consistently low and have remained unchanged over the years. San Joaquin River carries seleniferous agricultural drainwater and has historically much higher concentrations of dissolved selenium. Much of San Joaquin River flows are currently diverted before entering the Bay.	**Dissolved selenium**: Sacramento River - selenate (50 – 70%) selenite (10 – 20%) organic selenide (10–20%) San Joaquin River - selenate (60 – 70%) selenite (3 – 10%) organic selenide (15–20%) **Particulate selenium**	**3940** (annual average) (1110 - >11000) **770** (part. Se annual average) (170 -1660)
	Urban and non-urban runoff	Urban and non-urban runoff from local tributaries – includes both agricultural and urban stormwater runoff, and may be a significant source of selenium during the wet season	Speciation not measured but assumed to be similar to Sacramento R.	**350-840** (>1500)
	Atmospheric deposition	Atmospheric deposition includes both dry and wet deposition to the Bay water surface, and is considered as a small selenium source	Wet deposition (selenite) Dry deposition	**20** (120) <**10** (130)
Internal	Erosion and sediment transport in the Bay	Can be either a source or a sink of selenium. Input from Bay sediments may include net sediment erosion, resuspension and diffusion. Dredging activities can also potentially contribute selenium to the Bay water column	**Particulate selenium**	**280**

[a] Unless noted, loads are expressed as total selenium. Values in bold represent the best estimate, values in parenthesis show the range and/or the highest estimate. Estimates are rounded to the nearest 10 kg

5.1 External Sources

Municipal and Industrial Wastewater Dischargers

Figure 11 shows locations of municipal and industrial facilities discharging treated effluent directly or indirectly to the North Bay. Among them, there are 22 Publicly Owned Treatment Works (POTWs), 6 minor industrial facilities and 5 petroleum refineries.

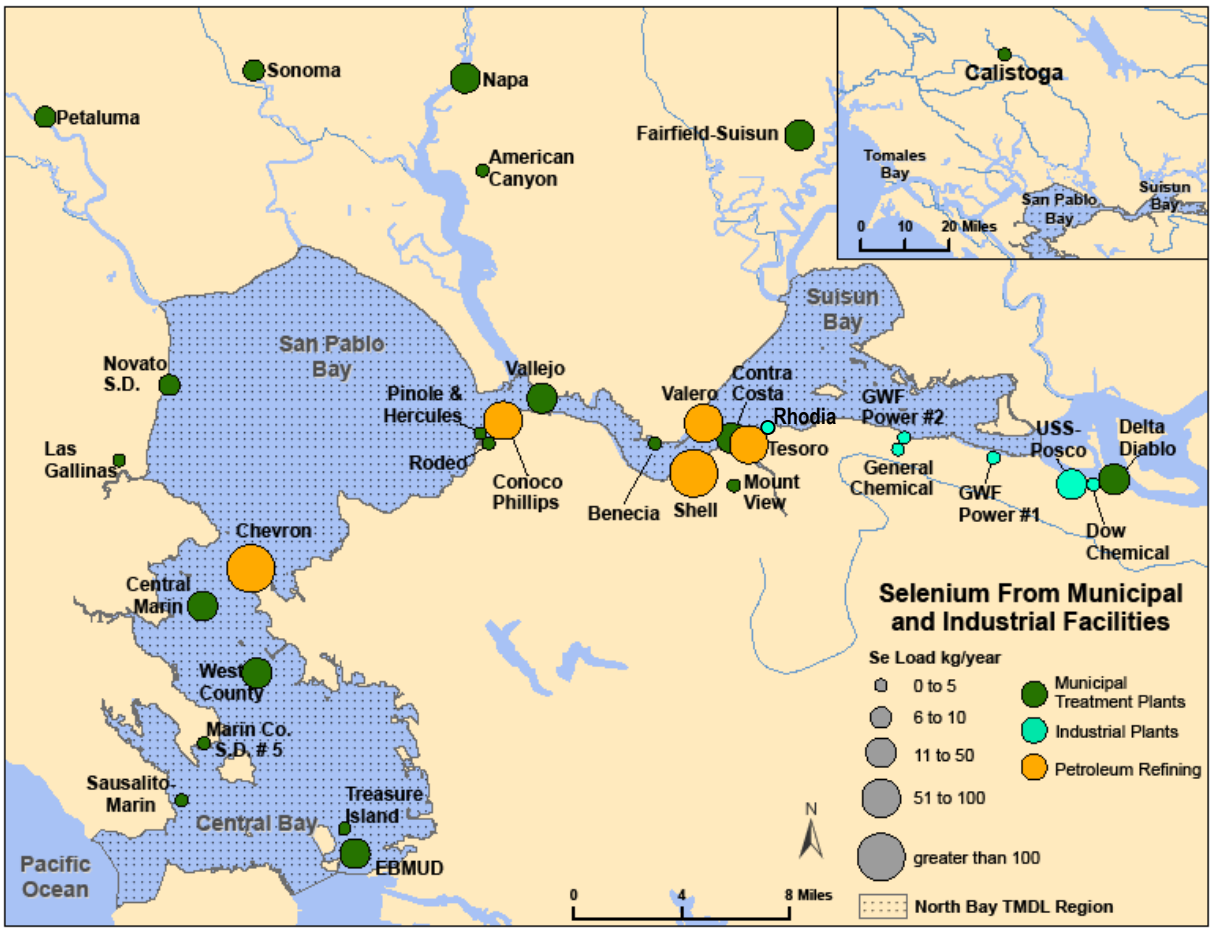


Figure 11: Point source dischargers in the North Bay

Publicly Owned Treatment Works (POTWs)

All most recent flow and effluent concentration data (1998 – 2007) reported by the POTWs as part of their permit requirements were used to evaluate the magnitude of selenium loads

(Table 11). Most municipal wastewater facilities treat effluent to the secondary level with the exception of City of American Canyon, Calistoga and Napa Sanitation District which have advanced level treatment. Discharge from these facilities generally follows a seasonal pattern of higher flows during wet season, most likely due to contribution from stormwater runoff.

Daily flow data and monthly selenium concentrations are usually available to compute loads. The average flow ranges from less than 1 million gallons per day (mgd) (City of Calistoga) to over 74 mgd (East Bay Municipal Utility District, EBMUD) with the maximum flow exceeding 150 mgd. Selenium concentrations in effluent are generally below 1 µg/L, with many samples below the detection limit (Table 11, Figure 12). Concentrations at two facilities with the largest discharges, EBMUD and Central Contra Costa Sanitation District (CCCSD), average 0.34 ± 0.19 µg/L and 0.34 ± 0.50 µg/L respectively. These most current concentrations are similar to the dissolved selenium concentrations observed by Cutter and San Diego-McGlone (1990) during 1987-1988 sampling of effluent at monthly intervals (EBMUD: 0.37 ± 0.10 µg/L, CCCSD: 0.53 ± 0.11 µg/L). This study also determined that the speciation of selenium in effluent from municipal wastewater was dominated by less bioavailable selenate (60%), followed by selenite (25%) and organic and elemental selenium (15%).

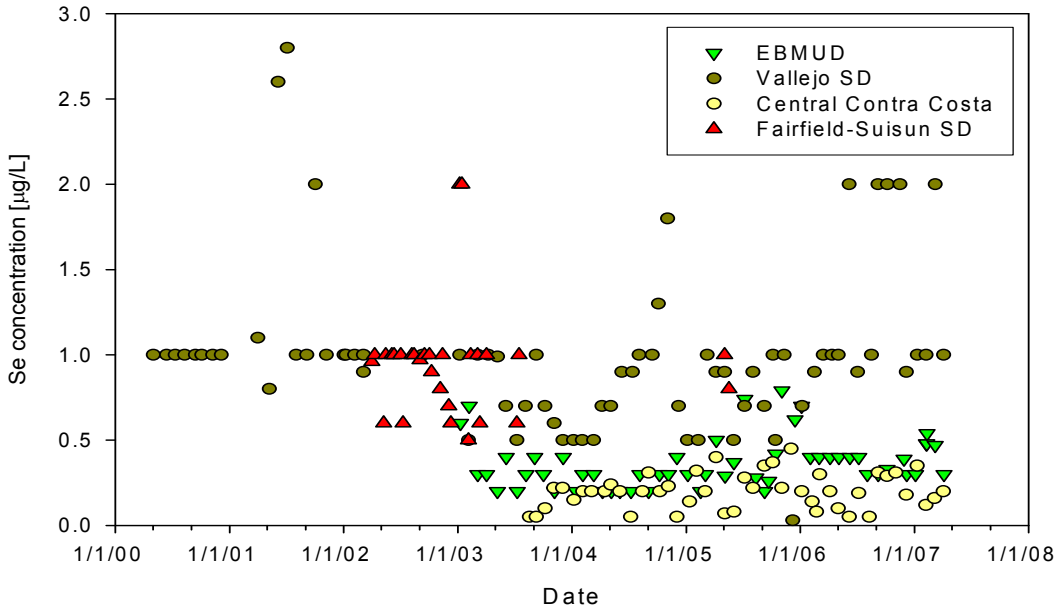


Figure 12: Selenium concentrations in effluent from selected largest POTWs

Two methods were used to estimate daily loads from POTWs. In the first method, the overall average daily maximum concentration for each facility was multiplied by overall average daily flow. In the second method, daily loads were first estimated based on daily flow and reported concentrations for all the available dates. Afterward, these estimates were used to compute an average daily load which was then extrapolated to an annual load. For concentrations reported below the detection limit, concentrations were assumed to be half of the detection limit. Sonoma Valley County Sanitation District reported selenium concentrations using a very high detection limit of 5 µg/L, therefore loads were not calculated for this facility.

Both computation methods resulted in similar load estimates (Table 11). POTWs on average discharge into the North Bay approximately 260 kg of selenium per year. The largest selenium load of 64.5 kg was calculated for Delta Diablo Sanitation District, where in early May 2004 for eight consecutive days, effluent selenium concentrations averaged above 28 µg/L. The duration and magnitude of high selenium concentrations suggested a problem within the wastewater facility or a spill incident. When these extreme concentrations are excluded from the assessment, the average selenium load from Delta Diablo SD is reduced to approximately 34 kg per year. Likewise, selenium load for Sonoma Valley Sanitation District could be extrapolated based on the performance of the City of Petaluma POTW that represents a comparable treatment technology, magnitude of discharge, and service area. The approximate load from this facility calculated with Method 1 and using the average selenium concentration of 0.65 µg/L is 3.7 kg per year. Taking into account the above adjustments (reduction of Delta Diablo SD and Sonoma Valley SD) the total average annual load generated by all POTWs is approximately 226 kg.

Table 11: Summary statistics of daily maximum effluent concentrations and estimated loads

Municipal dischargers	Time Period	No of samples	Effluent Concentrations µg/L				Average flow (mgd)	Estimated Loads kg/year [1]	
			Mean[2]	S.D.	Min	Max		Method 1	Method 2
City of American Canyon	2003-05	32	1.16	0.59	0.2	2	0.9	2.9	3.0
City of Benicia	1999-07	97	0.81	0.51	<0.3	5	3.0	3.5	3.4
City of Calistoga	2000-06	19	0.51	0.54	0.25	2.5	0.76	-	0.2
Central Contra Costa Sanitation District	1998-07	99	0.34	0.50	<0.05	4	45.8	21.8	15.0
Central Marin Sanitation Agency	1998-07	98	0.75	0.68	0.17	6.4	11.0	12.3	10.7
Delta Diablo Sanitation District [3]	1999-06	100	4.21	7.54	<1	37	11.5	64.5 (34)	64.1 (34)
East Bay Municipal Utility District	1998-07	294	0.34	0.19	<0.2	1.6	74.6	34.8	36.9
Fairfield-Suisun Sewer District	1998-03	95	0.75	0.38	0	2	17.0	19	18.5
Las Gallinas Valley SD Permit	2001-03	10	0.64	0.17	0.5	0.9	3.5	3.3	4.0
Marin Co. S.D. no 5	2000-07	47	1.93	1.4	0.5	6.0	1.0	2.7	1.9
Mount View Sanitary District	1999-06	37	0.62	0.60	<0.02	5	2.0	2.3	1.5
Napa Sanitation District (dry)	2002-04	13	0.57	0.21	<0.5	1	3.8	2	2.9
Napa Sanitation District (wet)	1999-04	26	0.27	0.25	0	<1	14	2.6	10.3
Novato Sanitation District (Ignacio dry)	1999-04	4	0.48	0.05	0.4	0.5	4.0	2.6	2.9
Novato Sanitation District (wet)		4	0.83	0.32	0.4	1	2.2	2.6	3.2
City of Petaluma	1999-07	60	0.65	0.23	0.35	1.4	7.6	6.9	8.3
City of Pinole and Hercules	2000-07	47	0.91	0.66	<0.1	4	3.2	4.0	4.2
Rodeo Sanitary District	2000-07	30	0.80	0.61	<0.1	3	0.8	0.9	0.9
Sausalito-Marin Sanitary District	1999-07	85	1.36	0.91	0.5	17.5	1.6	5.5	4.9
Sewerage Agency of South Marin	1999-04	133	1.39	2.01	0.15	12	3.3	6.4	5.1
Sonoma Valley County Sanitation District	1999-02	27	<5.00	0.00	<5	<5	4.1	3.7[4]	3.7[4]
US Navy Treasure Island	2000-04	46	0.29	0.17	<0.25	8.9	0.5	0.4	0.3
Vallejo Sanitation and Flood Control District	2000-07	79	0.84	0.52	<0.7	10.6	8.0	20.3	23.2
West County Agency /City of Richmond	2002-07	60	1.73	0.97	0.25	9	14.1	33.7	30.7
							Total	***258.7***	***260.2***

[1] Method 1: Loads computed based on overall average concentration and average daily flow; Method 2: Loads based on flow and concentrations for all available dates

[2] For values below detection limit, half of the detection limit was used in mean calculations

[3] Compliance monitoring data and the 13267 study data were used to estimate loads for this facility because of high variability in Se concentrations

[4] High detection limit of 5 µg/L, load in extrapolated based on average concentrations measured at City of Petaluma with Method 1

Industrial Wastewater Discharges

Loads from industrial facilities in the North Bay were calculated in a similar way to the second method used for POTWs. These loads are minor compared to other sources and average about 17 kg/yr (Table 12).

Table 12: Estimated selenium loads from industrial wastewater dischargers in the North Bay

Industrial Facilities	Daily load g/day	Annual load kg/yr
Dow Chemical	6.5	2.4
General Chemical	4.8	1.8
GWF (I)	1.1	0.4
GWF (V)	0.4	0.1
USS-Posco	31.0	11.3
Rhodia	2.8	1.0
Total	***46.6***	***17.0***

North Bay Petroleum Refineries

Petroleum refineries are the largest permitted source of selenium in the North Bay that tend to dominate selenium load during periods of low flow. The total refinery emissions estimated based on the 1998-2007 data exceed 530 kg/year. Mean selenium concentrations at the refineries vary from 11.9 µg/L (Tesoro) to 27.7 µg/L (Shell Martinez; Table 13) and show relatively large variations over time (Figure 13).

Table 13: Summary statistics of effluent concentrations at petroleum refineries

Refineries	Time Period	No of samples	Median	Mean	SD	Min	Max
Chevron	1999-05	308	11.2	12.1	5.9	2.3	48.0
ConocoPhillips (at Rodeo)	1999-07	448	14.0	15.5	8.5	1.0	49.0
Shell Martinez	1998-07	266	27.0	27.7	9.4	4.0	82.0
Tesoro	2000-07	367	11.0	11.9	5.1	1.0	41.0
Valero	1999-07	447	26.1	26.6	7.4	8.0	50.0

SD – standard deviation

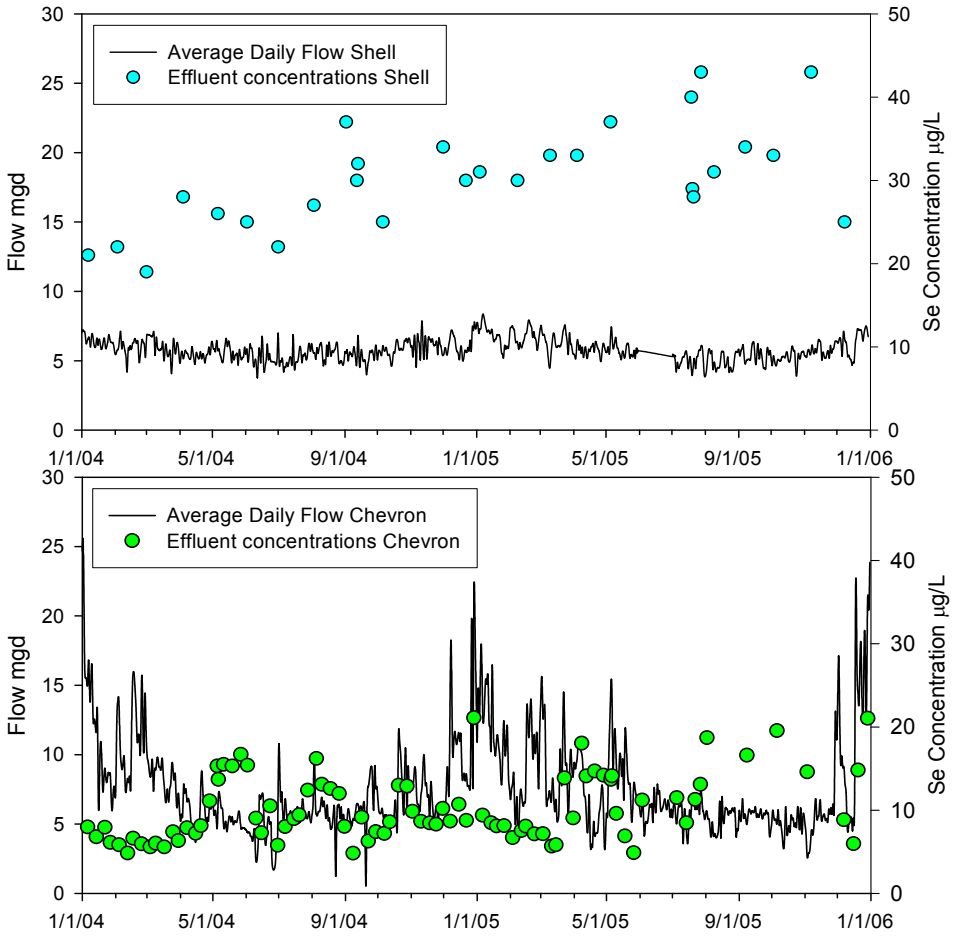


Figure 13: Effluent selenium concentrations and daily flow in Shell Martinez and Chevron refineries

Daily flow measurements at the refineries indicate some seasonal high flows, probably due to stormwater runoff. Similarly to municipal and other wastewater discharges, selenium concentrations in the effluents from refineries generally show no correlation with flow.

For the five petroleum refineries located in the North Bay, daily loads were estimated based on the continuous daily measurements of flow and the effluent daily maximum concentrations reported on a weekly basis. Mean daily maximum selenium concentrations for the refineries range between 12 and 28 µg/L. The estimated total daily load from these refineries is 1.47 kg/day or an average of 537 kg/yr during 1999-2007 (Table 14). Current loads are significantly lower than the previous years (1,407 – 3,382 kg/yr in 1986 – 1992) following the improvement in waste water treatment practices at the refineries (Presser and Luoma 2006).

Seasonal changes in loads from refineries were also evaluated by totaling the daily loads according to dry and wet season. The wet season was defined as October 1st to April 30th. The dry season was defined as May 1st to September 30th. Estimated annual selenium loads are relatively constant throughout the years (Figure 14). Average dry season loads are generally 62-78% of the average wet season loadings at four of the refineries. Average dry season loads at the Tesoro refinery are only 35% of the wet season loadings. Yearly loading does not appear to be affected by dry vs wet years.

The petroleum refinery effluents are dominated by selenate (56%) and organic selenide (30%), with selenite accounting for only 14% on average (compared to 64% of selenite in 1987-1988, Cutter and Cutter, 2004). Selenium speciation in refineries is similar to that found in municipal wastewater effluents.

Table 14: Estimated total selenium loads from petroleum refineries in the North Bay

Refinery	Flow mgd	Mean daily load[1]	Mean daily load[2]	Annual load[1]	Annual load[2]
		in kg/year			
Chevron	7.1	0.31	0.33	112.6	120.7
Conoco Philips	2.3	0.16	0.16	57.9	58.0
Shell Martinez	5.8	0.61	0.59	224.1	214.9
Tesoro	4.1	0.19	0.19	70.2	69.3
Valero	2.0	0.20	0.20	71.9	75.1
			Total	***537***	***538***

[1] Calculated as continuous daily flow multiplied by weekly concentrations and extrapolated to the rest of the week

[2] Calculated based on daily flow and concentrations on sampling dates only

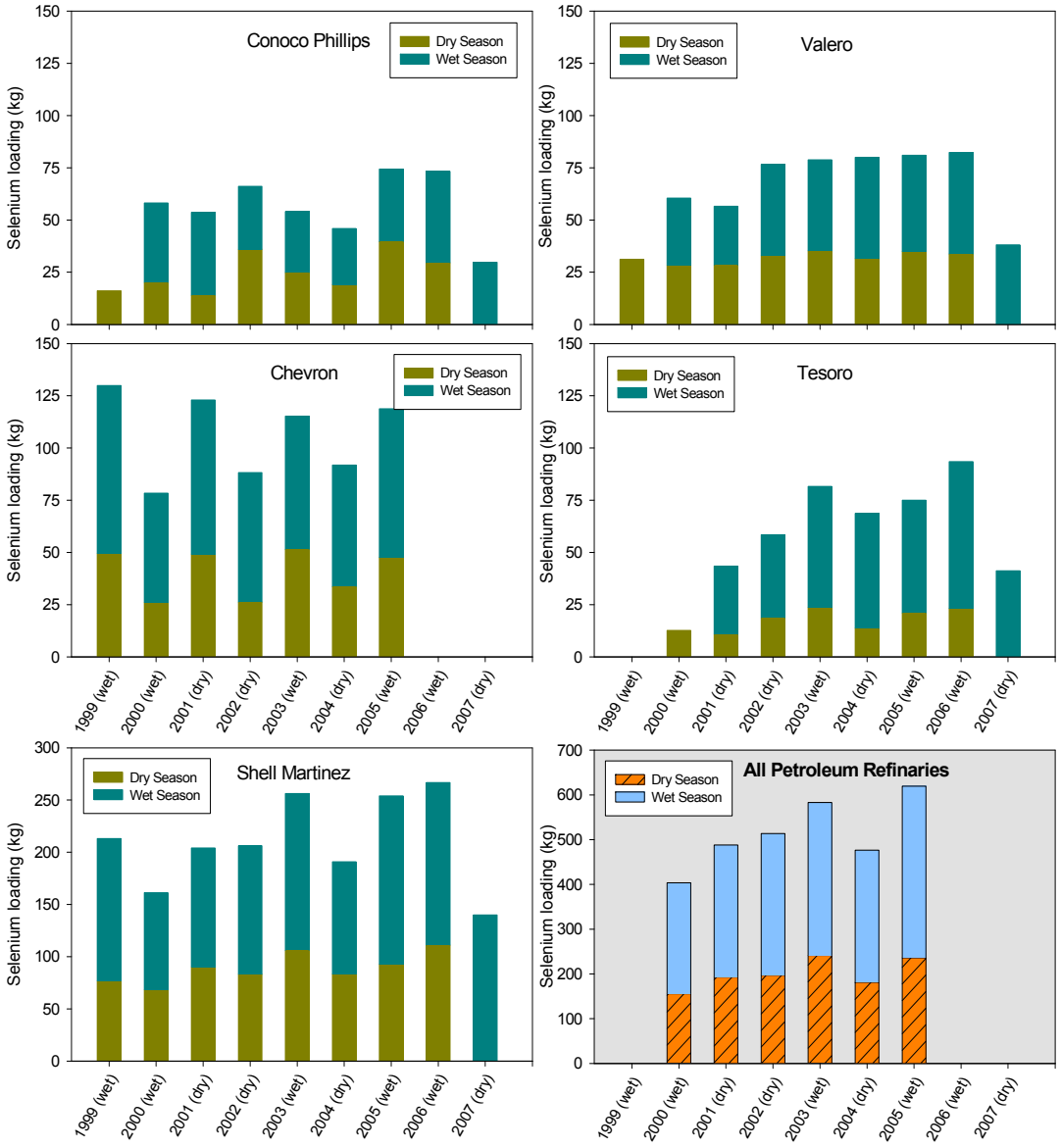


Figure 14: Dry and wet season selenium loads from refineries from 1999 through 2007

Urban and Non-Urban Stormwater Runoff from Local Tributaries

Local tributaries, that is, streams that discharge directly into the North Bay (Figure 15), can potentially contribute elevated selenium loads due to the presence of agricultural, urban and industrial land uses in their watersheds. Although these tributaries generate less than 4% of the total freshwater flow to the Bay, the relative proximity to the local sources of pollution, soil disturbances associated with urban development, and the dense stormwater conveyance

system could amplify the delivery rate. McKee *et al*. (2003) have found that sediment export from small local tributaries averages approximately 100 t km^{-2}, which is much higher than the export from Central Valley (~14 t km^{-2}).

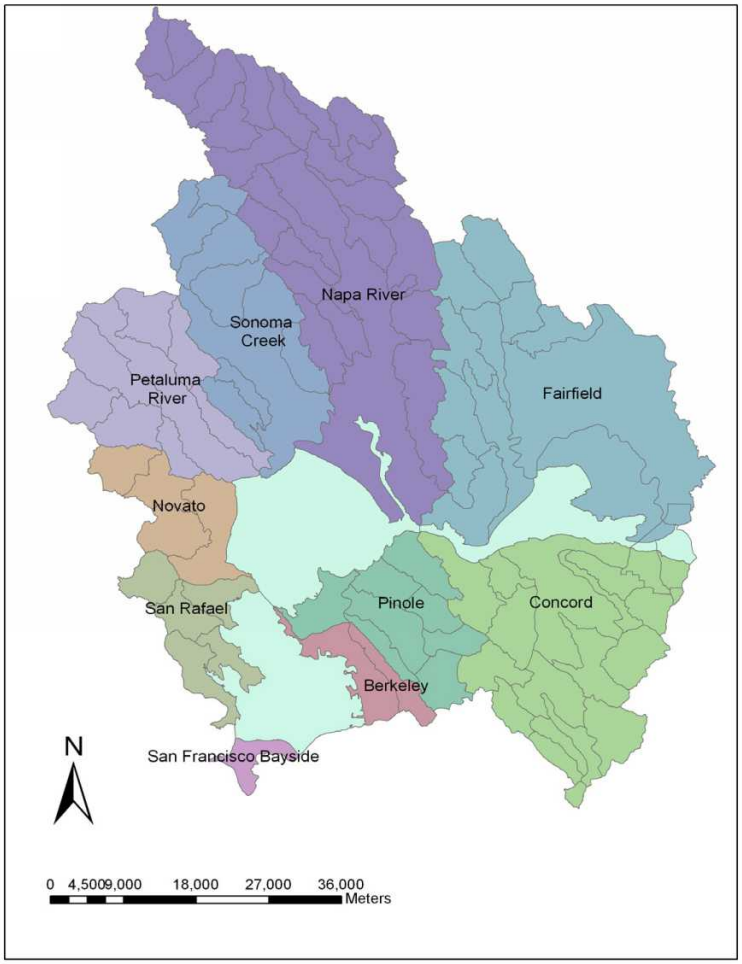


Figure 15: Hydrological areas surrounding the North Bay
(*Source: San Francisco Bay Institute*)

The available selenium concentration data for tributaries are limited and highly variable (Table 15). In 2001 – 2002 Surface Water Ambient Monitoring Program (SWAMP) monitored selenium in five tributaries in the North Bay and reported concentrations of 0.18–3.39 µg/L (median 0.94 µg/L) during the dry season, and 0.39–3.14 µg/L (median 0.90 µg/L) during the wet season (SFBRWQCB 2007a). Total selenium concentrations as high as 1.7 and 4 µg/L during wet and dry seasons of 2003-2004 were observed in Petaluma River (SFBRWQCB 2007b). Table 15 shows data available for the most downstream locations within the

tributaries draining into the North Bay. These sites are considered to be indicative of the conditions within the entire watershed and therefore most suitable for the purpose of load estimates.

Table 15: Selenium concentrations at the SWAMP downstream monitoring locations collected during wet, spring and dry seasons

Water Body	Site	Season	Year	Total Se [µg/L]
Kirker Creek	KIR020	Wet	2003-2004	1.26
		Spring		1.30
		Dry		2.50
Mt Diablo Creek	MTD010	Wet	2003-2004	2.00
		Spring		0.40
Petaluma River	PET010 San Antonio Ck	Wet	2003-2004	1.30
		Spring		0.20
	PET310	Wet	2003-2004	1.70
		Spring		1.30
		Dry		4.00
San Pablo Creek	206SPA020	Spring	2001-2002	2.74
		Dry		1.60
Suisun Creek	207SUI020	Spring	2001-2002	0.90
		Dry		0.32
Wildcat Creek	206WIL020	Spring	2001-2002	0.39
		Dry		1.33
Average		***Wet***		***1.57***
		Spring		***1.03***
		Dry		***1.95***
		All Data		***1.45***

Bay Area Stormwater Management Agencies Association (BASMAA) collected selenium concentration data during a 1988-1995 monitoring study. The sampling sites in that assessment were mostly located in the Alameda County (16) with two sites located in the Contra Costa County. The monitoring program focused on measuring concentrations of pollutants in stormwater and was designed to determine pollutant loads in stormwater runoff dominated by different land uses (BASMAA 1996). Automated monitoring equipment was placed within the stormwater conveyance system to record runoff and to collect flow-weighted composite water samples. These monitoring stations received runoff from areas that were not larger than 1.5 square mile. Samples were also collected from selected waterways, including San Lorenzo, Alameda, Walnut and Dry Creek, to evaluate the quality of receiving waters during storm events. The waterway drainage areas varied in size from approximately 10 square miles (Dry Ck) to over 600 square miles (Alameda Ck).

Selenium concentrations reported by BASMAA are generally lower than values reported in subsequent SWAMP studies. Median concentrations were 0.40 µg/L during dry weather (n = 7) and 0.33 µg/L for storm event sampling (n = 28). By land use, median selenium concentrations were 0.29 µg/L, 0.35 µg/L and 0.30 µg/L for residential, open and industrial locations, respectively. However, the range of concentrations (0.06 – 0.90 µg/L) detected during the later period of data collection, which coincided with introduction of analytical methods with lower detection limit (< 0.05 µg/L), indicates that higher concentrations exceeding 0.1 µg/L were common. A wide range of selenium concentrations was detected in the monitored creeks that ranged from below detection limit to 9.9 µg/L. Concentrations exceeding 5 µg/L were recorded in all waterways during wet weather events.

Real time flow measurements and selenium concentrations in runoff from local tributaries are limited, thus the load assessments based on the available data are associated with large uncertainty. Therefore, to provide a better insight into the variability and magnitude of loads delivered into the North Bay, we used three methods to evaluate selenium tributary loads. The methods, data requirements and assumptions are summarized here.

Load Estimates Using Simple Model with SWAMP Data (Method 1)

This mass loading assessment employs a concept of a simple model to predict runoff volumes and the SWAMP data collected at the local tributaries. The volume of runoff is predicted using empirical runoff coefficients for discrete land use categories, rainfall, and the area of each land use. Pollutant loads are then calculated as the product of mean pollutant concentrations and runoff depths over specified period of time. The validity of the runoff model was tested and compared against the local data by Davis *et al*. (2000).
The contaminant load is calculated as follows:

$$Load = \sum_{j=1}^{n} (v_j * i * A_j) * C_{ave}$$

where ***v*** is runoff coefficient for land use *j*; ***i*** is the average rainfall for hydrologic unit and ***A*** represents the area of land use *j* in the hydrologic unit. $\boldsymbol{C_{ave}}$ is the average measured runoff contaminant concentration for the hydrologic unit.

Runoff volumes calculated by Davis *et al*. (2000) and concentrations measured in the SWAMP study were used to estimate loads from each watershed surrounding the North Bay (Table 16, Figure 15). Selenium was sampled during wet, spring, and dry seasons at four out of ten hydrological areas surrounding the North Bay. For those areas where site-specific data were not available, the average concentration from all the available monitoring locations was

used to estimate loads. The average annual load of total selenium from local tributaries to the North Bay exceeds 900 kg/yr, with the Napa River and Concord watersheds identified as the largest sources. Higher total selenium loads from these watersheds are most likely due to larger watershed areas and high annual runoff.

Table 16: Annual runoff and selenium loads from local watersheds

Hydrologic Area	Total Annual Runoff (Mm^3/yr)[1]	SWAMP Stations	Mean Total Se Concentrations ($\mu g/L$)[2]	Total Se Load (kg/yr)
San Rafael	56		1.45	81.2
Berkeley	25		1.45	36.3
San Francisco-Bayside	8.8		1.45	12.8
Novato	47		1.45	68.2
Petaluma River	60	Petaluma River	1.7	102.0
Sonoma Creek	68		1.45	98.6
Napa River	180		1.45	261.0
Pinole	35	Wildcat, San Pablo	1.5	52.5
Fairfield	129	Suisun Creek	0.6	77.4
Concord[3]	106	Mt. Diablo Creek	1.2	127.2
Concord[4]	6.7	Kirker Creek	1.7	11.4
Total	***721.5***			***929***

[1] From Davis *et al*. (2000)

[2] Data collected by SWAMP (SFBWQCB 2007a, b); 1.45 µg/L is the mean concentration for all sites

[3] Concord hydrologic area: subunits 220731, 220732, 220733

[4] Concord hydrologic area: subunit 220734

These large watershed loads expressed on a per unit area basis do not differ significantly form other drainage areas. It is the most developed and highly urbanized watersheds of San Rafael, Berkeley and San Francisco Bayside that contribute on average well above 4 grams selenium per hectare (1.2 kg mi^{-2}), while Petaluma, Napa and Concord generating less than 3 grams per hectare (0.7 kg mi^{-2}).

Runoff in the Bay area is governed by the inter-annual variability in rainfall, which subsequently affects the magnitude of pollutant loads. The estimates of the 10th and 90th percentiles of rainfall could be indicative of load range for dry and wet years respectively. Davis *et al*. (2000) evaluated rainfall variability in the Bay area for the record period of 1961-1990. Taking into account these rainfall values and assuming average selenium runoff

concentration of 1.45 µg/L (Table 15); the selenium load from local tributaries could vary from 686 kg in a dry year to 1750 kg in a wet year.

Load Estimates Using Available Measured Flow and SWAMP Data (Method 2)

The long-term average monthly flow measured by USGS and the seasonal selenium concentrations from the SWAMP study were used to estimate long-term average selenium loads at available gauging stations. Loads were calculated by multiplying flow and concentrations data for the same river. For tributaries without observed selenium concentrations, the overall average wet and/or dry concentration for all the North Bay sites was used (Table 15).

Long-term average monthly flow records at the USGS stations indicate that the majority of the flow is discharged during the wet season defined as October 1st through April 30th. Flow during the dry season (May 1st to September 30th) amounts to only a small fraction of the wet season flow (0.2 – 3.5%) with the exception of Walnut Creek and Pinole Creek for which the dry season flows could reach 13.1% and 5.8% of the wet flows, respectively. Similarly, the majority of the load is delivered to the Bay during wet season. Figure 16 shows a typical monthly pattern of selenium loads from representative tributaries in the North Bay. The highest annual load was estimated for the gauging station at Napa River near Napa (288.9 kg/yr) followed by Sonoma Creek at Aqua Caliente (97.1 kg/yr). Dry season loads are very small and average between 0.2 and 3.0% of the wet season loads for 6 of the 8 gauging locations (Table 17). A scaling factor based on the annual areal loading was used to extrapolate loads from the gauging location to the entire watershed area for each of the tributary. An areal loading from a nearby watershed was applied for the hydrological areas without data.

Estimated total selenium loads for the North Bay by hydrological area are summarized in Table 18. The total selenium loads calculated using the available USGS flow data and the SWAMP concentration data exceed 1510 kg/yr and are higher than the estimates based on modeled runoff described as Method 1. Once again, a large portion of the total tributary load was estimated to originate from Napa and Sonoma hydrological areas. Due to the lack of selenium concentrations for these two areas in the SWAMP dataset, an overall mean concentration of the whole North Bay tributaries was used to compute loads. Thus, these estimates are highly uncertain. Flow records for the Napa and Sonoma rivers also suggested higher runoff from these two areas compared to the rest of the North Bay (337 and 422

mm/yr for Napa and Sonoma, compared to ~200 mm/yr for the other tributaries). This will also contribute to the higher selenium loads than observed in other locations.

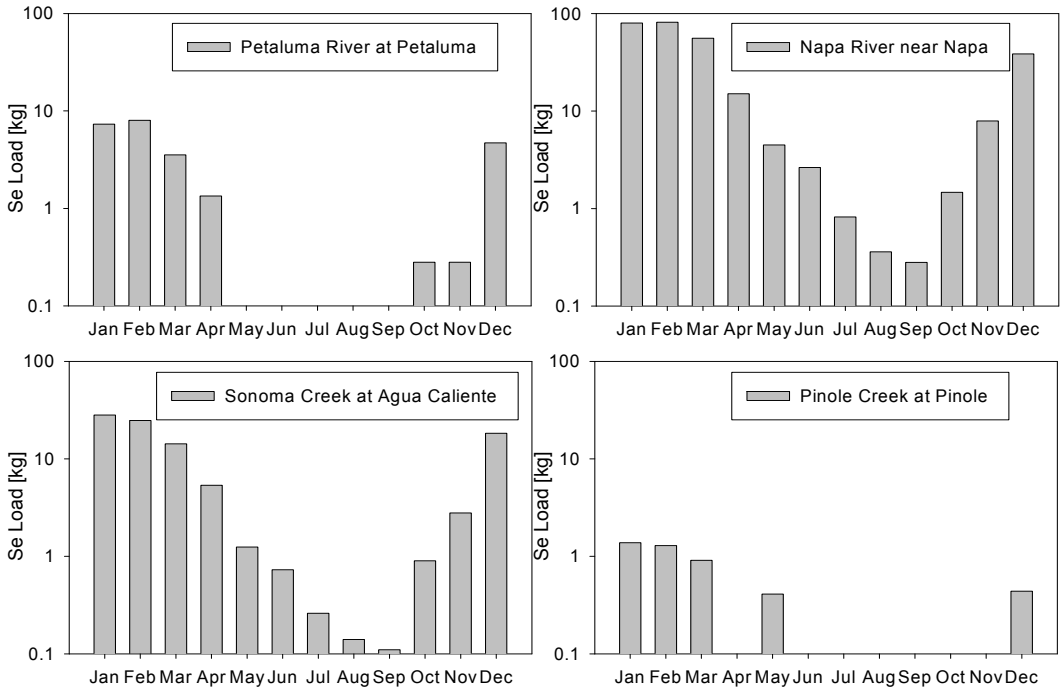


Figure 16: Average long-term monthly selenium loads at selected gauging locations

Table 17: Summary of selenium loads at the USGS gauging stations

	USGS Gauging Stations							
	11459500 Novato Ck at Novato	11459300 San Antonia Ck nr. Petaluma	11459000 Petaluma R. at Petaluma	11458500 Sonoma Ck at Agua Caliente	11458000 Napa R. nr. Napa	11181400 Wildcat Ck at Richmond	11183600 Walnut Ck at Concord	11182100 Pinole Ck at Pinole
Drainage area (mi^2)	17.6	28.9	30.9	58.4	218	8.7	85.2	10
Dry season load (kg)	0.5	< 0.1	<0.1	2.5	8.6	0.1	7.0	0.3
Wet season load (kg)	16.9	18.9	25.4	94.6	280.4	6.7	56.3	4.6
Dry as wet %	2.6	0.2	0.2	2.6	3.1	1.7	12.5	5.7
Total Load (kg/year)	17.4	19.0	25.5	97.1	289	6.8	63.3	4.9
Areal load (kg/mi^2)	*0.99*	*0.66*	*0.83*	*1.66*	*1.33*	*0.78*	*0.74*	*0.49*

Table 18: Estimated wet and dry season loads from local tributaries (*Method 2*)

Hydrological Areas	Area (mi^2)	Dry (kg)	Wet (kg)	Total Load (kg/yr)
San Rafael	60.9	1.6	58.8	60.3
Berkeley	33.8	0.4	26.0	26.4
San Francisco Bayside	11.1	0.3	10.7	11.0
Novato	71.03	1.8	68.6	70.4
Petaluma	145.8	0.3	120.2	120.5
Sonoma	165.9	7.1	268.6	275.9
Napa	362.1	14.3	465.7	480.0
Pinole	58.9	1.5	26.9	28.4
Fairfield	339.0	27.9	223.9	251.8
Concord	250.3	20.6	165.3	185.9
Total		***76***	***1435***	***1511***

Land Use-Specific Loads with Modeled Runoff and Concentration Data from BASMAA and SWAMP Studies (Method 3)

This assessment focused on evaluation of selenium loads generated by individual land uses in each hydrologic area. The method employs the simple model to estimate stormwater runoff associated with each land use within the drainage area and land use distribution (see Method 1, Davis *et al.* 2000). The model links contaminant emissions to rainfall and land use allowing for evaluation of potential differences in generated loads between years of different rainfall and types of land uses. It is assumed that mass loads are generated predominantly from diffuse sources and are representative of a long-term average runoff. As such, loads generated during dry weather conditions and resulting from, for example, bank erosion or groundwater inflows are not well represented in the assessment. Moreover, degradation or adsorption of pollutants while they are being transported downstream is not explicitly accounted for. However, this approach is widely accepted and tested against measured data with good results.

Loads are estimated for five broad land use categories (open space, agricultural, residential, industrial and commercial) based on estimated runoff from each land use type and land-use specific mean selenium concentrations. For the purpose of this assessment, urban land use includes industrial, commercial and residential areas. The “best estimates” of runoff coefficients and the mean selenium concentrations indicative of a particular land use are shown in Table 19. Land use specific concentrations were derived from BASMAA (1996) and

SWAMP studies (SFBRWQCB 2007a, b). Concentrations for agricultural land uses were assumed to be the same as open space. Due to the differences in concentrations reported by the two monitoring programs, values from the BASMAA project were used as the lower bound of concentrations from local tributaries, while SWAMP data were used as the upper bound.

Table 19: Land use specific runoff coefficients and mean selenium concentrations *(Tetra Tech 2008a)*

	Land Use					Source
	Residential	Commercial	Industrial	Agricultural	Open Space	
Runoff coefficient (best estimate)	0.35	0.9	0.9	0.1	0.25	Davis *et al*. (2000)
Selenium concent. (low) µg/L	0.36	0.58	0.58	0.50	0.50	BASMAA (1996)
Selenium concent. (high) µg/L	1.55	1.55	1.55	0.85	0.85	SWAMP

The estimated loads range from 354 to 838 kg/yr depending on the mean concentration data used (Table 20). Open space and residential areas are among the major single contributors of selenium (301 and 250 kg/yr, respectively) mainly because they occupy a large proportion of every watershed. Many of the watersheds surrounding the North Bay experience very high level of urbanization. Urban areas that for the purpose of this assessment combine residential, industrial and commercial uses account for more than 50% in Pinole, San Rafael, Concord, Berkeley and San Francisco Bayside drainage areas. The estimated stormwater runoff from all urban areas is 316.8 Mm^3/yr that is approximately 44% of the total runoff. The loads from urban areas estimated based on the SWAMP concentration data exceed 490 kg/yr, or 59% of loads from all land use types. When BASMAA concentrations data are used the loads are reduced to 148 kg/yr, or about 43% of the total load from all land use areas. The land use specific loads for each hydrologic area are shown in Table 20.

Despite observed variability, Methods 1 and 3 provide similar results that are generally lower than that of Method 2 with the exception of the smallest and most urbanized drainage areas, such as Pinole or San Rafael (Figure 17). All three methods show similar load estimates for the highly urbanized drainage areas. This is not surprising as both methods (1 and 2) rely on the same approach to determine runoff volumes. Method 3 attempts to increase the estimate resolution by making the best use of the available concentration and land use data. All

calculation methods show that one of the largest loads is generated by the Napa watershed for which the concentration data are not available. This may suggest that the load estimate is subject to greater uncertainties. Concurrently it could be seen that the highest selenium loads per unit area correlate positively with the level of development and the selenium generation rate for Napa watershed closely resembles other tributaries with similar land use composition (Figure 17).

Table 20: Selenium loads derived based on land use composition in local tributaries

Hydrological area	Land Use Load (kg/yr)					Total Load (kg/yr)
	Residential	Commercial	Industrial	Agricultural	Open Space	
San Rafael	42.4	17.4	2.2	0.0	13.6	*76*
Berkeley	14.4	10.4	11.7	0.0	0.9	*37*
San Francisco Bayside	4.8	8.3	0.4	0.0	0.0	*14*
Novato	19.2	15.1	2.2	1.7	18.4	*57*
Petaluma River	19.7	3.6	7.2	7.7	26.4	*65*
Sonoma Creek	13.7	4.4	4.4	9.7	36.3	*69*
Napa River	40.1	30.9	10.3	15.1	97.3	*194*
Pinole	15.9	6.2	14.9	0.0	9.3	*46*
Fairfield	18.8	20.3	16.1	11.5	67.0	*134*
Concord	60.7	30.5	24.6	1.1	31.6	*149*
UB[1] Load (kg/yr)	***250***	***147***	***94***	***47***	***301***	***838***
LB[2] Load (kg/yr)	***58***	***55***	***35***	***28***	***178***	***354***

UB[1] Load estimated using the upper bound mean selenium concentrations from the SWAMP data
LB[2] Load estimated using the lower bound mean selenium concentrations from the BASMAA data

The methods used to determine selenium loads from local tributaries into the North Bay take into account underlying data limitations, year-to-year and seasonal variability, and uncertainties in flow calculations. All these uncertainties are reflected in the estimated selenium load that according to the best available information could range from 354 to 838 kg/yr. We estimate that approximately half of this load originates from urban runoff.

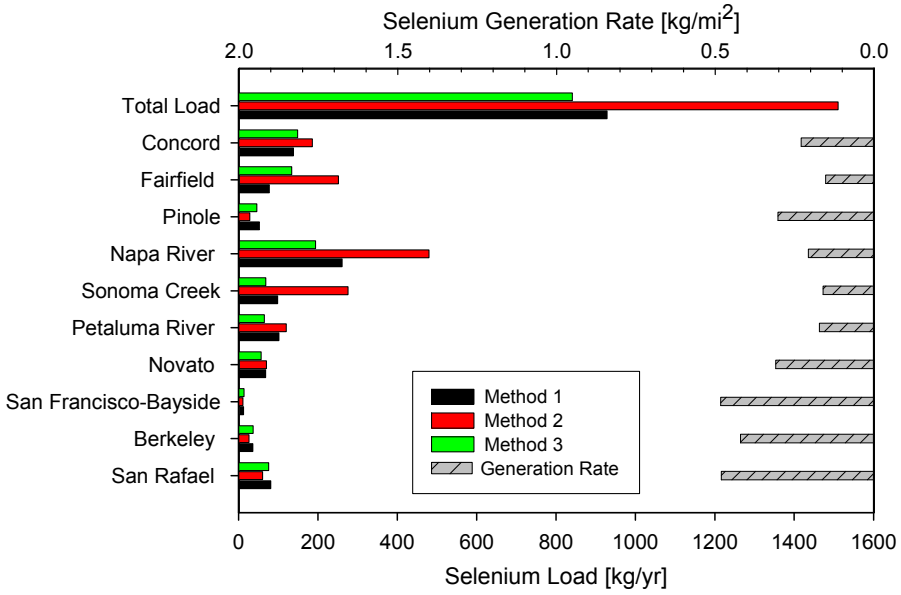


Figure 17: Comparison of load estimates from local tributaries using different calculation methods

(Se generation rates for each drainage area calculated using Method 3)

Direct Atmospheric Deposition

Atmospheric deposition of selenium occurs in dry and wet forms. Selenium is emitted to the atmosphere naturally as volatile dimethyl selenide, or as selenium dioxide and elemental selenium from fossil fuel combustion (Cutter and Church 1986). Deposition of selenium is part of a global cycle as gaseous selenium bound to particulate materials can be transported over long distances (USEPA 2002). Selenium in wet deposition consists of selenate, selenite, and elemental selenium. Rainwater samples from coastal California indicated that selenite is the major species in wet deposition for the region (Cutter 1978).

Dry and wet deposition of selenium has not been measured in the San Francisco Bay and estimates were made using data from other studies. However, similarly to other studies (USEPA 2002), it is likely that atmospheric deposition represents only a small load. Reported concentrations of selenium in precipitation are <0.1 - 0.4 µg/L in urban areas (Mosher and Duce 1989). Concentrations in precipitation measured in the Chesapeake Bay atmospheric deposition study are in the range of 0.07- 0.17 µg/L (USEPA 1996).

Given selenium concentrations of 0.07-0.4 µg/L ,an approximate annual rainfall of 450 mm/yr, and a water surface area of 648 km^2 in the North Bay (including Central Bay), direct wet

deposition of selenium is in the range of 20.4 – 116.6 kg/yr. Wet deposition of selenium could be relatively bioavailable as selenite is the dominant form.

Dry deposition was calculated from air-phase concentrations of selenium. Reported concentrations in the air exhibit a large variation from 0.3 to 2.4 ng/m^3. Concentrations measured in the Chesapeake Bay range from 1.4 – 1.8 ng/m^3. Different deposition velocities were used to estimate dry deposition fluxes for the Great Lakes (0.1 cm/s, Sweet *et al*. 1998) and the Chesapeake Bay (0.26 cm/s low, 0.72 cm/s high; USEPA 1996). Selenium in the air is mostly associated with fine particles; therefore a lower deposition velocity is expected. Based on a concentration range of 0.3 – 2.4 ng/m^3 and deposition velocities of 0.1 cm/s and 0.26 cm/s, estimated dry deposition is in the range of 6.1 – 127.5 kg/yr. Considering the fact that the largest single source of airborne selenium is combustion of coal the atmospheric deposition of selenium in the Bay area is likely to be at the lower end of the estimated range.

Loads from San Joaquin and Sacramento Rivers Delivered via Delta

Selenium loads discharged from San Joaquin and Sacramento rivers remain highly variable despite water storage and extensive flow management taking place in the Delta watershed. Changing patterns of precipitation and runoff together with water diversions and complex interactions occurring at the Delta – Bay interface add to difficulties in estimating the loads. The relative flows from the rivers and other main components of the Delta water budget for an average flow year 2000 are depicted in Figure 18.

Despite San Joaquin River inflows to the Delta being an order of magnitude smaller than those of Sacramento River, San Joaquin River loads are consistently higher. This is because San Joaquin conveys selenium enriched agricultural drainage from Central Valley resulting in elevated selenium concentrations (0.68±0.02µg/L dissolved Se). Still, because of diversion and reverse flows in the Lower San Joaquin River, much of the agricultural drainage does not reach the lower estuary. Sacramento River selenium concentrations are much lower (0.07±0.02µg/L dissolved Se) and more typical of background concentrations in the region.

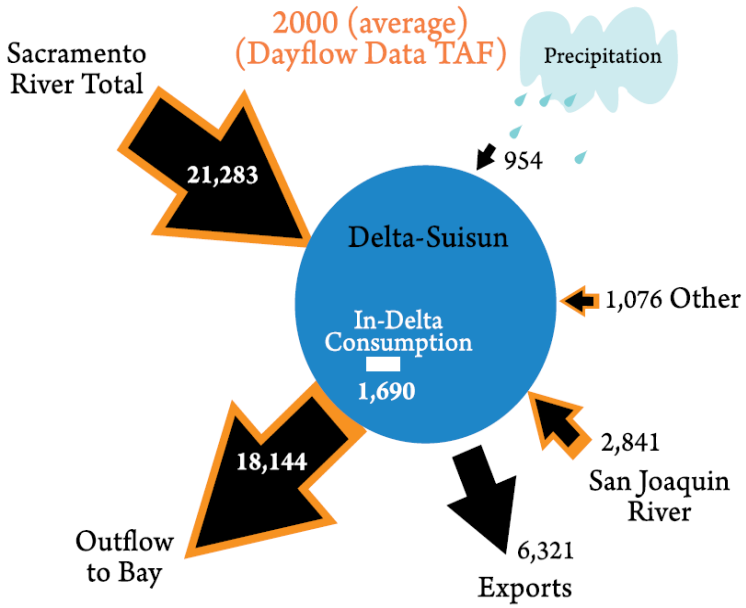


Figure 18: Water balance in the Delta for an average flow year 2000
Flow in thousand acre-feet (*From URS 2007*)

Three methods were used to estimate the relative contribution of the Sacramento and San Joaquin rivers to the Delta and to examine seasonal and annual load patterns from the Delta to the North Bay. The first method calculates selenium load discharged through the Delta using average dry and wet season concentrations measured at the two RMP stations (BG20 and BG30) above Mallard Island and the tidally corrected net Delta outflow generated by the Dayflow program. This approach was used in the past to estimate various pollutant loads from Central Valley to the Bay (for example see Davies *et al.* 2000).

The second method uses dissolved selenium concentrations measured by Cutter and Cutter (2004) in the Sacramento River at Freeport and data collected in the San Joaquin River at Vernalis to estimate individual loads contributed by both rivers. Then a “Delta removal constant” of 60% similar to the one described in Meseck (2002) is applied to the San Joaquin River load to account for complex interactions and likely selenium losses in the Delta. In the third method selenium loads from the Central Valley through the Delta are determined by estimating loads from the two rivers as described above and subtracting the load lost to the diversion of much of San Joaquin flow thru the aqueducts. This last approach is particularly effective for examining relative selenium load contributions of the two rivers to the North Bay.

The explanation of the load calculation methods and the concentration data are described in detail in Tetra Tech (2008a).

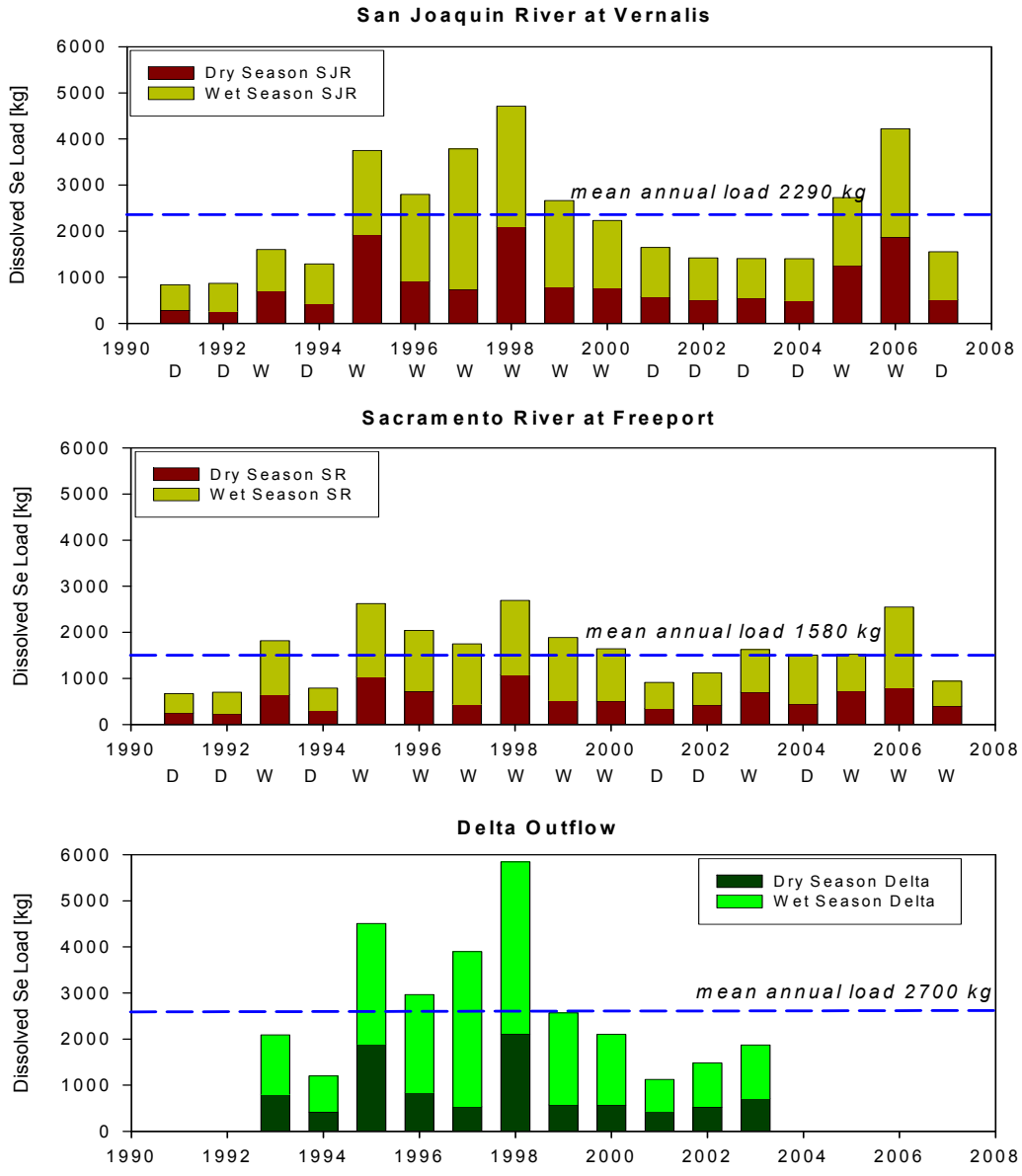
Table 21 shows a summary of load estimates using different calculation methods and data sets and Figure 19 illustrates relative variability in the load delivered to the North Bay by season and year. Based on the dissolved selenium concentrations only, the estimated riverine loads range between 670 – 2690 kg/y for the Sacramento River at Freeport, and 840 – 4710 kg/y for the San Joaquin River in Vernalis. Dry season loads for both rivers on average do not exceed 40% of the annual load (Figure 19). The annual loads will also vary with water years. For example the San Joaquin River annual load may be higher than 4000 kg/y during wet years (e.g. 1998, 2006) and less than 900 kg/y in dry years (e.g. 1991, 1992). However, selenium loads that reach the North Bay through the Delta are likely to be more affected by flow diversions and water management than the overall hydrologic conditions.

Table 21: Dry and wet season loads to the North Bay from the Central Valley watershed

Source	Average Selenium Load [kg]			Assumptions and data used
	Dry	Wet	Annual	
Delta outflow	1007	2931	3938	Total Se load; RMP data, 1994-2006 (Method 1)
Delta outflow	910	1583	2493	Dissolved Se load, 60% removal constant for SJR (Method 2)
Sacramento River at Freeport San Joaquin River at Vernalis	564 863	1013 1426	1577 2289	Dissolved Se load, 1993-2003 concentration data (Cutter and Cutter, 2004)
Export through aqueducts Delta outflow	665 856	842 1840	1506 2596	Dissolved Se load, 1993-2003 concentration data (Cutter and Cutter, 2004) Method 3
Tributaries	76	1435	1511	Measured flow and SWAMP data

Estimates of dissolved selenium load originating from the Central Valley watershed using either the “Delta removal constant” or taking into account selenium export through the aqueducts are very similar and range between 2500 and 2600 kg/y. To account for particulate selenium load we employed the annual suspended sediment data at Mallard Island for water years 1995-2003 (McKee *et al.* 2006) and limited particulate concentration data from both rivers (Doblin *et al.* 2006). For the range of reported suspended sediment loads from 0.26 Mt/y (2001) to 2.6 Mt/y (1995) and the average particulate concentration (n=10) of 0.64 µg/g the estimated particulate load varies from approximately 170 to 1660 kg/y

and the average annual load is 768 kg/y. The total average selenium load calculated as a sum of particulate and dissolved loads (estimated with Method 1 or 2) corresponds well to the first assessment method of total selenium load based on the RMP data and tidally corrected flow, which estimated the average annual load from the Central Valley watershed as 3938 kg/y (Table 21).



D - overall year classified as Dry; W – overall year classified as Wet

Figure 19: Estimates of dry and wet season riverine loads to the Delta and the Delta Outflow to North Bay

Considering the complexity of the Bay-Delta system, all three methods result in selenium loads that are fairly consistent. Method 1 with the different set of concentration data and flow independently ascertains that average dissolved and particulate loads are accurate and in general do not exceed 4000 kg/y. However, a large interannual variability could be expected depending on hydrologic conditions, magnitude of flow and water exports through the aqueducts.

5.2 Internal Sources

Erosion and Transformations of Selenium in Bottom Sediments

Conditions such as pH, oxidation-reduction potential, and the presence of metal oxides are among the key factors affecting the partitioning of selenium in the aquatic environment and controlling selenium transformations at the water column/sediment interface (USDHHS 2003b). In the North Bay bottom sediments, average selenium concentrations in samples from the depth of 5 to 15 cm range between 0.22 – 0.41 µg/g (G. Cutter, ODU, *pers. comm*.) and the mean sediment concentration based on RMP data is 0.25 µg/g. These levels of selenium are at the lower limit of the concentrations measured in 66 marine sediments from the northwest Pacific Ocean that ranged from 0.1 to 1.7 µg/g with a mean of 0.63 µg/g (Ihnat 1989). Recent RMP coring data show that unlike some other contaminants in the Bay sediments (e.g. Hg, Cu, PCBs) selenium concentrations stay relatively constant with depth and have remained unchanged for decades (Yee *et al.* 2010). Selenium in the bottom sediments is dominated by elemental selenium, which is considered insoluble, less mobile than other forms of selenium, and much less bioavailable. In a study by Doblin and others (2006) it was observed that Bay-Delta sediments averaged as high as 53-57% of elemental selenium. Selenium in bottom sediments can be mobilized to the water column through resuspension, erosion, diffusion and bioturbation. It can be also eroded and discharged through the Golden Gate to the ocean. Hence, the presence of elemental selenium in water column may indicate its origin from bottom sediments.

In previous Bay-wide TMDLs a top 15-cm layer of sediment was assumed to form an active layer that is in contact with biota or that can be resuspended into the water column. Sediment volumes are converted to sediment dry mass assuming that the Bay sediments are 50 percent solid by weight (range from 40 to 80%), and using densities of water and sediment of 1.03 kg/L and 2.65 kg/L respectively. The surface area of the North Bay extends for approximately 648 km^2. Using the mean sediment selenium concentration of 0.25 µg/kg, we

estimate that a selenium mass in the active sediment layer is just over 18,000 kg with more than half of this mass being elemental selenium.

Localized sediment erosion also occurs due to decreases in sediment supply from the surrounding watersheds. Net sediment erosion was found to occur both in the Suisun Bay (~1.27 Mm^3/yr) and San Pablo Bay (~0.22 Mm^3/yr) (USGS 2001a, b). This rate of bed erosion will result in selenium load of approximately 277 kg/yr that can be potentially released to water column or exported into the ocean.

6 LINKAGE ANALYSIS – RELATIONSHIP BETWEEN SOURCES, TARGETS AND BENEFICIAL USES

Selenium impairment in the North Bay is related to elevated concentrations found in fish tissue. In order to evaluate assimilative capacity of the Bay and determine the most effective load reductions, it is critical to understand the important factors and sources causing selenium bioaccumulation in fish.

Selenium bioaccumulation is site-specific and driven by feeding habits of fish and differences in choice of prey. Particulate selenium and dietary uptake is the most important exposure pathway for aquatic organisms, especially predators, and that some types of food webs bioaccumulate selenium more efficiently than others. A conceptual representation emphasizing key factors affecting selenium transfer in two common food web types, benthic bivalve-based and pelagic crustacean-based in San Francisco Bay is shown in Figure 20.

In the North Bay adverse impacts of selenium bioaccumulation have been detected only in the benthic food web, and are particularly evident where the invasive clam *Corbula amurensis* dominates. A significantly slower rate loss exhibited by *C. amurensis* as compared to native clams and crustaceans, results in high tissue concentrations ranging from 4.3 to 14 µg Se/g dw (data collected in November 2008). This in turn poses a risk to the predators feeding on these clams, mainly white sturgeon and diving ducks.

6.1 Importance of Particulate Selenium in Managing Ecological Exposure

Although dissolved selenium dominates in the water column, the relatively small fraction (2-18.5%) that is particulate is far more available to bivalves and zooplankton, and is therefore of special significance to bioaccumulation observed in the North Bay. The direct intake of selenium by bivalves and higher level predators from the dissolved phase is extremely limited and, in fact, the pathway for nearly all selenium transfer to higher trophic levels is dietary exposure through particulate material (Luoma and Rainbow 2008). Estimates of invertebrate bioaccumulation with biodynamic modeling show that uptake of dissolved selenium is responsible for less than 2% of selenium found in tissue of bivalves (Presser *et al.* 2008). Only phytoplankton and bacteria are able to take up and concentrate aqueous selenium and this uptake varies widely across species.

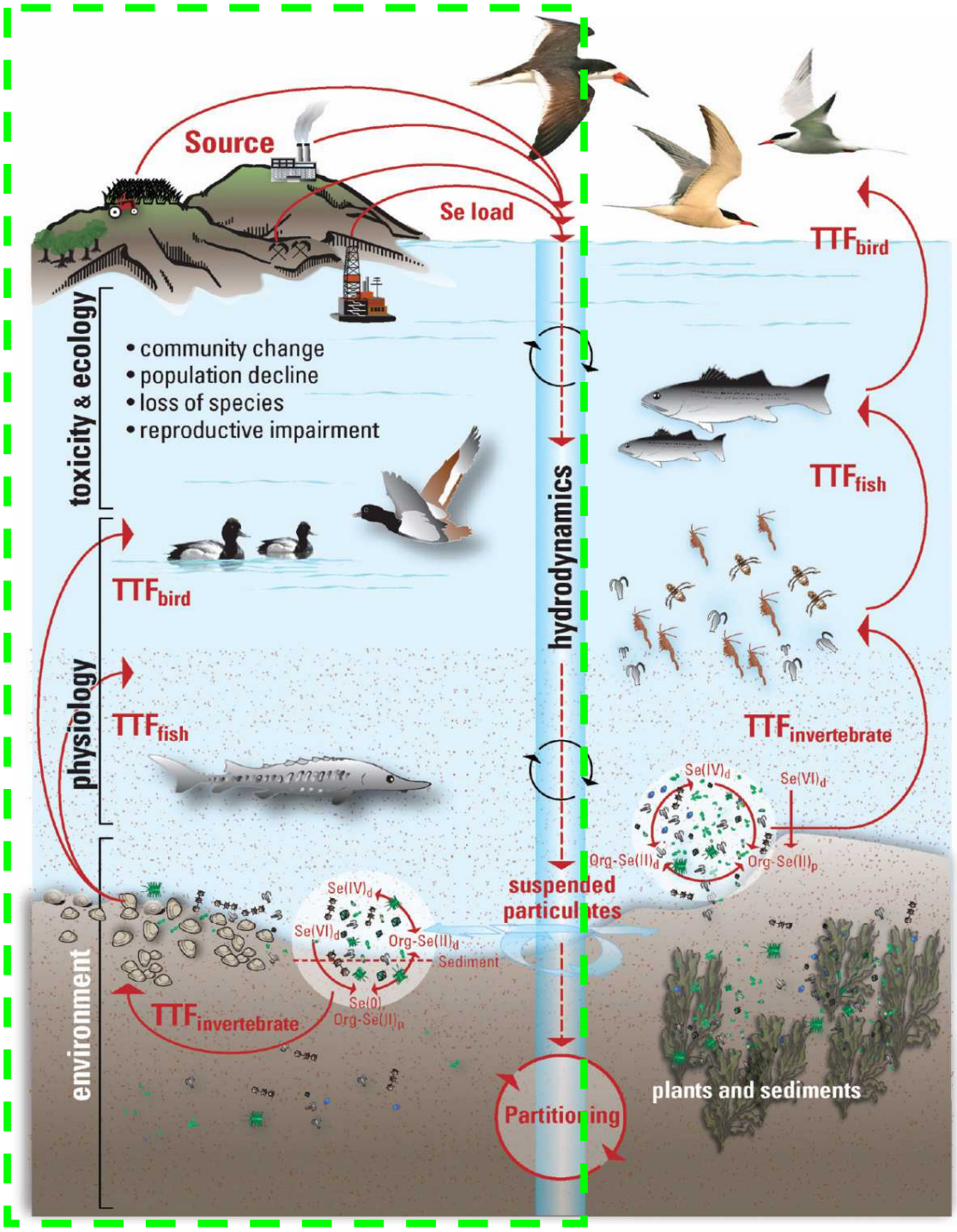


Figure 20: Conceptual model showing selenium biotransformations and implications for a benthic bivalve-based food web (*left panel*) and a water column food web (*right panel*)

(p - particulate, d-dissolved; from Luoma and Presser 2009)

Baines and Fisher (2001) demonstrated in laboratory experiments that marine algae cellular concentrations may exceed more than 100-fold ambient dissolved concentrations. These organisms will preferentially take up dissolved selenite and organo–selenide and rapidly convert it to organic selenides within their cells, thus becoming a rich source of particulate selenium to bivalves and other organisms that consume live and senescing algae. Uptake of selenate by algae is inhibited by sulfate content in water column (N. Fisher, Stony Brook University. *pers. comm*), hence, since the sulfate concentration in sea water is several orders of magnitude higher than that of selenate, under conditions in the North Bay uptake will be limited. Scientists now agree that the highest bioaccumulation takes place at the base of the food web (primary producers – algae, bacteria, fungi and plants) while the subsequent transfers to higher trophic levels, although biologically significant, tend to be much smaller (Chapman *et al.* 2009, Figure 21).

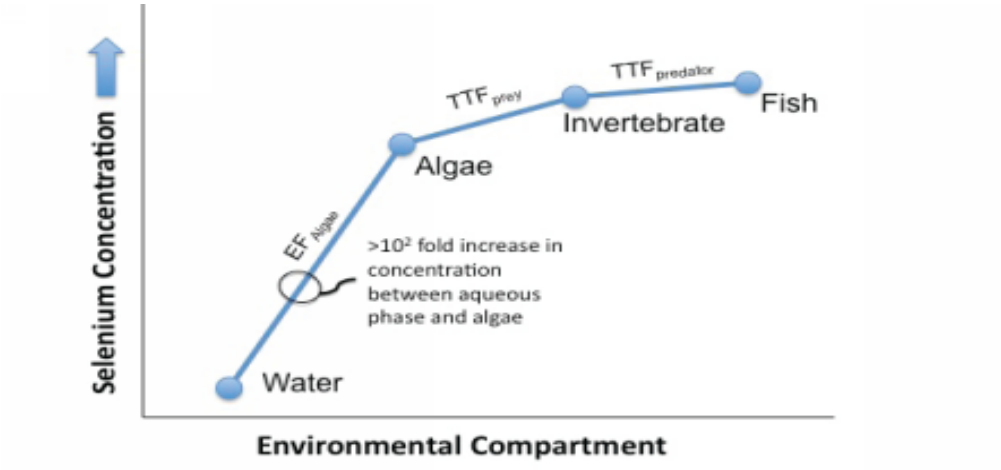


Figure 21: Selenium enrichment and trophic transfer in aquatic food web
(*Chapman et al. 2009 - SETAC Pellston Workshop*)

Particulate selenium in the estuary originates mainly from riverine input, with a smaller proportion of selenium coming from sediment resuspension, and in-situ transformations. Riverine inputs of particulate selenium can be a significant source of selenium to the North Bay as large amounts of sediments and living and non-living particulate organic material enter the Delta from Sacramento and San Joaquin watersheds. Particulate river load was estimated to range from 170 to 1660 kg per year (see Chapter 5 for discussion of selenium sources and loads). In riverine inputs, particulate selenium is mainly present as particulate elemental selenium, adsorbed selenite and selenate and particulate organic selenide.

6.2 Modeling Framework

We explored the available mathematical and empirical models to help identify conditions that could potentially exacerbate selenium associated risks and explain processes that affect relationships between environmental and anthropogenic loads of selenium in the North Bay and bioaccumulation in biota. Figure 22 shows a modeling framework comprising a numerical estuary model and a bioaccumulation DYMBAM model selected to simulate transformations and biological uptake processes in the North Bay (Tetra Tech 2008c, 2008d).

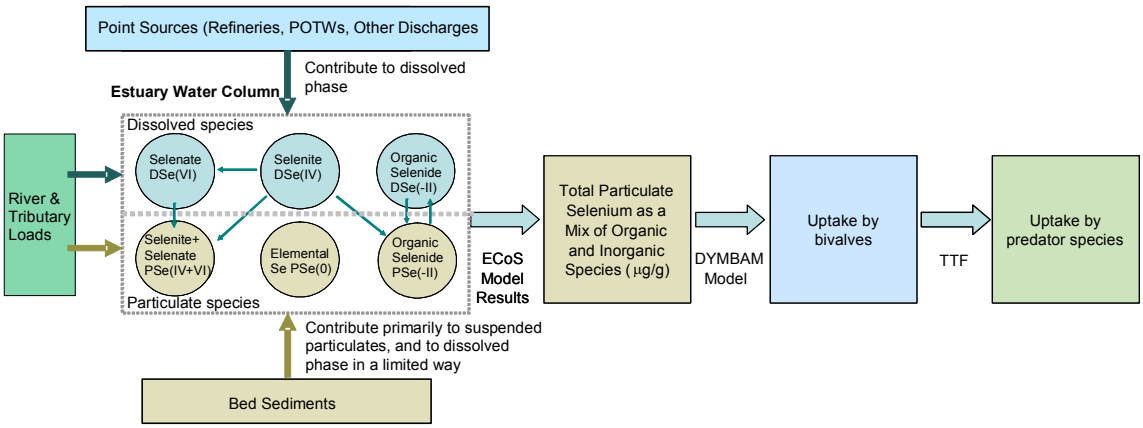


Figure 22: Schematic representation of the modeling framework linking selenium in water column and suspended particulates to bivalves, and then to predator species

The estuary model was developed using the ECoS3 framework and built upon the previous work of Meseck and Cutter (2006). The model was applied in a one-dimensional form with a daily time step. The estuary model simulates the biogeochemistry of selenium, including transformations among different species of dissolved and particulate selenium, salinity, total suspended matter (TSM), phytoplankton and water column concentrations, and the subsequent bioaccumulation of selenium in the North Bay. The aggregated output of the estuary model is subsequently used to evaluate selenium concentrations in bivalves and bioaccumulation of selenium through the food web by applying the empirical DYMBAM model (Presser and Luoma 2006) in a steady state mode.

The modeling framework, described only briefly in this report, provides a means to integrate and synthesize the existing information and offers a platform for evaluation of adaptive approaches to management of ecological exposure to selenium. The models were run to demonstrate how selenium discharges and other inputs can be related to the release

mechanisms, secondary sources, and exposure pathways. For details on model application, assumptions, calibration and testing see Technical Memorandum 6: *Application of ECoS3 for Simulation of Selenium Fate and Transport in North San Francisco Bay* prepared by Tetra Tech (2010).

ECoS3 Estuary Model

The estuarine modeling framework ECoS3 was originally developed by the Center for Coastal and Marine Sciences at the Plymouth Marine Laboratory, UK, and subsequently used to simulate biological productivity, total suspended material, salinity, nutrients, and trace metal behavior in a range of European estuaries. As described in Harris and Gorley (1998), the ECoS3 framework contains modules that simulate transport and dynamics of different dissolved and particulate constituents in an estuary and can be applied in a 1-D or 2-D form.

It was first applied to model selenium in the North Bay by Meseck and Cutter (2006). In that application, equations to simulate transport and transformations of different species of selenium were formulated and the North Bay was modeled as a 1-D well-mixed estuary divided into 33 segments. The model domain starts from the freshwater end member at the Sacramento River at Rio Vista (X = 0 m; head) and extends to the mouth of the estuary at the Golden Gate (total length = 101,000 m). The head of the estuary is modeled as a closed boundary with seawater as an open boundary. The same spatial representation was also used in this project (Figure 23).

Salinity – Along the estuary gradient, salinity is governed by freshwater inflows, wind and tides, and simulated using advection and dispersion equations. During the high flow season, freshwater advection dominates and lower salinity is observed through the estuary. During low flow, salinity in the estuary increases as a result of reduced freshwater inflows. Water velocities are computed with cross section areas derived from the Uncles and Peterson model.

Sediment Transport – Potential sources of sediments to the Bay include the Delta input, local tributaries, in situ resuspension and erosion, and in situ production due to phytoplankton growth. In ECoS3, total suspended material (TSM) is represented as three different components: permanently suspended particles (PSP), bed exchangeable particles (BEPS) and phytoplankton (B).

PSP is defined as suspended material that does not sink and does not interact with the bottom sediments, and is modeled in a manner analogous to a dissolved solute (Harris and Gorley 1998; Meseck 2002). BEPS originates from sediment resuspension. A small portion of BEPS also originates from the riverine input. BEPS is modeled as a function of sediment

resuspension and deposition, as well as advection and dispersion. The dispersion of BEPS is proportional to mixing that occurs due to both freshwater inflows and tides.

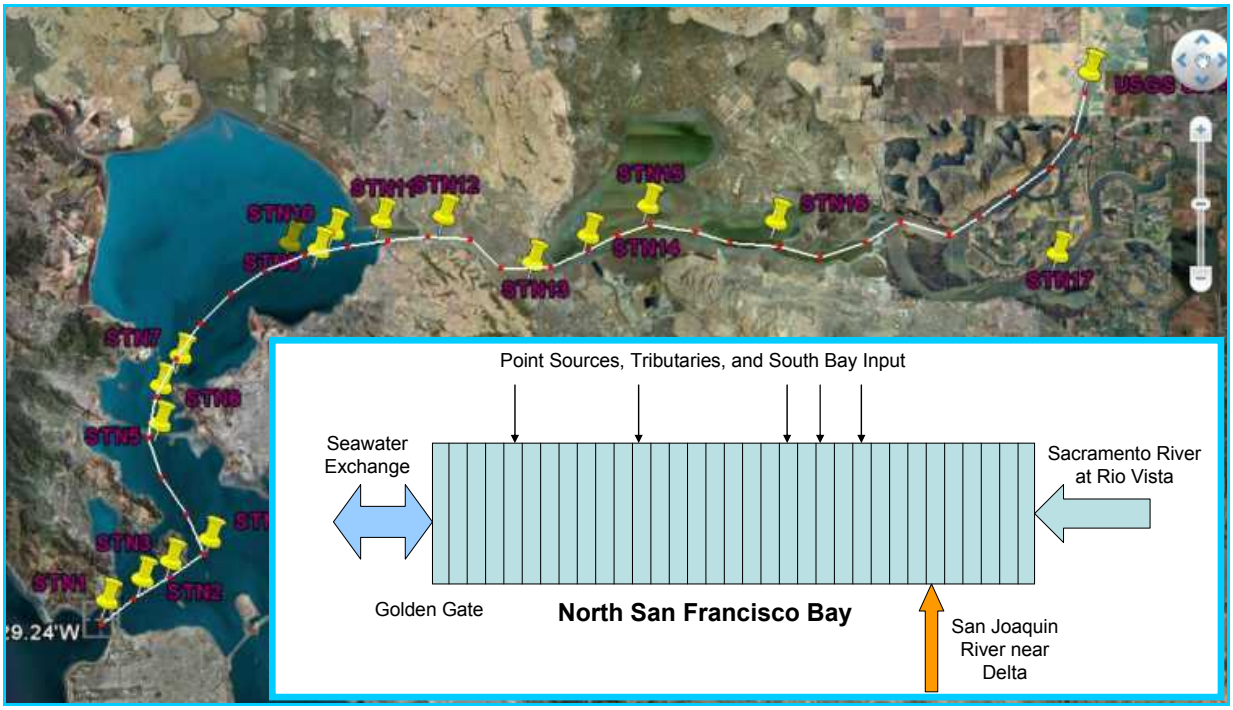


Figure 23: Spatial location of 33 model segments (*red dots*) **and schematic representation of the estuary showing boundary conditions and point source inputs**

Phytoplankton – The dynamics of phytoplankton play the key role in regulating selenium transformations. Dissolved selenium can be taken up by phytoplankton to form particulate organic selenium, which is bioavailable to higher trophic level organisms (Luoma *et al.* 1992). Phytoplankton is particularly affected by transport, growth and grazing by zooplankton and benthic organisms as well as settling and respiration (Meseck 2002) and modeled as a function of different sources and sinks. Benthic grazing can be a controlling factor in phytoplankton biomass as in laboratory experiments grazing rates observed for *C. amurensis* were found to exceed the specific growth rate of phytoplankton. Evident decreases in chlorophyll *a* concentrations observed in the Bay until recently, have been commonly linked to the invasion of *C. amurensis*. For further discussion of grazing effects and other limiting factors see Chapter 2 in Technical Memorandum 6 (Tetra Tech 2010).

Dissolved selenium – enters the North Bay from the Delta, local tributaries, refineries, municipal and industrial wastewater discharges, and diffusion from sediment. Speciation of

selenium from these sources is generally dominated by selenate (Se^{6+}), followed by organic selenide (Se^{2-}) and selenite (Se^{4+}). In the water column, these different species of selenium can undergo biological and chemical transformations.

Transformations of dissolved selenite include oxidation to selenate, uptake by phytoplankton and adsorption and desorption from minerals. Transformations of dissolved organic selenide include oxidation to selenite and uptake by phytoplankton. Dissolved organic selenide is also generated through mineralization of particulate organic selenide. For selenate, the transformation includes uptake by phytoplankton and microbes. Oxidation of selenite to selenate was found to be a slow process which can take hundreds of years, while oxidation of organic selenide to selenite occurs over a timeframe of weeks (Cutter 1992). Similarly, phytoplankton uptake of dissolved selenite and organic selenide was found to occur relatively rapidly (Riedel *et al.* 1996; Baines *et al.* 2004). Transformations between species are simulated as first-order kinetic reactions. Uptake and transformation processes of dissolved selenium are shown schematically in Figure 24.

Particulate selenium – can originate from riverine input, sediment resuspension, and in-situ production (e.g., phytoplankton uptake of selenium). Different species of particulate selenium are assumed to be associated with PSP and BEPS. Phytoplankton selenium is assumed to be present only as organic selenide. Riverine inputs of particulate selenium are specified as selenium content on riverine loads of particulates (PSP, BEPS, and phytoplankton). Although phytoplankton can be measured as part of the TSM, for this project phytoplankton and phytoplankton-associated particulate organic selenium are modeled separately. Particulate organic selenium associated with PSP is assumed to be selenium associated with organic carbon other than living phytoplankton (e.g., detritus of phytoplankton, plant material, and bacteria).

In the model selenium content on riverine PSP is determined with calibrated parameters that are bounded by values reported in Doblin *et al*. (2006). Particulate selenium associated with BEPS is subjected to exchange with particulate selenium in bed sediments at the same rates as sediment resuspension and deposition. Seawater end member concentrations of particulate selenium are specified as constants (as selenium concentrations of PSP in seawater) for an open boundary. The transfer from dissolved selenium to particulate selenium includes mineral adsorption (mostly for selenite) and phytoplankton uptake of dissolved selenium for all three dissolved selenium species.

Selenium in sediments is modeled as a combination of initial concentrations modified by resuspension and deposition through sediment-water interaction, as well as some riverine input. Due to the balanced resuspension and deposition rates of sediment, the changes in selenium concentrations in bottom sediments are small.

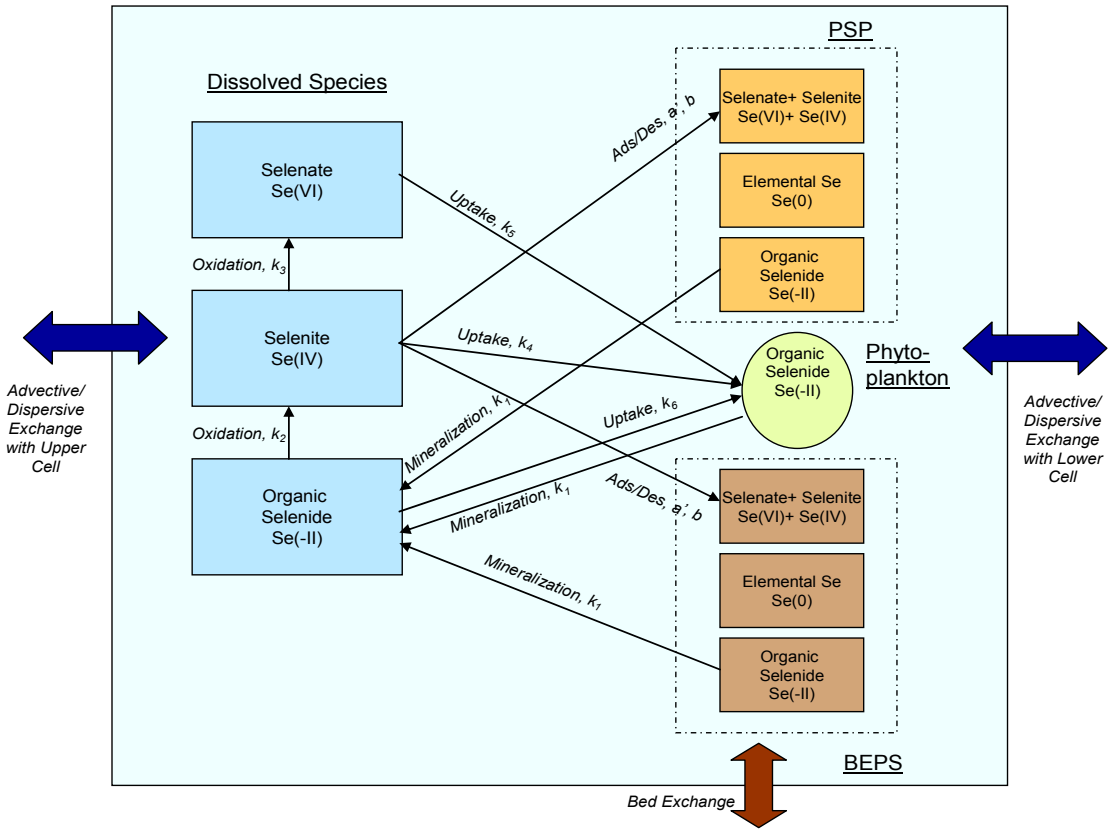


Figure 24: Interactions and transformations of dissolved and particulate selenium between different compartments in each cell of the ECoS3 model

DYMBAM Bioaccumulation Model

A dynamic multipathway bioaccumulation model (DYMBAM) describes contaminant accumulation and loss as a function of energy requirement in the lower trophic level organisms. DYMBAM uses species-specific empirically developed physiological rate parameters and environmental data representative of system conditions to assess and compare risks from metal exposure. In a steady-state application contaminant concentrations are expressed as a sum of waterborne and dietary uptake routes (Presser and Luoma 2006):

$$C_{ss} = \underbrace{\frac{k_u * C_w}{k_e}}_{\text{Water (dissolved)}} + \underbrace{\frac{AE * IR * C_p}{k_e}}_{\text{Food (particulate)}}$$

Where:

C_{ss} - steady state tissue Se concentration in clams
k_u - rate constant of Se uptake from water
C_w - Se concentration in water
AE - Se assimilation efficiency
IR - food ingestion rate
C_p- Se concentration in particulate material
k_e - the rate constant of loss

DYMBAM has been tested to be especially effective in determining selenium bioaccumulation in bivalves, copepods and polychaetes, and sufficient data exist to support assessments for benthic-based food webs with *C. amurensis* in San Francisco Bay. Applications of DYMBAM provide good compatibility with field observations despite simplifying assumptions and limited representation of bioenergetic responses in the model (Stewart *et al.* 2004). Model parameters to simulate selenium uptake by bivalves under a range of conditions are shown in Table 22. The ECoS3 model is used to determine concentrations of particulate selenium (organic selenide, selenite and selenate, and elemental Se) available on a daily basis. Then the species composition in the daily food intake by bivalves is assumed to be the same as simulated by the ECoS3 model, and used to compute average selenium concentrations in bivalve tissue according to the equation above.

Table 22: Parameters for DYMBAM model

Ingestion Rates	Assimilation Efficiency (%) for Particulate Selenium		
	elemental selenium	adsorbed selenite and selenate	organic selenide
0.45	0.2	0.45	0.8
0.25	0.2	0.45	0.8
0.45	0.2	0.45	0.54
0.85	0.2	0.45	0.80

Model Calibration and Evaluation

The basic physical functions of the model (salinity, total suspended material and phytoplankton) were calibrated using USGS data from 19 monitoring locations in the North Bay (http://sfbay.wr.usgs.gov/access/wqdata/). The main calibration time periods for these parameters are from January 1999 to December 1999. Water year 1999 was selected for calibration of the model because of the availability of detailed selenium speciation data sampled during both low and high flow periods. Water year 1999 also represents conditions for which detailed refinery discharge data are available. One-day time step was used in model runs, and the warm-up time was set to approximately 180 days starting from June 1, 1998.

The model calibration was done with a least squares minimization approach, using a fitting program provided by Dr. John Harris, the developer of the ECoS code. For every iteration, the sum of square deviation between observed and simulated values was calculated by the program and the parameters were adjusted for the next iteration to minimize the sum of square errors. After calibration the model was run to simulate the conditions in the Bay and the simulation results were validated for two hydrologicaly distinct years 1986 and 2001. Running a model for the year preceding the calibration time (hindcast mode) is considered to provide a good insight into the capability of the model to simulate conditions different from the calibration period in terms of hydrology and selenium loading. The results of these runs were compared with the observed data and the model performance was evaluated with two measures: correlation coefficient between predicted and observed values, and goodness of fit.

After initial evaluation of the model formulation and performance against the existing data, a series of model runs were conducted to gain more confidence in the model's ability to simulate selenium transformations across a range of conditions. The model was run under different input conditions and with different parameter values to assess the impact to selenium species concentrations. These tests offer better understanding of the functioning of the model by identifying processes and variables especially sensitive to the inputs, and point to the key variables where greater uncertainties may exist. The scope of the additional testing and the significance of each test are summarized in Table 23.

In general, the testing of the calibrated model demonstrated the ability of ECoS modeling framework to represent the key characteristics relevant to selenium fate and transport in the North Bay. The model performs particularly well in simulation of physical features of the Bay

such as salinity. Although poorer match was achieved between the observed and simulated results for suspended sediments and phytoplankton, numerous runs clearly have shown that the model is able to adequately simulate selenium in various compartments. For all the parameters modeled, the model is able to represent average conditions better than spatial and temporal peaks in concentrations, and longer-term evaluations capture phytoplankton transformations reasonably well.

Table 23: Testing performed to assess model performance

Testing Performed	Significance
Sensitivity analyses	The calibrated model parameters are perturbed from their base case values to assess whether specific dependent variables respond significantly. Future model development and/or data collection must be targeted at the most sensitive parameters.
Changing Chlorophyll a	The model calibration and evaluation shows that chlorophyll *a* concentrations were sometimes poorly fitted with the ECoS framework. Additional model runs were conducted with varied chlorophyll *a* concentrations to better understand the importance of chlorophyll *a* to the predicted values of particulate selenium.
Changing uptake rates of dissolved selenium species	The uptake rates for selenate, selenite, and dissolved organic selenide are based on literature reports and calibrated to fit the data. Testing was performed to explore the impact of varying the rates over a wide range, from 10 to 100 times the rates in the base case calibration.
Different boundary conditions for riverine and seawater input	Particulate selenium concentrations in the riverine and seawater boundary have a significant impact on the concentrations in the Bay and the subsequent estimates of selenium levels in bivalves. Data to define these boundaries are scarce. Exploratory runs were performed over a wide range of values for both boundary conditions to evaluate simulated concentrations in the Bay.
Relative contribution of different sources of particulate selenium	Particulate selenium concentrations are the single most important constituent with respect to bivalve uptake, thus understanding of relative contributions from sources into the Bay: riverine, in-Bay sediment erosion or phytoplankton, and their effect on estuary concentrations is necessary for developing management options.
Spatial trends in particulate selenium	Spatial distribution of particulate selenium varies across the estuary. The model allows examining the main processes responsible for the small increases in particulate selenium observed towards higher salinities.
Mass balance	A mass balance of inputs and outputs provides a higher level check of the overall numerical representation. Selenium sources, outflows, and changes in stored mass in the water column are presented.

The fact that peaks in flow and flow-controlled attributes cannot be fully captured is commonly observed in many models used to simulate environmental conditions. The value of these models lies in their ability to link complex environmental processes and reproduce longer term trends. The ECoS-based modeling framework gives consideration to speciation effects and simulates temporal and spatial variations in selenium concentrations that compare well with the available field observations. It also offers a means to predict changes in selenium uptake by phytoplankton and bivalves and therefore to evaluate the effect of reduction strategies for the TMDL.

6.3 Effects of Load Change in the North Bay

Load Change Scenarios

The calibrated and validated ECoS3 model coupled with DYMBAM was used to evaluate the effects of hypothetical changes in point and non-point loads on the dissolved and particulate selenium concentrations in water column and bivalves to evaluate linkages to sources and to better understand the potential for system recovery. The selenium speciation and loads were varied and compared to the existing conditions. The effects of changing the most prominent selenium sources: San Joaquin River and petroleum refineries are shown in Figure 25 and discussed below.

The results show that the model is able to forecast even small changes in particulate selenium but other forms of selenium are less important in the North Bay system. Thus if selenium speciation in refinery effluent was hypothetically altered to include 10% of particulate selenium (see Figure 25, scenario 3), it would trigger the increase in selenium levels in biota. It was also confirmed that a potential for adverse impacts resulting from speciation change is especially prominent during low flow conditions. The hypothetical addition of 10% particulate selenium would also contribute to significant increases in selenium concentrations in bivalves during the dry season. Contrary to this scenario, even a 20% decrease in petroleum refineries' dissolved load, i.e. a hypothetical reduction by more than 110 kg Se per year (see Figure 25 scenario 4) based on the current selenium speciation that is all in dissolved form and dominated by selenate, will have no discernible effect on bivalve concentrations, nor will it contribute to a significant decrease in particulate selenium levels. This leads to a conclusion that reductions in dissolved selenium loads do not result in proportional change in particulate concentrations, hence the less significant than expected response observed in the Bay following petroleum refineries cleanup in 1999.

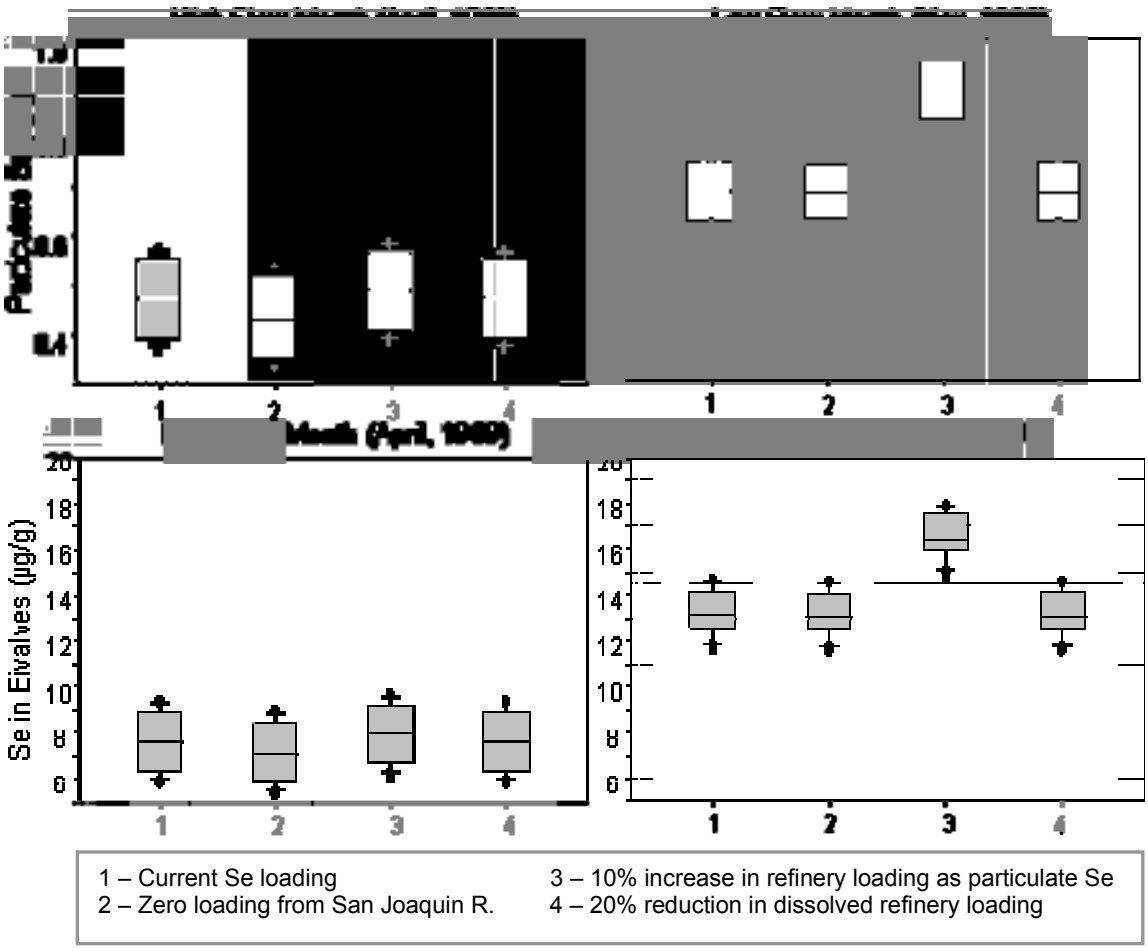


Figure 25: Predicted selenium concentrations for different loading scenarios

Complete elimination of the San Joaquin River dissolved load (e.g. see scenario 2) shows limited impact on dissolved and particulate concentrations. This is caused partly by the fact that most of the San Joaquin River inflow is diverted from entering the Bay and any changes in selenium loads are relatively small compared to the contribution of the Sacramento River load. However, if there is no continued reduction of San Joaquin River flow due to the State Water Project operations and other upstream diversions, significant increases in dissolved and particulate selenium concentrations in the North Bay may result.

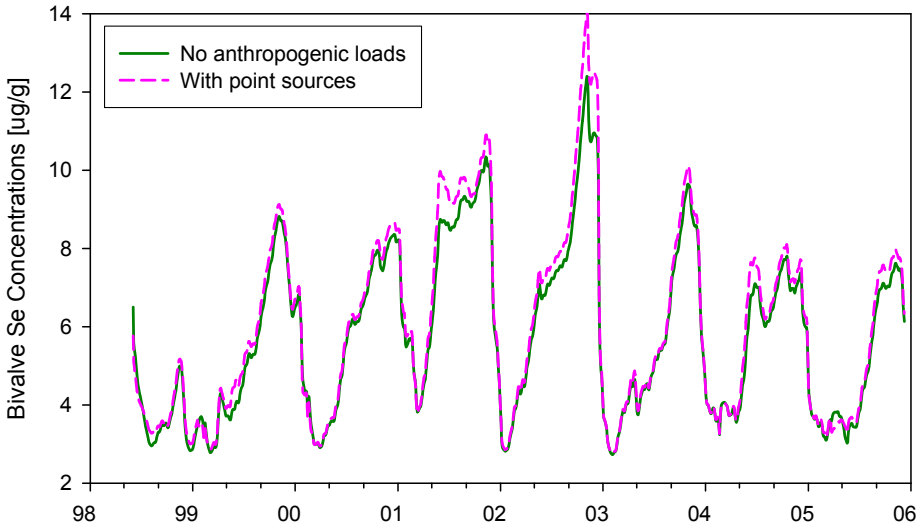
The overall sensitivity of the estuary to load changes from local tributaries and point sources is greater during dry months, especially during a dry year, i.e., for a given load change factor, greater change is observed during the dry periods, which relates to the overall lower inflow from Sacramento River and the longer residence times in the Bay.

Background Conditions

The natural baseline concentrations in the North Bay are defined by selenium inflow from Sacramento River mixing with selenium from the ocean. The inflow from Sacramento River at the background level selenium concentrations (~0.07 µg dissolved Se/L) carries on average 4.3 kg Se per day or 3.1 to 5.5 kg/day during dry and wet seasons, respectively. The maximum daily load during high flows may be as high as 7 kg/day, while the average refinery load is relatively small and stable throughout the year at 1.5 kg/day.

A scenario was run to evaluate the effect of background conditions on selenium levels in *C. amurensis*. This was defined as selenium loads that originate from natural background only without significant anthropogenic influences (e.g. refinery discharges, agricultural drainage, and POTW discharges), and assuming conservatively the Sacramento River concentrations as the natural background for the entire region (0.07 µg dissolved Se/L) including tributaries draining to the Bay and San Joaquin River, which is known to have higher background selenium concentrations (0.2 – 0.5 µg/L). On the other hand, in this scenario the impact of San Joaquin River discharge remains somewhat diminished because the model run reflects current (1999 – 2006) flow conditions with only a small proportion of San Joaquin River flow reaching the Bay. Discharges from petroleum refineries and POTWs were set to zero.

The results in Figure 26 show that under background load conditions the concentrations of selenium in *C. amurensis* may reach highs similar to those currently seen in the North Bay indicating that this invasive species plays a key role in amplifying available dietary selenium in the benthic food web. Much lower selenium concentrations are found in native clams due to low ingestion rates and higher loss rates. The results also indicate that for very short periods of time in low flow conditions (October – November) anthropogenic loads may be at levels that potentially can impact concentrations in bivalves. However, there is no evidence to suggest that this is really occurring. High selenium concentrations found in bivalves at the end of low flow/dry period may also reflect the growth cycle of *C. amurensis*. For example, in San Pablo Bay, they usually reproduce in spring and depend on phytoplankton blooms for food during spawning and growth, reaching their highest size in fall. Thus, selenium concentrations found in the bivalve tissue may also result from the overall longer accumulation period (see section 6.4 for further discussion).



Ingestion rates (IR) = 0.45, AE = 0.2, 0.45, 0.8 for elemental, inorganic, and organic particulate Se

Figure 26: Model predicted selenium concentrations in bivalves under background load conditions and with point source loads

Although a simulation with all point sources of selenium removed is essential to our understanding of selenium bioaccumulation potential, these predictions are associated with large uncertainties. For the calibration of the model we relied on the best available data and scientific judgment in defining boundary conditions at the freshwater end member in Sacramento River.

Due to the lack of measured particulate data in the freshwater reach, the available data from the nearest suitable location (Rio Vista) were selected that allowed for the best fit with the measured concentrations in the Bay. The salinity of these samples was at zero or near zero signifying that at the time of the measurement the freshwater flow was prevalent. While this is a valid approach, the Rio Vista area is known to be tidal, hence some uncertainty still remains as to whether the origin of particulate material was in fact the Sacramento River or the Bay. Validation of baseline particulate conditions in the Sacramento River is vital and cannot be resolved without collecting new data.

6.4 Predicted Concentrations in Bivalves and Sturgeon

Figure 27 shows predicted selenium concentrations in bivalves for an array of ingestion rates and assimilation efficiencies. The results are calculated using the DYMBAM model with the assumption that the composition of particulate selenium species in the daily input of food

ingested by clams is the same as simulated by the ECoS3 model. The observed peaks in concentrations are influenced mainly by seawater/freshwater mixing and chlorophyll levels, which change from year to year. The clam feeding rates (biodynamic model parameters) are based on studies with *C. amurensis* in the laboratory, and represent the high end of the experimental values (Lee *et al.* 2006).

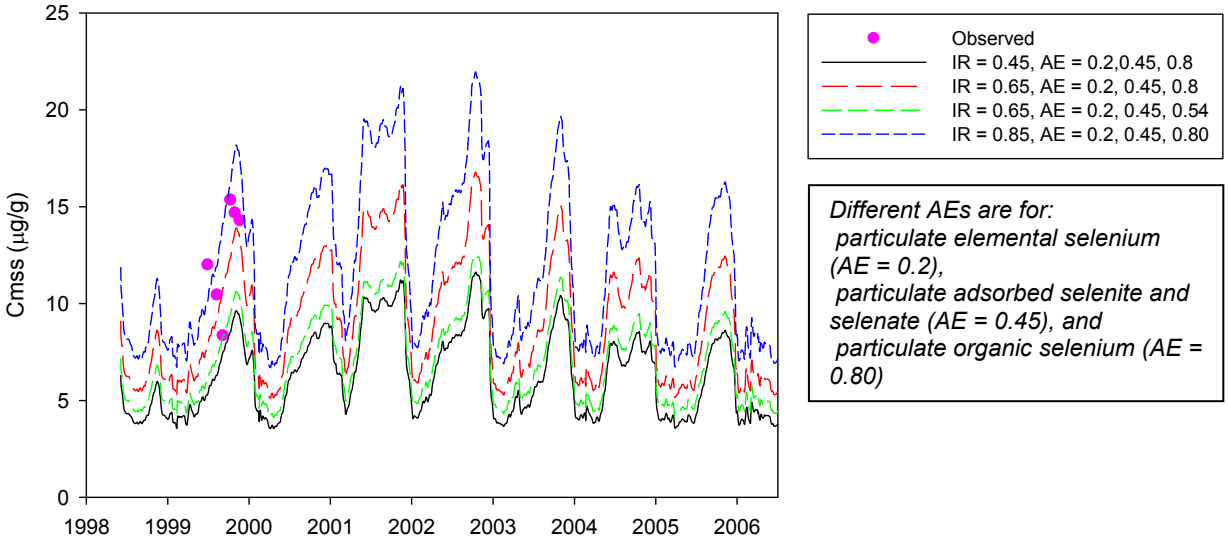


Figure 27: Simulated selenium concentrations (Cmss) in bivalve *C. amurensis* near the Carquinez Strait compared to observed values at the USGS station 8.1

For 1999 – 2006 the predicted ranges in bivalve selenium concentrations are between 3 and 22 µg/g and compare well with the measured concentrations (Stewart et al. 2004). After reaching the apparent peak in 2001-2003 the forecasted bivalve concentrations show a considerable decline, which has been also confirmed by the recent measured data showing concentrations below 10 µg/g from 2004 through 2006 (USGS 2010, Figure 28).

However, the levels of selenium in these clams are likely to fluctuate and stay elevated compared to other benthic organisms. Not only do these clams exhibit a high propensity to bioacumulate selenium based on their bioenergetic characteristics but they also appear not to differentiate between food sources of selenium, like other bivalves. For example, in laboratory experiments the Asiatic clam *C. fluminea*, more efficiently assimilates selenium associated with algae (66–87%) than selenium associated with oxic sediments (20–37%), but no consistent difference was found between assimilation efficiencies from organic and sedimentary food types (19–60%) for *C. amurensis* (Lee *et al*, 2006). In addition, it appears that other factors such as rainfall and Delta flows that control salinity particularly in the North

Bay, may alter conditions in which *C. amurensis* could thrive from year to year and thus affect selenium levels.

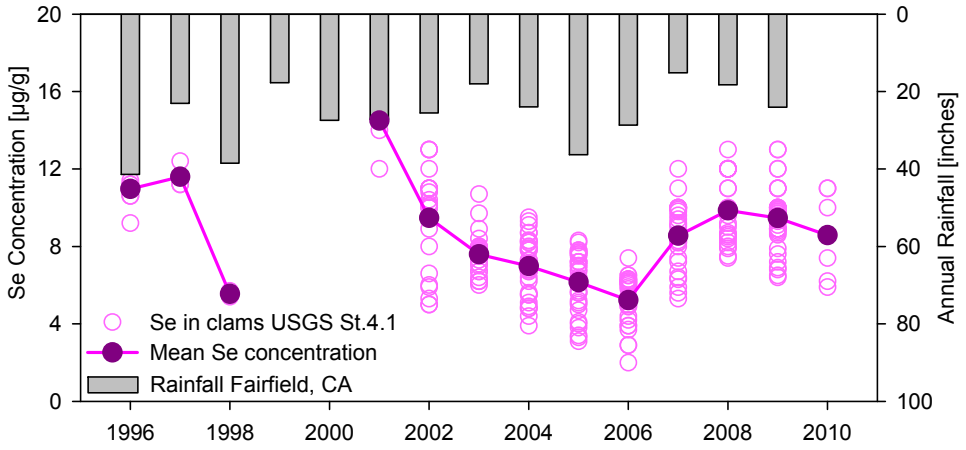


Figure 28: Concentration of selenium in *C. amurensis* measured at USGS station 4.1 and annual rainfall

The DYMBAM approach could also be used to forecast selenium bioaccumulation in fish except that kinetic uptake parameters for sturgeon are not known. Instead, transfer of selenium from food (e.g. bivalves) to fish can be represented by relationships between concentrations in fish tissue and concentration in dietary items (Luoma and Presser 2009). This ratio is called the Trophic Transfer Factor (TTF) and combines three biodynamic constants: assimilation efficiency, ingestion rate and efflux rate. For each species a TTF can be derived from laboratory experiments, literature estimates or with greater uncertainty from field data.

Selenium bioaccumulation in sturgeon ($C_{sturgeon}$) is then simply expressed as:

$$C_{sturgeon} = TTF_{sturgeon} * C_{clams}$$ ***Box 1***

$$C_{clams} = K_d * Se_{dissolved} * TTF_{clams}$$

Where:

K_d is a distribution coefficient [L/kg] that describes a relationship between selenium concentrations in particulate and dissolved phases;

C_{clams} represents selenium concentration [µg/g] in sturgeon dietary items and for this computation is conservatively assumed as equal to selenium concentrations in *C. amurensis*

The available TTFs for white sturgeon are regression estimates in the range from 1.0 to 1.7 based on extremely limited data collected in the 1990s (Presser and Luoma 2006). Since then Presser and Luoma (2009) compiled TTFs for fish derived from experimental studies and sets of matching field data and calculated the average TTF for generic fish to be 1.1 and the 75th percentile of 1.34 which also corresponds to the average of the sturgeon TTF range.

Using the default recommended TTF for fish of 1.1 and the typical range of concentrations measured in *C. amurensis* (~5 to 11 µg/g) we can estimate the projected concentration in sturgeon to likely vary from 5.5 to 12.1 µg/g. The upper end of the predicted concentrations is higher that the proposed target range for the TMDL (6 - 8.1 µg/g) and the draft 2004 USEPA criterion of 7.91 µg/g. Yet the above evaluation assumes that sturgeon diet consists entirely of bivalves or includes other food items that have similarly high selenium concentrations and that all selenium is retained by sturgeon. In fact, other components of sturgeon's diet in the North Bay exhibit much lower selenium concentrations from ~ 1 to 3 µg/g (Stewart *et al.* 2004) and there is new evidence to suggest that the diet of white sturgeon may comprise only 40% of *C. amurensis* (T. Presser, USGS, *pers. comm*. May 12, 2010).

Moreover, Poulton and others (2004) investigated spatial and seasonal patterns of clams and found that densities of *C. amurensis* at six sites in San Pablo Bay declined dramatically over winter (mean= 152 m^{-2}) while other clams were still abundant. The highest density among more than 1700 core samples was only 2206 m^{-2} which is far lower than those commonly found in 1987-88 (>10000 individuals per m^{-2}). An approximately 20-fold decline in the bivalve abundance in San Francisco Bay after 1998 has been also linked to the increased predation by Crangon shrimp, juvenile Dungeness crab and English sole which have persisted at high densities since 1999 (Cloern *et al.* 2007).

Therefore, it may be considered that white sturgeon is not exposed to as much selenium in its diet as previously thought. We cautiously assumed that sturgeon's diet includes 50% of *C. amurensis* and thus the selenium dietary intake is approximately 7 µg/g, which is in all likelihood higher than the overall selenium concentration in food items consumed by sturgeon. The subsequent tissue concentrations calculated with the TTF of 1.1 will be in the range of 8 µg/g. A TTF of 1.3 could result in tissue concentrations reaching 9.1 µg/g. In the North Bay-Delta in 2002-09 the mean selenium concentration found in 53 samples of sturgeon muscle was 6.6 µg/g dw (Figure 29). Only 8% of samples collected since 2002 exceeded the upper value of the numeric target range and 9 out of 53 samples had selenium concentrations above 10 µg/g.

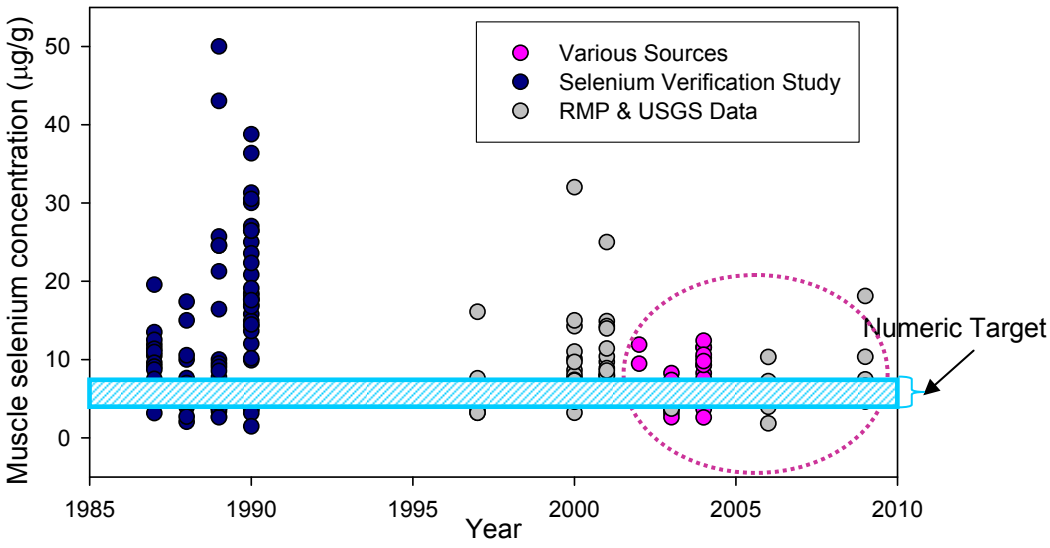


Figure 29: Observed selenium concentrations in white sturgeon in the North Bay

Linking Fish Tissue Target to Water Column Concentrations

Although aqueous selenium concentrations could not be linked directly to bioaccumulation in sturgeon, transformation from dissolved forms to living organisms takes place at the base of the food web and for that reason it has a bearing on the amount of selenium available for higher level predators. In addition, knowing the threshold dissolved selenium concentration in the North Bay that could potentially limit the adverse effects on sturgeon provides means for monitoring these concentrations as part of routine water quality measurements in the Bay and, in the future, could be used to track reductions of selenium due to source control efforts or implementation of best management practices. Water column concentrations can also offer a starting point for an initial risk characterization and assessment.

In the calculation of the water column concentration of selenium from the desired sturgeon tissue concentration of 8.1 µg/g we followed the general approach developed by Presser and Luoma (2009, 2010) that was first used for the San Diego Creek and Newport Bay TMDL (*in preparation*). Table 24 shows methodology steps and the assumptions used in the translation process. By rearranging the equations in ***Box 1*** above, the dissolved selenium ($Se_{dissolved}$ in µg/L) can be calculated as follows:

$$Se_{dissolved} = \frac{C_{sturgeon}}{TTF_{sturgeon} * TTF_{clams} * K_d} * 1000$$

Where: $C_{sturgeon}$ – fish tissue criterion/numeric target

Table 24: Selection of parameters for translation of sturgeon tissue numeric target to water column concentration

Methodology steps	Assumptions
Determine the target species	Sturgeon
Choose toxicity guideline (numeric target) for fish	Numeric Target: 6 - **8.1** µg/g
Choose species-specific TTF_{fish} or use default TTF_{fish} of 1.1	$TTF_{generic\ fish}$ = 1.1 $TTF_{sturgeon}$ = **1.3**
Identify appropriate food web(s) for selected fish species based on fish-specific diet	Benthic – dominated by *C. amurensis* Benthic – with a mixed diet of *C. amurensis (50%) and M. balthica (50%)*
Choose TTF_{clams} for invertebrates in selected food web or use default TTF_{clams} for class of invertebrate	$TTF_{C.\ amurensis}$ = range 4.0 – 8.5 => **6.25** $TTF_{M.\ balthica}$ = 4.5
Choose K_d based on source of selenium and receiving water conditions	Computed from modeled data
Translation assuming a single invertebrate diet Translation assuming a mixed invertebrate diet	$C_{water} = (C_{sturgeon}) \div (TTF_{fish})(TTF_{clam})(K_d)$ $C_{water} = (C_{sturgeon}) \div (TTF_{fish})(K_d)$ $[0.5(TTF_{C.amurensis}) + 0.5(TTF_{.M.balthica})]$

Partitioning of selenium between water and particulate material is a dynamic biogeochemical process and the distribution coefficient (K_d) which describes the proportion of selenium associated with particulate matter at any given time and location may vary by many orders of magnitude (Presser and Luoma 2009). In fact, K_d varies more widely than any other parameter used in the translation process and careful consideration should be given while selecting the appropriate values. By definition K_d values greatly depend on selenium speciation in the water column. For translation of sturgeon tissue target to a water column concentration we derived the K_d values from the ECoS3 model simulations of transport and dynamics of different dissolved and particulate selenium species throughout the North Bay.

The modeling results verify that large spatial and temporal variability in selenium partitioning exists, which signifies that even the monitoring data, after all representing instantaneous conditions, may not be adequate to fully describe selenium transformations occurring in a complex ecosystem such as the North Bay. However, the ECoS3-based modeling framework helps establish a first-order understanding of relevant transformation conditions that are

linked to specific hydrodynamic regimes and reflective of ecological factors making it especially effective in K_d determination.

The model estimated K_{ds} (particulate/dissolved selenium) at five locations for the period of 1999-2007 were used to compute the K_d statistics. K_d values generally increased from Suisun Bay to San Pablo Bay and to Central Bay, largely as a result of the organic enrichment of particulates that takes place from the riverine boundary to the ocean boundary (Table 25). The calculated K_{ds} range from 2000 to just over 17000 L/kg and are generally within the array of values found in estuaries.

Table 25: Selenium partitioning coefficient (K_d) as a function of location in the North Bay and the North Bay average

	Rio Vista	Suisun Bay	Carquinez Strait	San Pablo Bay	Central Bay	North Bay
MIN	2719	2598	2235	2577	4930	2954
MAX	9461	12059	14634	17214	16541	12785
MEAN	5326	4791	5379	7939	14116	6676
75th Percentile	6145	5373	6606	10111	15301	7581

Although in the North Bay the change in dissolved selenium concentrations is small, the particulate concentrations increase with distance from the Delta resulting in higher values of K_{ds}. These are caused by an increase in the chlorophyll *a* to total suspended material (TSM) ratio across the North Bay. The higher particulate selenium values also appear to result in higher clam concentrations at greater distances from the Delta, where higher salinities offer more favorable habitat conditions. The changing mix of particulate selenium across the North Bay, with increasing proportion of organic selenium, is shown in Figure 30.

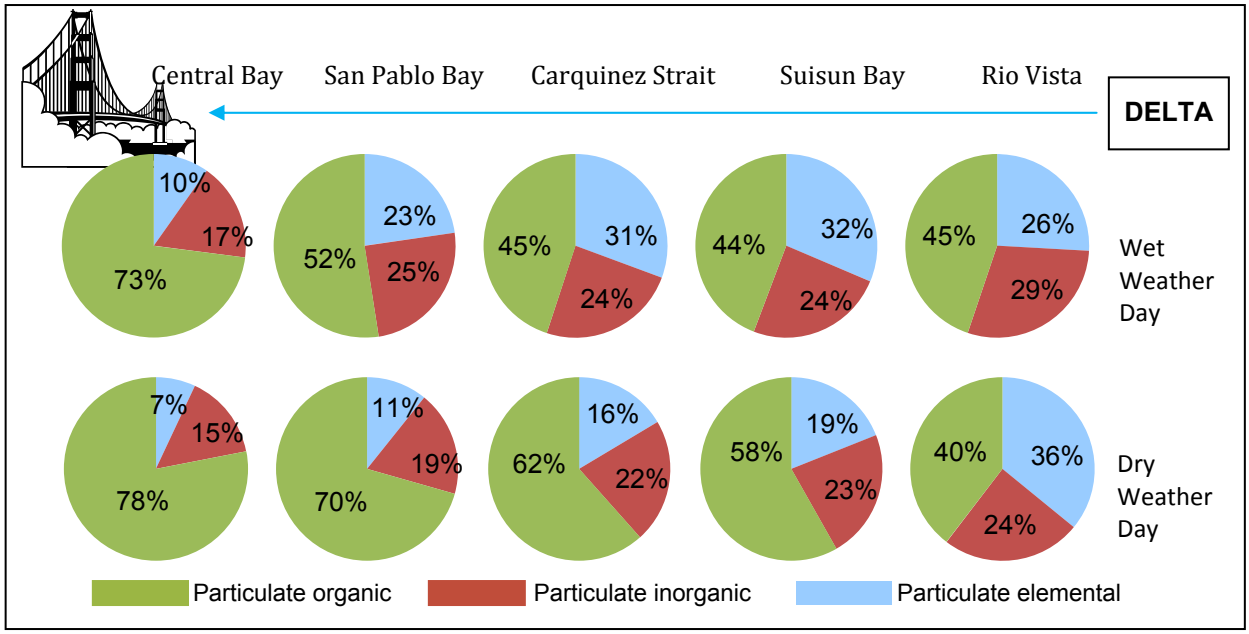


Figure 30: Changing mix of particulate selenium from the Delta to the Golden Gate

Table 26 shows the water column concentrations translated from the upper sturgeon tissue target of 8.1 µg/g for the computed statistics of the K_d values and the TTF values in Table 24. Estimated target concentrations based on mean K_d values and the sturgeon-specific TTF of 1.3 range from 0.21 µg/L in Suisun Bay to 0.07 µg/L in Central Bay with the North Bay–wide concentration of 0.15 µg/L. In random sampling of selenium in the North Bay (2002- 2008) the measured selenium concentrations varied from 0.04 to 0.44 µg/L (75th percentile = 0.125 µg/L) and the mean concentration was 0.10 µg/L. Considering conservative assumptions applied at each step of the target translation process these results tentatively suggest that the North Bay shows signs of at least a limited capacity to assimilate existing selenium loadings.

Table 26: Water column targets corresponding to the sturgeon target of 8.1 µg/g, clam to fish TTF of 1.1 and 1.3 and with clam TTF of 6.25

TTF_{fish} = 1.1		Rio Vista	Suisun Bay	San Pablo Bay	Central Bay	North Bay
		Dissolved Se concentration [µg/L]				
K_d	MIN	0.43	0.45	0.46	0.24	0.40
	MAX	0.13	0.10	0.07	0.07	0.09
	MEAN	**0.22**	**0.25**	**0.15**	**0.08**	**0.18**
	75th %ile	0.19	0.22	0.12	0.08	0.16

$TTF_{sturgeon}$ = 1.3		Rio Vista	Suisun Bay	San Pablo Bay	Central Bay	North Bay
		Dissolved Se concentration [µg/L]				
K_d	MIN	0.37	0.38	0.39	0.20	0.34
	MAX	0.11	0.08	0.06	0.06	0.08
	MEAN	**0.19**	**0.21**	**0.13**	**0.07**	**0.15**
	75th %ile	0.16	0.19	0.10	0.07	0.13

Knowing that sturgeon like most fish eat a diverse diet comprising at least an assortment of benthic organisms we also constructed a conservative scenario in which 50% of the sturgeon's *C. amurensis* diet is substituted with *Macoma balthica* another invertebrate common in San Francisco Bay and with high selenium TTF of 4.5. Following the translation steps (see Table 24 for assumptions) the allowable water column concentrations in the North Bay segments range from 0.08 to 0.24 µg/L for the estimated average K_d (Table 27). A mean selenium concentration of 0.17 µg/L is predicted as protective of sturgeon when the entire North Bay is considered, which again is higher than the monitored average water column concentration of 0.10 µg/L.

Table 27: Water column targets corresponding to the sturgeon target of 8.1 µg/g, mixed invertebrate diet and clam to fish TTF of 1.3

$TTF_{sturgeon}$ = 1.3		Rio Vista	Suisun Bay	San Pablo Bay	Central Bay	North Bay
		Dissolved Se concentration [µg/L]				
K_d	MIN	0.43	0.45	0.45	0.24	0.40
	MAX	0.12	0.10	0.07	0.07	0.09
	MEAN	**0.22**	**0.24**	**0.15**	**0.08**	**0.17**
	75th %ile	0.19	0.22	0.12	0.08	0.15

Seasonal Variations

The diminishing freshwater inflow from the Delta during dry weather season together with the increasing residence time could amplify the impact of in-the-Bay selenium sources, predominantly discharges from petroleum refineries, on selenium transformations and bioavailability. Therefore, the estimates of target concentrations for dry and wet seasons and different hydrologic regimes are useful to evaluate the linkages between selenium loading and the potential for adverse effects. The results in Table 28 show that for the evaluated set of conditions the water column concentrations would need to be lower during the dry season to reduce the potential for toxic exposure in sturgeon. However, only for the worst case

scenario (dry and wet season during a dry year) and the most conservative parameters are the computed target concentrations lower than the average selenium concentration measured in the North Bay of 0.10 µg/L (2002-2008).

To ensure protection of sturgeon from potentially harmful concentrations of selenium in the North Bay we propose that the water column target should be derived using the most conservative TTF_{clam} of 6.25, and TTF_{fish} of 1.3 (Table 28). In addition, based upon the characteristics of sturgeon, its long life-span, long-range and irregular spawning the appropriate spatial scale for assessing the compliance with the proposed target should be the entire North Bay rather than the individual Bay segments.

The clam trophic transfer factor of 6.25 represents the utmost value in the range estimated from laboratory experiments with *C. amurensis* and field data. Also this TTF is used by Presser and Luoma (2010) in the translation of selenium tissue guidelines to allowable dissolved selenium concentration for invertebrate-based food webs in San Francisco Bay. In the most recent study with radiolabeled food Lee and others (2006) measured assimilation and efflux parameters from which the calculated TTF varies from 3.6 to 5.4. Therefore, the TTF of 6.25 applied here is likely to overestimate selenium accumulation in clams providing for a reasonable margin of safety.

Table 28: Water column targets corresponding to wet and dry season and different type of hydrologic year

	TTF_{fish} 1.1 TTF_{clam} 6.25	**$TTF_{sturgeon}$ 1.3 TTF_{clam} 6.25**	**$TTF_{surgeon}$ 1.3 Mixed Diet TTF_{clam} 6.25/4.5**
1999 (Average Year)			
Wet Season	0.22	0.18	0.21
Dry Season	0.14	0.12	0.14
2001 (Dry Year)			
Wet Season	0.19	0.16	0.18
Dry Season	0.15	0.13	0.15
2005 (Wet Year)			
Wet Season	0.23	0.19	0.22
Dry Season	0.15	0.12	0.14

Sturgeon in San Francisco Bay are not only exposed to varying dietary concentrations throughout the year but also to different forms of selenium and these conditions are hard to replicate in the laboratory setup. In most studies fish are exposed to the most bioavailable forms of selenium at high concentrations so maximum transfer from diet to tissue would be

expected. Our preliminary estimates for dry seasons and a dry year indicate that water column concentrations of 0.12 – 0.16 µg/L are protective of sturgeon. For conservatively assumed mixed diet the water column concentrations during the dry year are 0.15 – 0.18 µg/L. This range of selenium appears to represent a foreseeable ambient concentration in the North Bay governed by mixing of the inflows from Sacramento River with the regional background concentrations of approximately 0.07 µg/L, San Joaquin River with concentrations of 0.2 to 0.5 µg/L and the North Pacific concentrations of 0.06 to 0.2 µg/L (Sugimura *et al.* 1976).

The array of water column concentrations computed with a conceivable range of parameters (Table 26, Table 27, Table 28) illustrates the importance of the values of the key parameters in identifying the targets. It is critical that these are calculated with credible data and/or well calibrated and validated models. Despite the greatly improved understanding of selenium processes and considerable amount of data used to develop the estuary model, in some aspects we had to rely on information more than a decade old. Therefore, additional monitoring data are necessary to validate model simulations for current flow and load conditions and, subsequently, to enhance the level of confidence in the translated water column targets.

Major Uncertainties and Next Steps

During the scientific review process of the modeling framework, crucial data needs and technical limitations were identified and discussed. It was agreed that the issues associated with defining the Sacrament River boundary conditions, riverine loading of organic selenium in phytoplankton and the rates at which different selenium species are converted to organic selenides could not be resolved without additional monitoring and research that may extend beyond the scope of this project.

One of the major concerns identified was lack of selenium particulate data which is essential to better quantify and confirm the role of the background selenium load entering the Bay. The model simulations discussed in Technical Memorandum 6 (Tetra Tech 2010) show that the selected particulate selenium concentrations at the system boundaries (Delta and Golden Gate Bridge) could have a significant effect on the predicted particulate selenium concentrations in the water column which, in turn, is critical to forecasting trophic transfer and bioaccumulation in predators. The modeling results are based on the existing data to characterize the boundary conditions. The lack of particulate selenium concentration measurements in the freshwater sections of Sacramento River (e.g. at Freeport) and in the

near-shore area beyond the Golden Gate Bridge is potentially a deficiency which also renders considerations of the appropriate remedial actions challenging.

The two main reasons for addition of data from recently conducted studies and for targeted new data collection are:

- to better understand and quantify the declines in selenium concentrations in bivalves and fish since 1999 and to confirm that selenium levels observed in the North Bay have food web and wildlife impacts
- to improve the accuracy of riverine selenium estimates and to clarify the effect of the background selenium load on conditions in the Bay

Three pertinent sources of data have been identified to accomplish the first purpose. These are: (1) RMP 2009 sport fish status and trends monitoring results; (2) USGS bivalve dataset (1995-2008), and (3) selenium tissue concentrations in archived (1997-2007) Largemouth bass from the Central Valley and Bay Delta. This new information is expected to be available later in 2010.

Systematic review of the additional information will strengthen the overall quality of the available data set and the subsequent findings for the TMDL. It is anticipated that the new data will facilitate verification of species of concern in the North Bay and help confirm that the recently observed decreases in concentrations in bivalves are representative of trends over time. Moreover, the RMP monitoring project will investigate the alternative non-lethal sampling (muscle biopsy) in white sturgeon, vital for implementing the TMDL and conducting future monitoring of this large, long-lived fish.

The second goal will be met when an “effluent and receiving water selenium characterization study” is conducted by the petroleum refineries, as required in their reissued NPDES permits.

The overall requirement of this study is to characterize: (1) the concentrations and speciation of selenium in effluent and receiving water, (2) the variability of selenium in the refinery discharge, (3) the potential for uptake and conversion of selenium to more bioavailable forms, (4) mixing and dilution in the receiving waters. The data collected to fulfill the NPDES permit provisions will include sampling of the freshwater reaches of Sacramento and San Joaquin Rivers and analyses of particulate selenium content. This will not only support the verification of riverine loads but will also be used to fine-tune the estuary model calibration thus enhance the accuracy of model predictions.

By extending the TMDL schedule we also anticipate to take advantage of the new assessment tools and guidelines that are being developed on regional and national scale, such as:

- California-wide selenium wildlife criteria (the interagency effort led by the USEPA Region IX in collaboration with US FWS, USGS and NOAA Fisheries)
- Nation-wide aquatic life criterion for selenium and guidance on how to adopt and implement criteria based on fish tissue concentrations (USEPA)

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EPA's Draft Tissue-Based Selenium Criterion:

A Technical Review

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Presented to U.S. Environmental Protection Agency, June 16, 2004

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Background

Selenium pollution of aquatic ecosystems is a significant global environmental safety issue. This is because selenium pollution is a common byproduct of several core economic activities including, but not limited to, irrigated agriculture, mining (coal, phosphate, uranium and numerous other sulfide minerals), coal-fired generation of electricity, and the refining of crude oil (1-6). Because selenium is often an unintended, but significant, component of commercial fertilizers (from the source rock used to make the fertilizer and/or from hazardous wastes, such as fly ash, legally disposed of in fertilizers) watersheds far removed from traditional sources of selenium pollution are also increasingly affected (7-9). Many aquatic ecosystems are sensitive to even low levels of selenium pollution and multiple toxic episodes have now been documented (10). Toxicity is typically expressed as impaired reproduction among populations of fish and/or aquatic-dependent birds (10). Due to these economic and environmental aspects, guidance for regulating selenium pollution is closely monitored by both the corporate-service scientific community (primarily, but not only, private-sector researchers and corporate-funded academia) and the public-service scientific community (primarily, but not only, government researchers and public-funded academia). Managers of commerce and managers of public-trust biotic resources (such as salmonids and waterfowl) both have vital interests that are directly influenced by the regulation of selenium pollution (11-13). The core regulatory guidelines for aquatic selenium pollution in the United States (U.S.) are the Aquatic Life Water Quality Criteria (Aquatic Life Criteria) derived by the U.S. Environmental Protection Agency (EPA) pursuant to the Clean Water Act (CWA) of 1977 (as amended). Because selenium is highly bioaccumulative and its

toxicity to fish and birds occurs primarily via dietary exposure, it is the long-term chronic criterion for selenium that is virtually always the controlling standard from a risk management perspective. EPA last promulgated an updated chronic criterion for selenium 17 years ago, in 1987 (14-15). EPA's current chronic criterion for selenium is 5 μg/L on an acid-soluble basis (16).

Controversy over the EPA chronic criterion emerges. During the past 17 years numerous researchers have estimated that the toxicity threshold for selenium lies below 5 μg/L (10, 17-23). In addition, three independently conducted studies funded by EPA since 1987 also reached the same conclusion (24-26). This body of work was produced predominantly by the public-service scientific community (27). More recently, a notable (11, 13) counter consensus predominantly from the corporate-service scientific community (27) has asserted that the current chronic criterion of 5 μg/L is overly restrictive (28-35). Critical reviews of the counter consensus focus on methodological deficiencies and the selective use of available literature and data (36-39). In another case (29), selective publication of their own analyses occurred after corporate-service authors were made aware that their full range of analyses provided strong support for toxicity guidelines endorsed by the public-service scientific community (40). Contributions from the corporate-service scientific community have sometimes been consistent with the public-service consensus regarding toxicity thresholds for lentic aquatic systems (28, 40), but not for lotic aquatic systems (28, 32-33). The core studies relied upon by the public-service scientific community are primarily from lentic systems (1, 3, 10, 21, 27, 39). Very recently, however, the first conclusive documentation of a toxic episode in a lotic system has been reported (41), and at modest levels of selenium pollution (6-32 μg/L waterborne selenium).

The paucity of lotic studies that match this recent study's (41) methodological rigor for detecting adverse effects suggests that our understanding of the vulnerability of lotic systems may be fairly uninformed, especially compared to the rich adverse effects databases from the much easier to study lentic systems (1, 10).

Even if lotic systems are less sensitive to selenium pollution; however, virtually all lotic systems serve either naturally (via floodplains) or artificially (via in-stream impoundments and off-stream water diversions) as source waters for lentic aquatic systems. From a risk management perspective, because of the hydrologic connections between lotic and lentic systems, it is the most sensitive system (lentic) that must dictate the controlling regulatory standards. A good illustration of this principle is provided by the hydrological system linking the Colorado River (lotic) to the Salton Sea (lentic) in southern California (10).

EPA prepares a draft updated chronic criterion. In 1997, EPA published a proposed set of Water Quality Criteria known as the California Toxics Rule, *aka* CTR (42). Pursuant to the Endangered Species Act (ESA) of 1973 (as amended), and prior to EPA promulgating the CTR, EPA was required to consult with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service (Services) and obtain the Services' concurrence that none of the proposed criteria in the CTR would jeopardize any ESA-listed species (43). Formal consultation between EPA and the Services was initiated in fall, 1997, and by spring, 1998, the Services had issued a draft "Jeopardy Opinion" based, in part, on the Services' evaluation that the 5 μg/L chronic criterion for selenium would likely jeopardize 15 ESA-listed species including species of fish, birds, amphibians, and reptiles. To avoid a final "Jeopardy Opinion" from the Services, EPA agreed to re-evaluate their CWA criteria guidance for selenium by 2002 (44). Re-evaluating the

selenium criteria guidance in the context of an ESA consultation raised new technical challenges for EPA.

EPA's normal procedure for setting Aquatic Life Criteria (45) does not directly consider toxicity data for aquatic-dependent wildlife (i.e., those species that depend on aquatic systems for food, but do not live and "breath" beneath the water's surface) and no separate Wildlife Criteria for selenium have been promulgated by EPA (13, 15, 46). Yet, the majority of the 15 ESA-listed species judged by the Services likely to be jeopardized by the current chronic criterion for selenium are aquatic-dependent wildlife (44). EPA's normal procedure is also much better suited for application to non-bioaccumulative pollutants, yet selenium is highly bioaccumulative (43, 46-47). Finally, for ESA-listed species, some of which are on the brink of extinction, both legally and biologically every individual of a population "counts" and therefore criteria guidance would need to be fully protective at an individual-effects level (43, 48).

EPA contracted with the Great Lakes Environmental Center (GLEC) to derive updated selenium criteria. To address the highly bioaccumulative nature of selenium, and concordant with expert consensus (15, 43, 47, 49), GLEC was instructed to derive the chronic criterion on a fish-tissue basis rather than on a water concentration basis. In March, 2002, EPA released the completed draft update document for selenium criteria (50). Largely, but not only, because the draft tissue-based chronic criterion was derived by GLEC employing an assumption that EC20 and LC20 levels of individual effects were acceptable, the draft chronic criterion of 7.9 μg/g, dry weight basis, was a nonstarter for ESA purposes (i.e., an LC20 level of allowable toxicity is far from fully protective). The U.S. Fish and Wildlife Service (FWS) immediately notified EPA of this and requested that EPA proceed no further with the draft criteria document (51).

The draft tissue-based criterion prematurely enters into decision-making arenas. For the past two years EPA has abided by the FWS request not to publish the draft criteria document in the Federal Register (13). However, during that period EPA also did not re-initiate the derivation of updated criteria on a basis that would be acceptable for ESA purposes and continued to make the draft criteria document available to the interested public. EPA has also created the appearance of supporting the draft document as sound science via public presentations before scientific professional societies (52-53) and via public statements (13). The draft tissue-based chronic criterion has been the subject of discourse in widely read scientific publications (12-13, 27), contributing to a developing perception within the regulated community of the draft guideline as quasi-officially sanctioned by EPA, i.e., but for a few bureaucratic formalities, the new chronic criterion for selenium. Consequently, EPA's draft criterion of 7.9 µg/g of whole-body fish tissue has prematurely made its way into environmental decision-making arenas and increasingly continues to do so. For example, West Virginia Senate Bill No. 353 was introduced January 30th, 2004, and seeks to replace West Virginia's current chronic criterion for selenium (5 µg/L) with the draft 7.9 µg/g tissue-based criterion effective September 1, 2004 (54). In Colorado, the draft tissue-based criterion has been introduced into the water standards regulatory arena (55). In California, water users within the federal Central Valley Project are citing the draft 7.9 µg/g tissue-based criterion as scientific support for seeking relaxed environmental terms and conditions on long-term water contract renewals that, once negotiated, would not be renewed again for at least 25 years (56-57). Decisions that may be irreversible for decades to come are being proposed based on the presumed scientific soundness of EPA's draft tissue-based chronic criterion for selenium.

Fundamental scientific flaws discovered in EPA's draft criterion proposal. Selenium standards and criteria recently emerged as a crucial issue among interest groups affected by the practice of mountain-top removal valley-fill coal mining (58-60). In this case, the difference between a 5 μg/L water criterion and a 7.9 μg/g tissue-based criterion is not trivial. One of us (JPS) was asked to conduct a detailed review of EPA's draft tissue-based criterion for selenium in response to questions emerging from the mountain-top mining controversy. As a result of that review and follow-up consultations with and amongst all co-authors of this paper, we discovered and confirmed several fundamental flaws that we believe are scientifically fatal for the draft criterion, not only for ESA purposes, but for any purpose. We discovered that the design implications of the controlling experiment from which EPA's draft 7.9 μg/g tissue-based criterion was derived had gone unrecognized by GLEC and EPA. We discovered that the crucial linear regression equation relating selenium concentrations in fish ovaries to concentrations on a whole-body basis was erroneously reported. We discovered that the assessments of risk to aquatic-dependent wildlife, if fish tissue were allowed to reach 7.9 μg/g selenium, were based on the 1995 draft of a wildlife toxicological benchmarks report rather than the much different 1996 final version. We discovered that the wildlife risk assessment was too narrowly focused on fish-eating birds. We discovered systematically incorrect wet-weight-to-dry-weight conversions of tissue concentrations for selenium. We discovered measures of selenium in aquatic invertebrates and fish liver tissue from a national database being erroneously plotted as data for selenium in whole-body fish tissue. In addition we discovered other less egregious errors. Most importantly, we found that all of the most egregious errors biased the final criterion recommendation in the same direction, toward dangerously overestimating the safely tolerable tissue-based number. Because this dangerously overestimated draft criterion has already taken on a quasi-official

status within scientific discourse (12-13, 52-53) and environmental decision-making arenas (54-57), we view as imperative the need for the fatal flaws we have discovered to be disseminated immediately and widely among scientists, natural resource managers, regulators, and policymakers. Therefore, we are submitting the following critical review for publication simultaneously with providing it to EPA.

Unrecognized Experimental Design of the Controlling Chronic Toxicity Study

GLEC's review of the scientific literature yielded 17 studies that were selected as the data pool from which an updated chronic criterion for selenium could be derived (50). GLEC followed EPA's standard procedures (45) as closely as possible and derived estimates of tissue-based chronic values for four genera of freshwater fish, including estimates of >11.64 μg/g for salmon and trout (*Oncorhynchus*), [<] 41.46 μg/g for fathead minnow (*Pimephales*), [<] 9.5 μg/g for bluegill sunfish (*Lepomis*), and < 17.50 μg/g for striped bass (*Morone*) (50) (where GLEC neglected to show a < sign that is, in fact, warranted, we have added it in brackets above). None of the genus chronic values could be estimated without substantial uncertainty (as indicated by the necessity of > and < signs). That outcome is a function of the available chronic toxicity data not being a very good fit for EPA's standard procedures (45).

A controlling chronic toxicity study is identified. However, GLEC noted that one of the 17 studies, Lemly's winter-stress study (20), was qualitatively distinct because in addition to a selenium treatment, the study included a simultaneous cold temperature stress similar to that faced in some degree by most natural fish populations during winter (winter stress). Because it was the only available study that incorporated the more realistic winter-stress design, and

because the study yielded an estimated chronic value lower than any of the uncertain genus chronic values noted above, GLEC quite reasonably chose to make Lemly's (20) experiment the controlling study for their criterion proposal. GLEC's draft tissue-based chronic criterion for selenium of 7.9 µg/g was adopted, unmodified, from the value Lemly reported for his selenium + winter stress treatment group, as measured at the end of the 180-day experiment (50). GLEC, following Lemly, associated that whole-body selenium concentration of 7.9 µg/g with 33.8 percent mortality of juvenile bluegill (50). GLEC did not clearly explain why there was no downward adjustment of the 7.9 µg/g concentration to bridge the gap between the attributed effects level of 30% mortality (on a control-adjusted basis) and the target effects level of 20% (EC20/LC20) that GLEC deemed appropriate for a criterion (50).

We support GLEC's decision to use the Lemly (20) winter-stress experiment as the controlling study for purposes of deriving a criterion. For more than 60 years it has been known that low winter temperatures substantively increase the toxicity of dietary selenium to birds (61-62), fish (20, 63-64), and mammals (65). Indeed, the selenium literature includes specific recommendations for considering and accounting for the effects of winter stress during hazard assessments (64).

Lemly's experimental design was more complex than GLEC recognized. Unfortunately, GLEC did not recognize the full complexity of Lemly's experimental design and its implications for estimating the magnitude of adverse effects. Lemly's study was a segmented time series experimental design that included periodic removal, without replacement, of surviving experimental fish (20). GLEC interpreted the study as if it were a much simpler experimental design, i.e., as if the selenium + winter stress treatments began with 210 fish (3 replicates of 70

fish each) which were all exposed to the treatment for 180 days, of which 71 died (71/210 = 33.8% mortality). GLEC may have been misled by the fact that Lemly reported only that same mortality quotient (20).

However, as clearly reported by Lemly, 30 of the 210 fish allocated to the selenium + winter stress treatments were removed before he initiated the experiment. The removed fish were used to establish baseline values for sublethal effects endpoints and tissue concentrations of selenium. Thirty additional surviving fish each were removed at days 60 and 120 of the experiment for intermediate measures of sublethal effects endpoints and tissue concentrations (20). Thus, unbiased direct measures of survivorship can only be derived within each distinct time segment of the experiment (i.e., days 1-60; days 61-120; days 120-180) because the number of fish entering each time segment was not the same as the number surviving the prior time segment. In other words, because 90 of the 139 fish that did not die during the experiment were exposed for less than the full 180 days of treatment (including 30 fish with zero exposure), the observed mortality count underestimated how many fish would have died had they all been exposed until they either died or survived the full 180-day treatment. A true effects estimate for the full 180-day treatment would account for the surviving fish that were removed periodically by the investigator and therefore were not available to suffer treatment-induced mortality. That can be accomplished by calculating the survival rates for each of the three time segments and then calculating the product of those three segment survival rates.

The true effects magnitude for the winter-stress selenium treatment was essentially 50% mortality. The relevant data are summarized in Table 1. For the selenium + winter stress treatment the time segment survival rates were 0.9167 (1-60 days), 0.6519 (61-120 days), and

0.8448 (121-180 days) respectively and the product of those three rates is 0.5048 (50.48% survivorship). Thus, the expected 180-day treatment mortality rate would be 49.52%. Similar calculations yield an expected 180-day control mortality rate of 4.19% (Table 1). Therefore on a control-adjusted basis, the effect level of Lemly's experiment was 47.31% mortality. Clearly, any tissue-based concentration associated with such a high level of mortality would constitute a fatally flawed criterion for protection of aquatic life and be scientifically inappropriate. Yet, because GLEC didn't recognize the complexities of Lemly's experimental design or the implications for assessing the true magnitude of toxicity, EPA has released a draft criterion that, at best (see next subsection), was essentially 50% lethal to juvenile bluegill fish.

The toxicologically controlling tissue value was probably 5.8 µg/g not 7.9 µg/g. It's likely that 7.9 µg/g is an overestimate of the tissue concentration necessary to cause the adverse effects observed in Lemly's study. Lemly (20) cautioned that the tissue concentration of 7.9 µg/g measured in fish from the selenium + winter stress treatment at day 180 was likely an artifact of severe lipid loss which reduced fish mass without reducing total selenium content of the fish (because lipids are essentially selenium-free; selenium is predominantly protein bound). Thus, the toxicologically controlling tissue concentration for risk assessment was the 5.8 µg/g reached by day 60 of exposure among fish in both the selenium + winter stress treatment and the selenium-only treatment. For fish in the selenium-only treatment, that is, in the absence of the severe lipid loss occurring after day 60 in the selenium + winter stress treatment, a whole-body selenium concentration of 5.8 µg/g was steadily maintained from day 60 to day 180. Therefore it was clearly established that 5.8 µg/g was the equilibrium tissue concentration to be expected

from consuming the 5 µg/g selenium feed used as the dietary exposure for both selenium treatments (20).

The clear implication from Lemly's discussion of his results is that a whole-body selenium concentration of 5.8 µg/g in juvenile bluegill as they enter the winter season (day 60 of the experiment) would be sufficient to cause 50% mortality and severe lipid depletion among fish still surviving by the end of winter (day 180 of the experiment). That severe lipid depletion in turn causes the selenium load in those surviving fish to become more concentrated. Accordingly, the terminal whole-body selenium concentration (7.9 µg/g) would be an artifact of toxic effects, triggered by the 5.8 µg/g of whole-body selenium the fish contained at day 60. In the absence of the 5.8 µg/g-triggered toxic effects (via lipid depletion), there would have been no increase in tissue selenium at day 180, as confirmed by the selenium only treatment. We agree with Lemly that this is the most parsimonious explanation of his experimental results and we expect that juvenile fish entering the winter season with 7.9 µg/g, as the current draft chronic criterion proposal allows, would result in even greater than 50% lethality.

Simple linear extrapolation [(7.9/5.8) x (47.3%) = 64.4%] yields an expectation of about 65% lethality. However, selenium toxicity response curves are distinctly nonlinear, and therefore linear extrapolation underestimates incremental increases in toxic effects to be expected from incremental increases in exposure (1, 4, 10, 41, 77). By comparison, for black-necked stilts (a species of shorebird) and the endpoint of selenium-induced embryo teratogenesis, the same proportional increase in exposure (1.36 times the 47.3% effects exposure concentration) causes the toxic response to increase from 47.3% to 90% (78). Consequently, we conclude that EPA's draft tissue-based chronic criterion for selenium of 7.9 µg/g would likely be associated

with the potential to cause on the order of 65-90% mortality of juvenile bluegill exposed to a winter stress challenge comparable to that simulated in the Lemly winter-stress study (20).

Correctly interpreted, EPA's controlling study indicates a tissue-based chronic criterion for selenium in the 4-6 µg/g range. Consequently, the controlling study for EPA's draft tissue-based chronic criterion, and the only study that incorporates a clearly demonstrated and environmentally widespread modifier of selenium toxicity (winter stress), is best interpreted as having demonstrated 50% lethality associated with a whole-body selenium concentration of 5.8 µg/g. The 50% lethality is not in question. Whether that effects level is judged by EPA to be associated with a tissue concentration of 5.8 or 7.9 µg/g is a matter of interpretation; however, either number would have to be substantially reduced to be an appropriately protective criterion, that is, to get the expected effects level down to the 0-10% level that is EPA's traditional goal for aquatic life water quality criteria (45, 50, 66). We believe that regardless of EPA's choice of interpretation, the appropriate criterion indicated by the Lemly winter-stress study (20) will likely need to be <5.8 µg/g on a whole-body fish tissue basis. For example, based on visual extrapolation from concentration-response curves available in the literature for whole-body fish tissue (50, 67-68), the ratio of the 50% effects whole-body concentration to the 10% effects whole-body concentration is roughly 1.75. Even 7.9 divided by 1.75 would yield a criterion estimate of 4.5 µg/g tissue selenium. Here it must also be considered that even 10% mortality may be unacceptable for ESA purposes. The public-service scientific community has identified 4-6 µg/g whole-body selenium in fish as the appropriately protective guidance for more than a decade (1, 4, 21, 39, 49).

Erroneous Presentation of a Crucial Regression Equation

The most sensitive endpoints for selenium toxicity in natural populations of fish and birds are measures of reproductive success. Therefore the preferred tissues for risk assessment are reproductive tissues such as eggs or ovaries (4, 15, 21-22, 47, 49-50), but reproductive tissues are available for sampling only seasonally and only at sites that support suitable breeding habitat. Consequently, whole-body tissue is a more practical measurement endpoint (15, 47, 49-50) making the relationship between selenium in whole-body tissues and reproductive tissues crucial for risk assessment (69). This is especially true for water bodies in moderate climates not subject to a strong winter-stress challenge. Where winter-stress is a strong challenge, the sensitivity of juvenile survivorship is comparable to more traditional reproductive endpoints (20). Clearly then, for a criterion based on a selenium concentration in whole-body tissue it is important to answer the question: "What will that whole-body chronic criterion translate to for eggs or ovaries?"

The erroneous regression equation presented in EPA's draft criterion document substantively misinforms risk assessment. GLEC developed a regression equation for translating between selenium concentrations in whole-body tissue and ovary tissue based on three sets of data (67, 70-71), although only two (67, 70) of the three sources for the data are identified in the applicable data appendix (50). A plot of the data is included in the draft criterion document and the regression equation of: [whole-body selenium] = 0.84 [ovary selenium] +0.45 is presented with the plot (Figure 4; 50). However, we observed that the plot showed the data pair (66 µg/g ovary selenium, 31 µg/g whole-body selenium) falling directly on the regression line. This would be possible only if either the regression equation had been

erroneously reported, or the data point had been plotted incorrectly. We re-calculated the regression equation using the same data listed in the data appendix and found that the correct regression equation for that data was: [whole-body selenium] = 0.45 [ovary selenium] + 1.32. For risk assessment purposes this difference is not trivial. Based on the erroneously reported regression equation, the proposed whole-body chronic criterion for selenium of 7.9 μg/g would translate to 8.9 μg/g in fish ovaries as opposed to an estimate of 14.8 μg/g from the correct regression equation. The former value would clearly be judged as safe and the safety of the later value would be a matter of interpretation. Alternative interpretations of the relevant literature have produced guidelines for reproductive toxicity thresholds ranging from 10-17 μg/g (22, 30). The public-service scientific community would consider 14.8 μg/g selenium in fish ovaries to exceed the threshold for reproductive toxicity among sensitive species.

Even the corrected regression equation is scientifically inappropriate. The corrected regression equation is valid only if the three data sets from which it was derived can be pooled together. Plotting each dataset separately we found that they yielded three clearly distinct regression relationships (Figure 1). There are straight forward reasons for the differences. The first dataset (70; Lemly 1982 in Figure 1) differed from the other two in that it is from a study that did not include a dietary exposure. Some authors suggest that the metabolic fate of selenium from water-only exposures is qualitatively different than that from exposures that include a dietary pathway (15, 43). With regard to the partitioning of selenium on a whole-body versus ovary basis that certainly appears to be true. Ovary selenium was always lower than whole-body selenium for Lemly's (70) water-only exposures. In clear contrast, ovary selenium was always higher than whole-body selenium for Coyle et al.'s study (67; Coyle et al. 1993 in Figure 1) that

included dietary exposures. GLEC had earlier reported in the draft criterion document that the scientific literature available for water-only exposures to selenium, and the associated whole-body toxicity thresholds reported in that literature, were excluded from consideration due to the lack of toxicological relevance of a water-only exposure pathway. We were therefore surprised to find water-only exposure data inappropriately pooled with data from dietary exposures for the purpose of calculating a regression equation relating whole-body selenium to ovary selenium. Clearly, the first dataset (70; Lemly 1982 in Figure 1) cannot be pooled with data from dietary exposures and must be excluded (just as all other water-only exposure data were excluded by GLEC).

Plotting the second dataset (71; Hermanutz et al. 1996 in Figure 1) required more effort. First, we did not believe it was appropriate to pool and average repeated measures of tissue selenium from within treatment groups (as done by GLEC) because doing so overestimates the strength of the regression (i.e., masks some of the variability in the raw data). Second, we used tissue-specific percent moistures reported specifically for bluegill (74% for whole-body tissue and 67% for ovary tissue; 72-75) to convert the Hermanutz et al. wet weight measures to a dry weight basis instead of the non-specific 80% "fish" percent moisture that GLEC applied to both types of bluegill tissue. The converted and plotted data revealed that although the Hermanutz et al. study included a dietary exposure pathway, it did not yield internally consistent results. Sometimes ovary selenium was higher than whole-body selenium (as would be expected for dietary exposure; 69) and sometimes it wasn't, thus the regression line falls mid-way between the internally consistent results of Lemly for water-only exposure and the opposite, but also internally consistent, results of Coyle et al. for exposure that includes a dietary pathway. We believe the mixed results follow from the Hermanutz et al. dataset representing a mix of data

from artificial streams that were being dosed with selenium on an ongoing basis and streams that were being allowed to recover (thus fish tissues were depurating) from prior dosing. Because portions of the Hermanutz et al. dataset are complicated by the differential depuration dynamics of whole-body versus ovary tissues, it also should not be pooled with the Coyle et al. dataset.

Appropriate translations of the proposed whole-body tissue-based chronic criterion to a reproductive tissue basis exceed all proposed toxicity thresholds. Of the three whole-body versus ovary datasets relied upon by GLEC, only the Coyle et al. dataset (67) represents an internally consistent equilibrium relationship between whole-body selenium and ovary selenium based on the predominant influence of dietary exposures as would be expected in nature. Based on the regression equation from the Coyle et al. dataset of: [whole body selenium] = 0.37 [ovary selenium] – 0.13, EPA's draft whole-body tissue-based chronic criterion of 7.9 μg/g would translate to 21.7 μg/g in ovary tissue. That estimate exceeds the entire range (10-17 μg/g) of alternative interpretations of the reproductive toxicity threshold for sensitive species of fish. For additional comparison, the most recent reproductive toxicity threshold rigorously documented in the published literature (for rainbow trout, based on field data) is 15.4 μg/g in eggs (41) [converted from 6 μg/g wet weight using the average percent moisture of 61.1% for rainbow trout and brown trout egg samples in the National Irrigation Water Quality Program's biota database (4, 76)]. Moreover, GLEC's data appendix includes a data pair from the Coyle et al. study (67) in which the whole-body selenium concentration (7.2 μg/g) in bluegill fish was very close to EPA's proposed draft tissue-based chronic criterion (7.9 μg/g). The ratio of ovary selenium to whole-body selenium for that data pair was 3.47 (50), a ratio very comparable to the factor of 3.3 recommended for generic hazard assessments (69). A ratio of 3.47 x 7.9 μg/g

translates to an ovary concentration of 27.4 µg/g. Employing the most scientifically appropriate translation factors, we estimate that a whole-body tissue-based chronic criterion for selenium of 7.9 µg/g would allow fish reproductive tissues to attain selenium concentrations (21.7-27.4 µg/g) exceeding even the most permissive toxicity threshold proposed to date (17 µg/g) by approximate 30-60% and to exceed the more cautious threshold (10 µg/g) recommended by the public-service scientific community by 117-174%. We believe that this outcome rises to the level of a second scientifically fatal flaw in EPA's draft chronic criterion proposal.

Inappropriate Basis for the Wildlife Risk Assessment

Although GLEC stated that their proposed draft chronic criterion was not developed with the intent of protecting wildlife, their draft criteria document contained a brief wildlife risk assessment. GLEC concluded from their risk assessment that the draft tissue-based criterion of 7.9 µg/g in fish would not cause unacceptable toxic effects for fish-eating birds (50). Aquatic life criteria are considered by EPA to be separate and distinct from wildlife criteria (43). Nonetheless, in the absence of promulgated wildlife criteria (as is the case for selenium), if the aquatic life criteria do not protect wildlife the purposes of the CWA are not being met (79). More critically, for waters of the United States supporting ESA-listed aquatic-dependent wildlife, the criteria would not be approvable for incorporation into state or tribal water quality standards (79). Thus, it would constitute more than just ecological folly to proceed with promulgation of an aquatic life criterion that demonstrably fails to protect aquatic-dependent wildlife.

GLEC's risk assessment was based on out of date information. The wildlife risk assessment presented in EPA's draft criteria document for selenium was based on information obtained from the 1995 revision of a U.S. Department of Energy report, *Toxicological Benchmarks for Wildlife* (80), and neglected the 1996 final revision of the same report (23). We refer to these two reports as Benchmarks 95 and Benchmarks 96. All of the information relied on by GLEC from Benchmarks 95 was updated in Benchmarks 96 and the updated information substantively alters the risk assessment outcomes and the conclusions that can be drawn from those outcomes. Here we focus on the risk assessment information in the Benchmarks reports that is based on toxicity data for selenomethionine because that is the form of selenium used in laboratory toxicity tests that is most relevant to avian dietary selenium exposures in nature (81).

Employing bioenergetic equations and allometric scaling between laboratory test species and risk assessment species the Benchmarks reports presented estimates for dietary NOAEL's and LOAEL's on a wet weight basis. GLEC focused on the Benchmarks 95 results for three fish-eating bird species. GLEC first converted the dietary NOAEL's and LOAEL's to a dry weight basis assuming 80% moisture for a fish diet. Then GLEC calculated the geometric mean of the NOAEL and LOAEL for each species which they equated to a maximum acceptable dietary toxicant concentration (MATC) for each species. Finally, the MATC's were compared to the draft fish tissue-based chronic criterion for selenium of 7.9 μg/g (50).

The three dietary MATC's reported by GLEC ranged from 10.61 to 12.20 μg/g (Table 2, first column). Because all of those estimates of the maximum acceptable dietary exposures to selenium exceeded 7.9 μg/g, GLEC concluded that the draft tissue based chronic criterion would protect wildlife (50). Using the same methods GLEC used, but employing the revised and more up to date information from Benchmarks 96 for the original three assessment species and an

additional species of aquatic-dependent bird included in Benchmarks 96, but not included in Benchmarks 95, we calculated a range for dietary MATC's of 3.73 to 20.31 μg/g (Table 2, second column). Two of our four estimated MATC's are lower than 7.9 μg/g. Finally, we calculated MATC's from Benchmarks 96 using a more realistic estimate of 75% moisture for a fish diet. A moisture content for whole-body fish tissue of 75% is the value commonly cited in selenium literature (22, 27, 41) and for 57 species of freshwater fish in the National Irrigation Water Quality Program biota database the median percent moisture was 74.5% [only 4 species averaged as high as 80% moisture (4, 76)]. The difference between using 75% moisture or 80% moisture is the difference between multiplying wet weight values by a factor of 4 or a factor of 5 to convert them to dry weight values. Thus, GLEC's use of 80% moisture introduced a systematic 25% bias in the direction of overestimating MATC's. Our final set of MATC's were 4.46 μg/g for belted kingfisher, 12.88 for great blue heron, 16.25 for osprey, and 3.34 for American woodcock (Table 2, third column). Our estimated MATC's for the American woodcock were calculated assuming a diet comprised predominantly of earthworms and therefore were based on the typical percent moisture of earthworms, not the percent moisture of fish (Table 2). Based on these four assessment species, the draft tissue-based chronic criterion for selenium of 7.9 μg/g would leave a substantive proportion of aquatic-dependent wildlife species unprotected; perhaps on the order of half the species.

The narrow focus on fish-eating birds as the assessment species neglects the more rigorous basis for wildlife risk assessment offered by other species. One of the weaknesses of relying on the Benchmarks reports for wildlife risk assessment is that there are numerous assumptions and uncertainties involved. The realism of the estimated MATC's is very difficult

to evaluate. Once it is realized that proposing to allow fish tissue to reach 7.9 µg/g selenium has implications for the rest of the aquatic food chain, wildlife risk assessment doesn't have to be confined to assessments based on fish-eating birds. That allows the risk assessment to move away from modeled (virtual) outcomes and toward empirical (real) outcomes documented for such species as the mallard duck. Additionally, fish-eating species of birds have not been found to be as sensitive to selenium as various species of ducks and shorebirds whose breeding-season diet is comprised primarily aquatic invertebrates (82-83).

It has been rigorously estimated for the mallard duck, based on multiple experimental feeding studies, that the dietary EC10 for selenium-induced reproductive effects is 4.87 µg/g with a 95% confidence interval of 3.56-5.74 µg/g (77). For the sake of providing the effects measure that GLEC would have used, the estimated EC20 is 5.86 µg/g (95% C.I. = 4.68-6.64 µg/g), but as previously noted a 20% effects level would not produce criteria estimates that meet ESA purposes. The estimated EC01, a more ESA-compatible reference point, is 2.82 µg/g (95% C.I. = 1.56-3.78 µg/g). To put these rigorous effects data for mallards to use, an estimate of how much selenium aquatic invertebrates would contain in environments sufficiently polluted to produce fish with 7.9 µg/g whole-body selenium is required?

The most rigorous experimental study of the relationship between aquatic invertebrate selenium and fish whole-body tissue selenium, which utilized radio-labeled selenium, concluded that the invertebrate-to-fish concentration factor was 0.5 across a range of foodborne (invertebrate) selenium concentrations (84). Other experimental studies have produced similar results (85-89). At a concentration factor of 0.5 the invertebrate food chain would have to contain about 15.8 µg/g selenium (i.e., 7.9/0.5) to produce fish with 7.9 µg/g. That would be equivalent to the dietary EC95 for reproductive toxicity to mallards (77). In other words,

allowing fish tissue to reach 7.9 μg/g would allow a level of contamination in the other parts of the aquatic ecosystem sufficient to cause nearly total reproductive failure among mallard ducks. As is the case for all lab studies, the realism of these lab-to-field extrapolations is fraught with uncertainty (10, 84, 90).

As a check on the realism of lab-generated invertebrate-to-fish concentration factors, comparison to field data is desirable. For this purpose we queried the biota database of the National Irrigation Water Quality Program (4, 76) and summarized the spatially and temporally matched samples of fish and aquatic invertebrates from sampling sites where whole-body fish tissue averaged between 5 and 10 μg/g selenium (a concentration range focused on the data that falls near the draft tissue-based criterion of 7.9 μg/g). The implied invertebrate-to-fish concentration factors from this dataset ranged from 0.67 to 1.36 (Table 3). These results suggest that the selenium content of aquatic invertebrates in ecosystems sufficiently contaminated to produce fish with 7.9 μg/g would fall in the range of 5.8-11.8 μg/g. Such a range of dietary exposure for mallards would correspond with an EC20 to EC85 range of toxic effects based on reproductive toxicity (77). The results of our database query also suggested a central tendency for the implied concentration factors of about 1.1 (Table 3). Thus, for wildlife risk assessment purposes, 7.9 μg/g in whole-body fish tissue might most reliably be considered to translate to about 7.2 μg/g in aquatic invertebrates. This estimate exceeds even the upper 95% statistical confidence boundary (6.64 μg/g) of the dietary EC20 for mallards and equals about the EC40 (77). In summary, allowing fish whole-body tissue to contain as much as 7.9 μg/g selenium would allow levels of aquatic food chain contamination highly likely (>95% statistical confidence) to exceed the dietary EC20 for reproductive toxicity in mallards, with a best-estimate likelihood of an EC40 level of adverse effects and the outside possibility of EC85-95

levels of adverse effects. We conclude that this clear lack of protection for aquatic-dependent wildlife provided by EPA's draft chronic criterion once again rises to the level of a scientifically fatal flaw.

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TABLE 1. EXPERIMENTAL RESULTS FROM LEMLY WINTER-STRESS STUDY (20).

	Cold-Control	Cold-Selenium
Fish Allocated to Treatment	70	210
Fish Removed for Baseline Samples Before Treatment	10	30
Fish Removed as Intermediate Samples:		
Day 60	10	30
Day 120	10	30
Day 180	10	30
Raw Number of Fish Deaths:		
Days 1-180	2	71
Fish Treated:		
Days 1-60	60	180
Days 61-120	49	135
Days 121-180	39	58
Fish Surviving:		
Days 1-60	59	165
Days 61-120	49	88
Days 121-180	38	49
Segment Survival Rates:		
Days 1-60	0.9833	0.9167
Days 61-120	1.0000	0.6519
Days 121-180	0.9744	0.8448
Days 1-180	**0.9581**	**0.5048**
Full Treatment Mortality Rates	**4.19%**	**49.52%**
Full Treatment Control-Adjusted Mortality Rates	**N/A**	**47.31%**

TABLE 2. COMPARISON OF WILDLIFE RISK ASSESSMENT OUTCOMES BASED ON DIFFERENT SOURCES AND METHODS
(Maximum Acceptable Toxicant Concentrations, MATC's, based on toxicity data for dietary exposure to selenomethionine)

Wildlife Species	MATC 1995 Benchmarks 80% Moisture (fish)	MATC 1996 Benchmarks 80% Moisture (fish)	MATC 1996 Benchmarks 75% Moisture (fish)
belted kingfisher	10.61 μg/g, dw	5.58 μg/g, dw	4.46 μg/g, dw
great blue heron	12.02	16.09	12.88
osprey	12.2	20.31	16.25
American woodcock	No Data	3.73	3.34

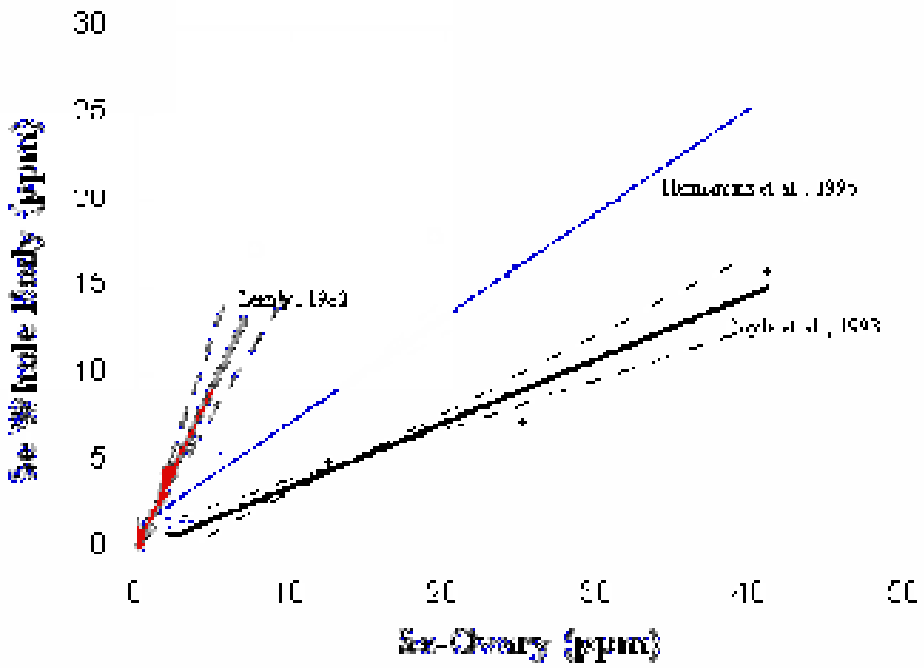
Note: MATC for American Woodcock in the last column is based on 77.7% moisture in worms. The estimate of percent moisture in earthworms is based on United States Fish and Wildlife Service file data; n = 83

TABLE 3. MATCHED SAMPLES OF FISH AND AQUATIC INVERTEBRATES FROM SAMPLING SITES WHERE THE FISH SAMPLES AVERAGED 5-10 μg/g SELENIUM, DRY WEIGHT

Location	Invertebrate Selenium	Fish Selenium	Implied Concentration Factor
Colorado	4.8 μg/g	5.3 μg/g	1.10
Utah	4.4	6.0	1.36
Utah	4.4	5.2	1.18
Utah	8.2	10	1.22
Utah	8.4	9.4	1.12
Utah	7.6	5.7	0.75
Utah	6.9	6.7	0.97
Montana	4.8	6.1	1.32
Montana	9.2	5.3	0.67
Median Concentration Factor			1.12
Average Concentration Factor			1.08

Source: National Irrigation Water Quality Program biota database (4, 76)

Figure 1. Regression lines for the three whole-body versus ovary datasets in Appendix G of EPA's Draft Criteria Document for Selenium (50). All three lines are statistically significantly different from each other ($p<0.05$). Lemly 1982, Hermanutz et al. 1996, and Coyle et al. 1993 are references 70, 71, and 67 respectively. The regression equation for Lemly 1982 is: $Y = 2.02X - 0.0325$; $R^2 = 0.970$. The regression equation for Hermanutz et al. 1996 is: $Y = 0.604X + 1.24$; $R^2 = 0.815$. The regression equation for Coyle et al. 1993 is: $Y = 0.369X - 0.126$; $R^2 = 0.970$. The Hermanutz data pairs were plotted individually (instead of pooling and averaging replicates as was done by GLEC) and were converted from wet weight to dry weight values using tissue specific percent moisture values for bluegill fish (74% for whole-body tissue; 67% for ovary tissue). (ppm = µg/g, dry weight)



Effects of Dietary Selenium and Methylmercury on Green and White Sturgeon Bioenergetics in Response to Changed Environmental Conditions.

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SF Bay-Delta is a multiply-stressed ecosystem

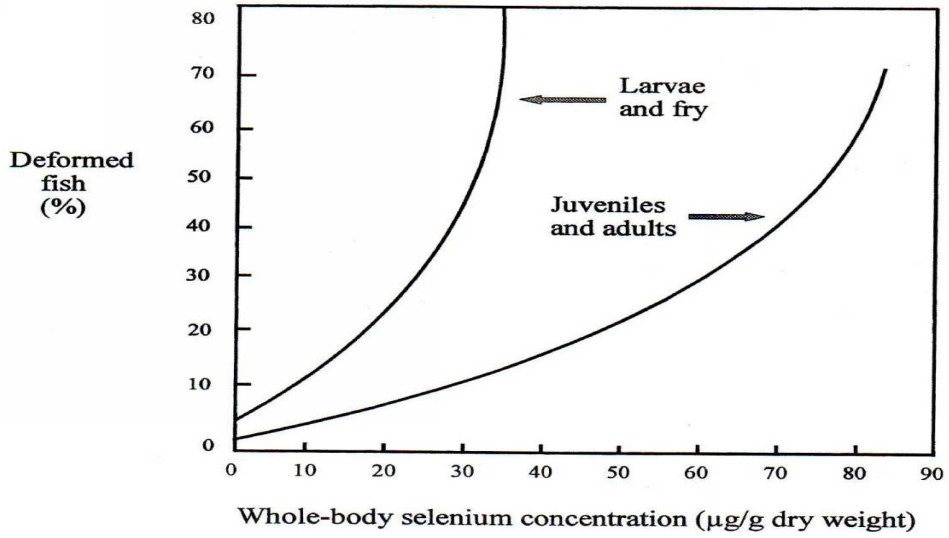
- Water diversions
- Salinity fluctuations
- Pollutants e.g., agricultural, industrial, storm-water runoff. Selenium (SeMet) and Mercury (MeHg) are toxicants of concern
- Introduced species, e.g., Asian clam
- Several species are currently imperiled e.g., POD & salmon
- Green and white sturgeon numbers are in decline
- Green sturgeon listed as threatened in 2006

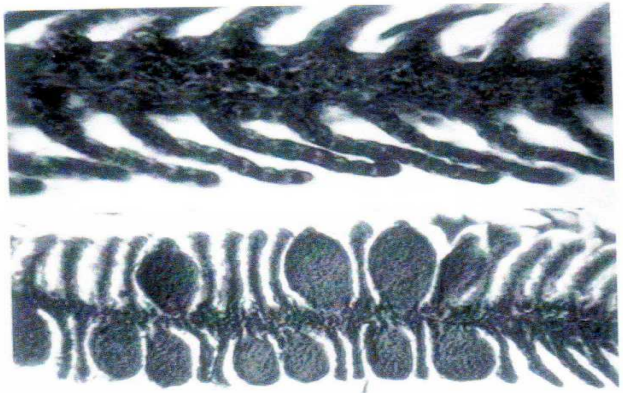
Problems

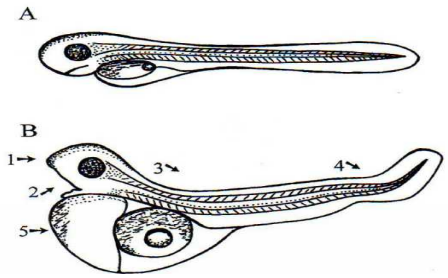
- Little is known of the effects of Hg on wildlife
- Aquatic food web recognized as the most efficient process of bioaccumulation of Hg
- Studies have shown reduced capacity for:
  - Reproduction when exposed as juveniles
  - Growth
  - Ability to avoid predators
  - Shoaling
  - Swimming performance

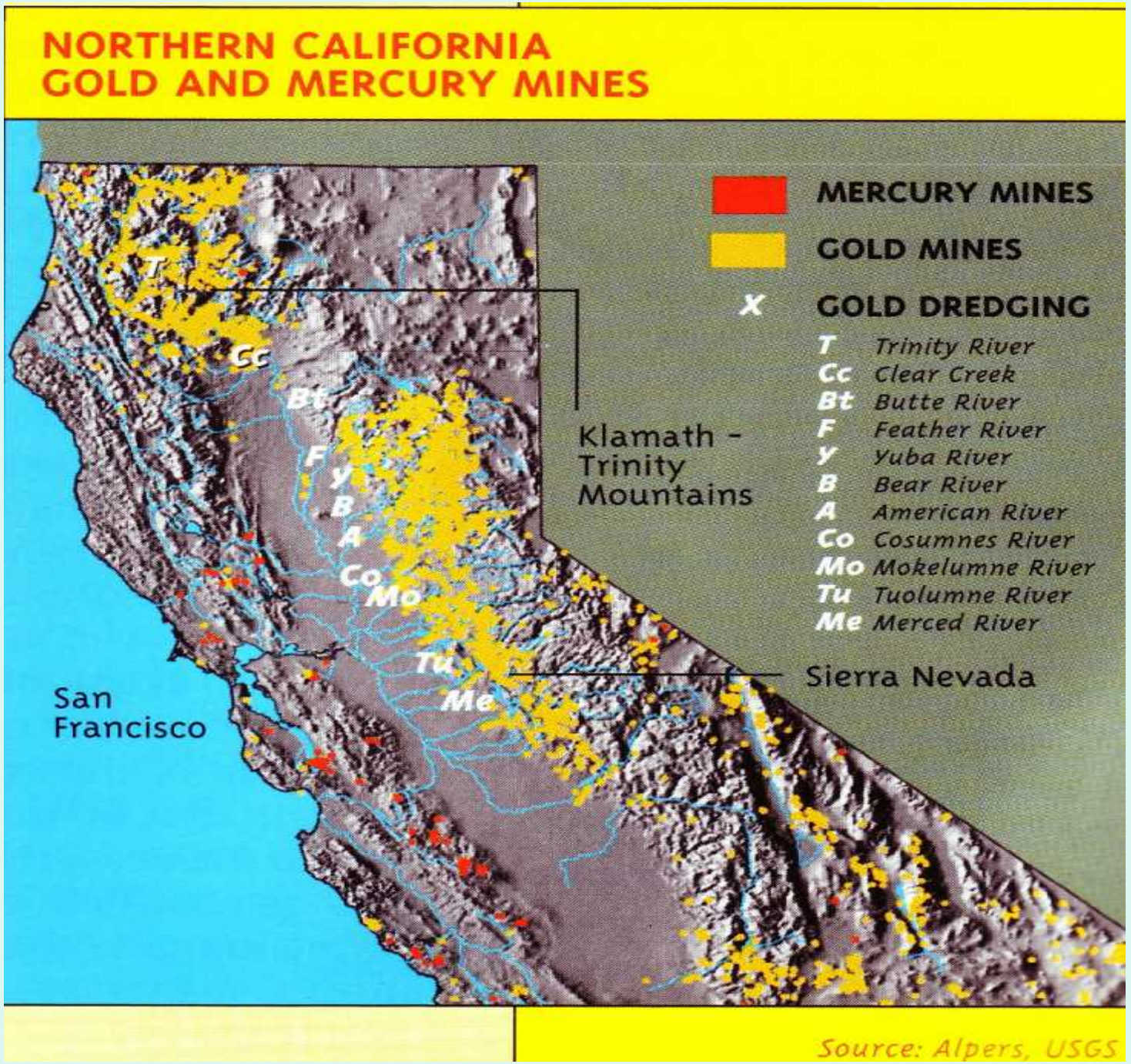
Problems cont.:

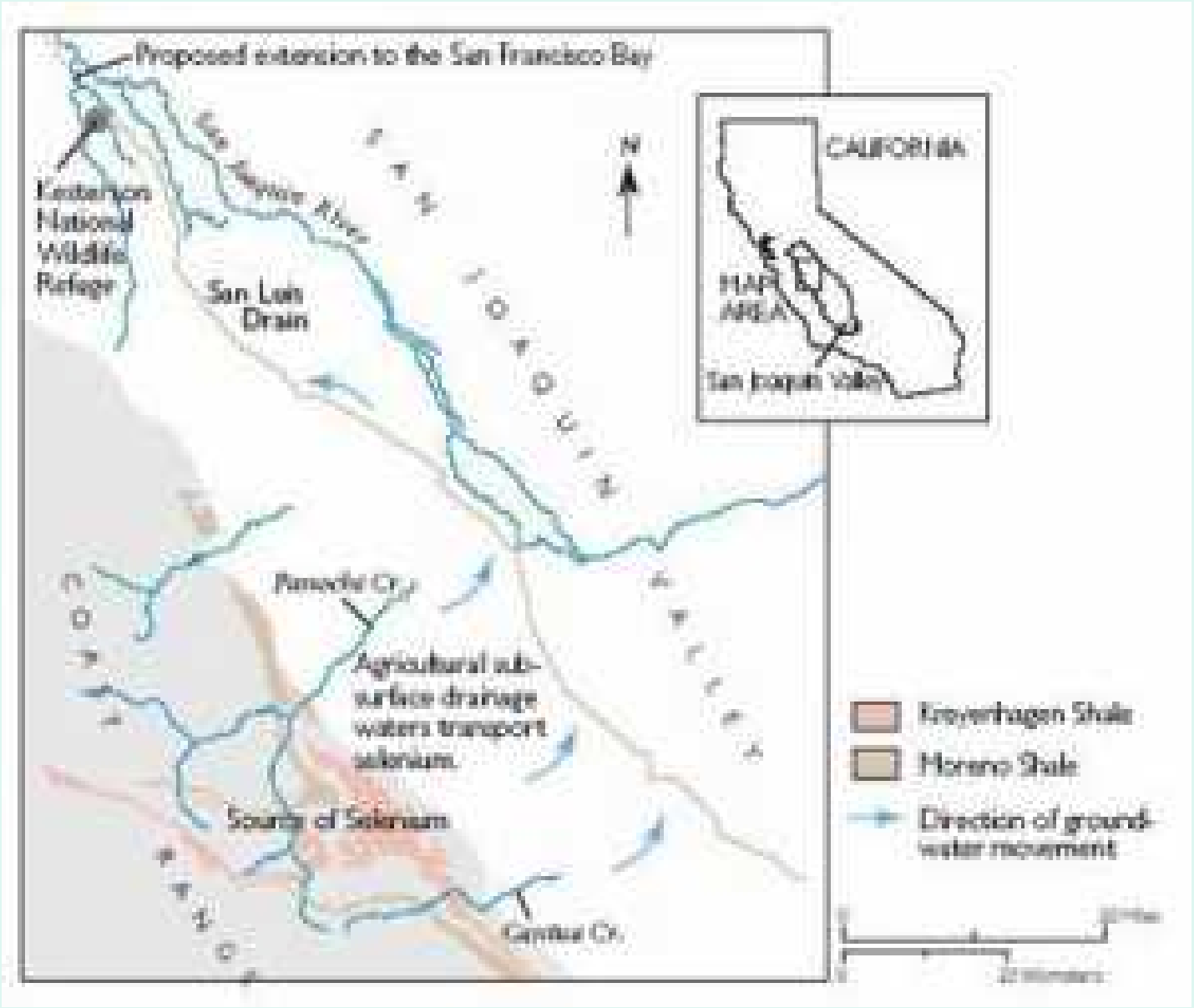
- Selenium: nutritional versus toxicity
- Effects on wildlife well documented
- Studies have shown that Se:
  - Teratogenic in fish and avian species e.g., Belews Lake, NC and Kesterson, CA.
  - Decreased reproduction
  - Concentrations in North SF Bay-Delta are a concern
  - Multiple sources of input e.g., agriculture and refining processes


Deformed fish (%)
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
Larvae and fry
Juveniles and adults
Whole-body selenium concentration (µg/g dry weight)




A
B
1
2
3
4
5

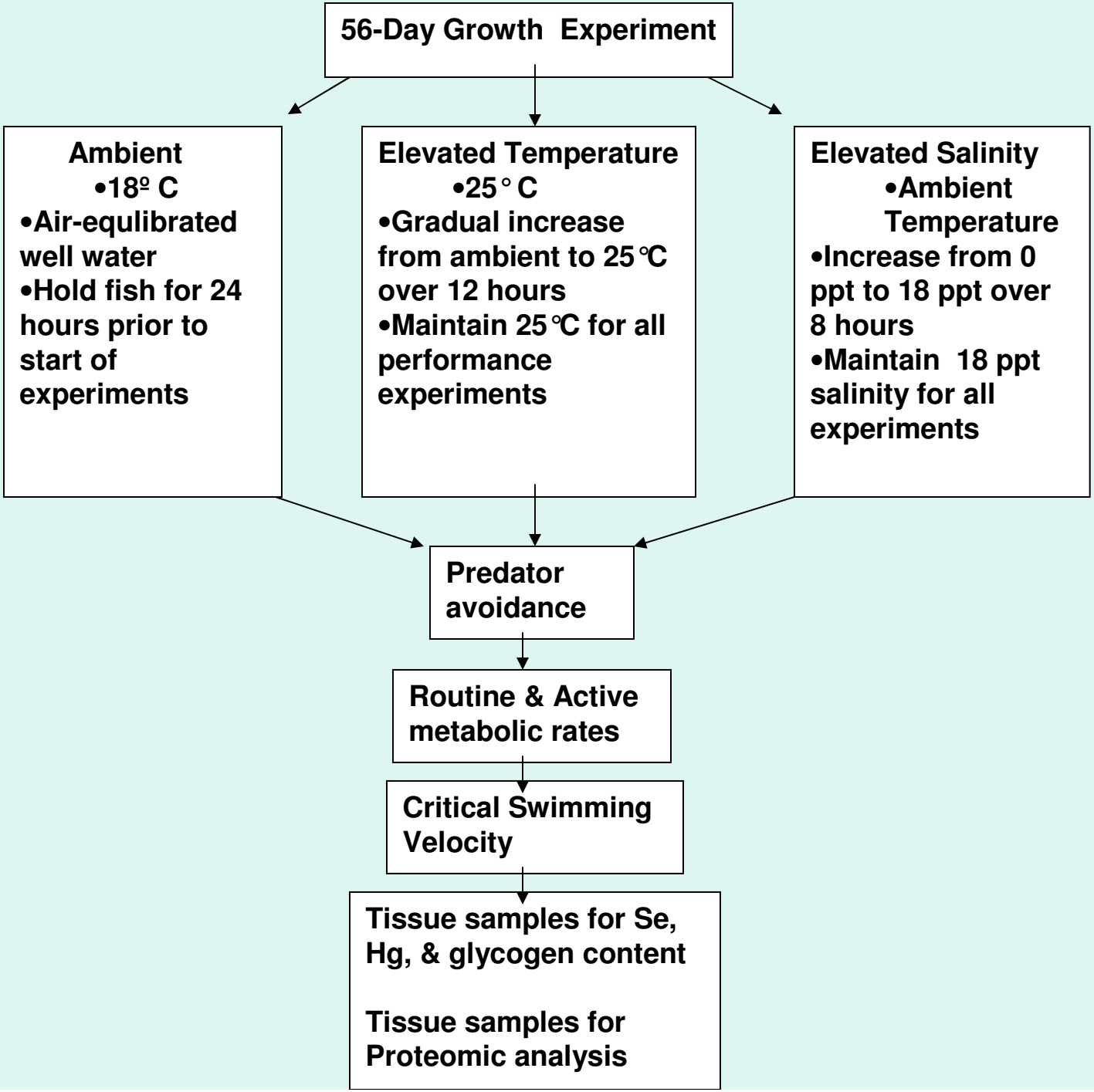

NORTHERN CALIFORNIA
GOLD AND MERCURY MINES
MERCURY MINES
GOLD MINES
X GOLD DREDGING
T Trinity River
Cc Clear Creek
Bt Butte River
F Feather River
y Yuba River
B Bear River
A American River
Co Cosumnes River
Mo Mokelumne River
Tu Tuolumne River
Me Merced River
Klamath - Trinity Mountains
Sierra Nevada
San Francisco
Source: Alpers, USGS



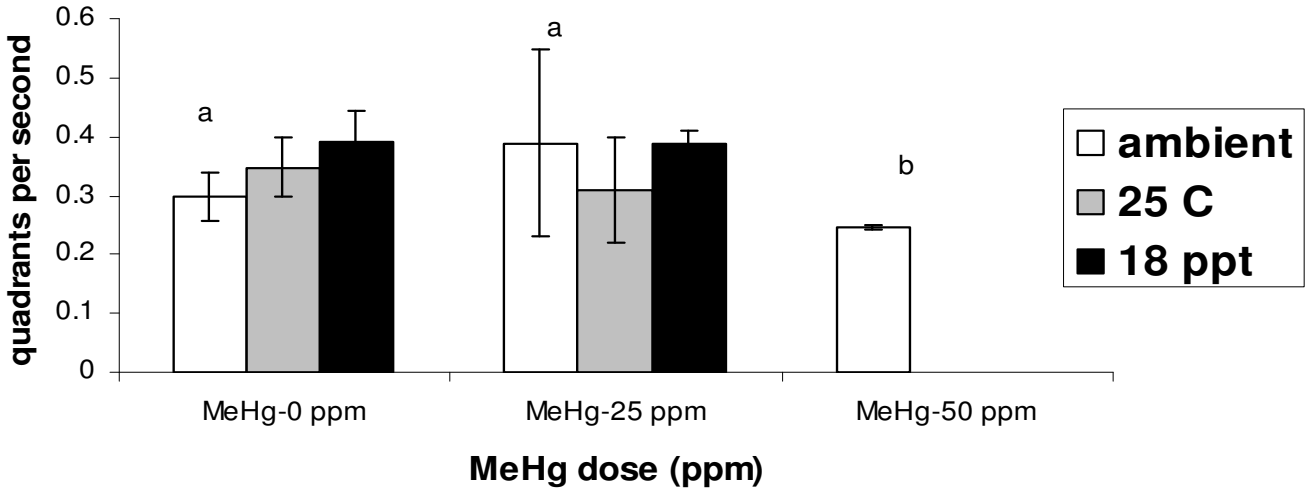
menlocampus.wr.usgs.gov/.../agriculture.html

Objectives

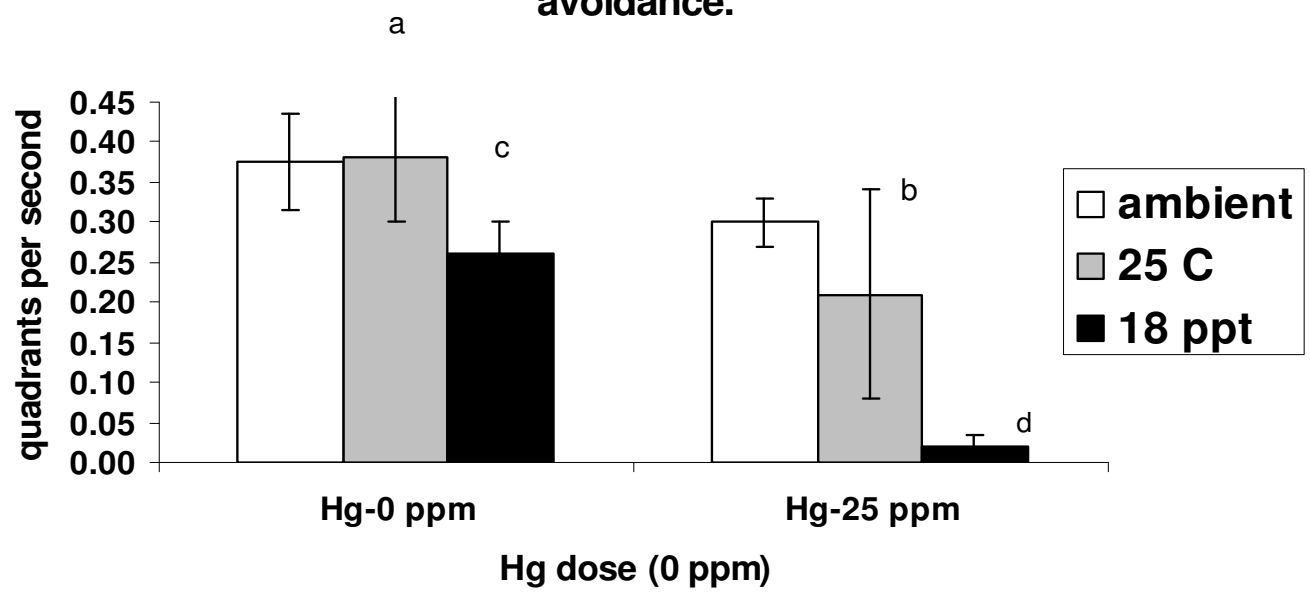
- Determine effects of SeMet and MeHg on sturgeon bioenergetics
- Determine the effects of environmental stressors, temperature and salinity, on previously exposed (SeMet & MeHg) individuals' bioenergetics
- Determine the feasibility of using non-listed, and domesticated white sturgeon as a surrogate for green sturgeon in toxicity testing

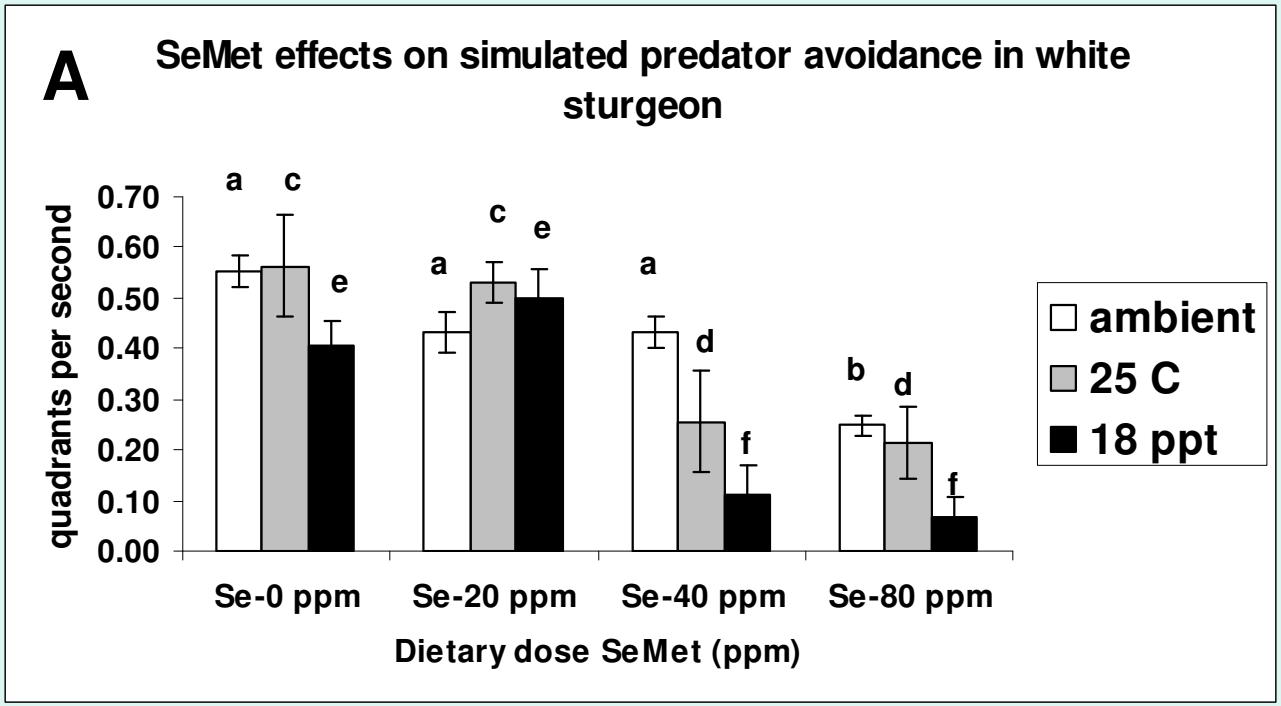

56-Day Growth Experiment
Ambient
•18º C
•Air-equlibrated well water
•Hold fish for 24 hours prior to start of experiments
Elevated Temperature
•25° C
•Gradual increase from ambient to 25°C over 12 hours
•Maintain 25°C for all performance experiments
Elevated Salinity
•Ambient Temperature
•Increase from 0 ppt to 18 ppt over 8 hours
•Maintain 18 ppt salinity for all experiments
Predator avoidance
Routine & Active metabolic rates
Critical Swimming Velocity
Tissue samples for Se, Hg, & glycogen content
Tissue samples for Proteomic analysis

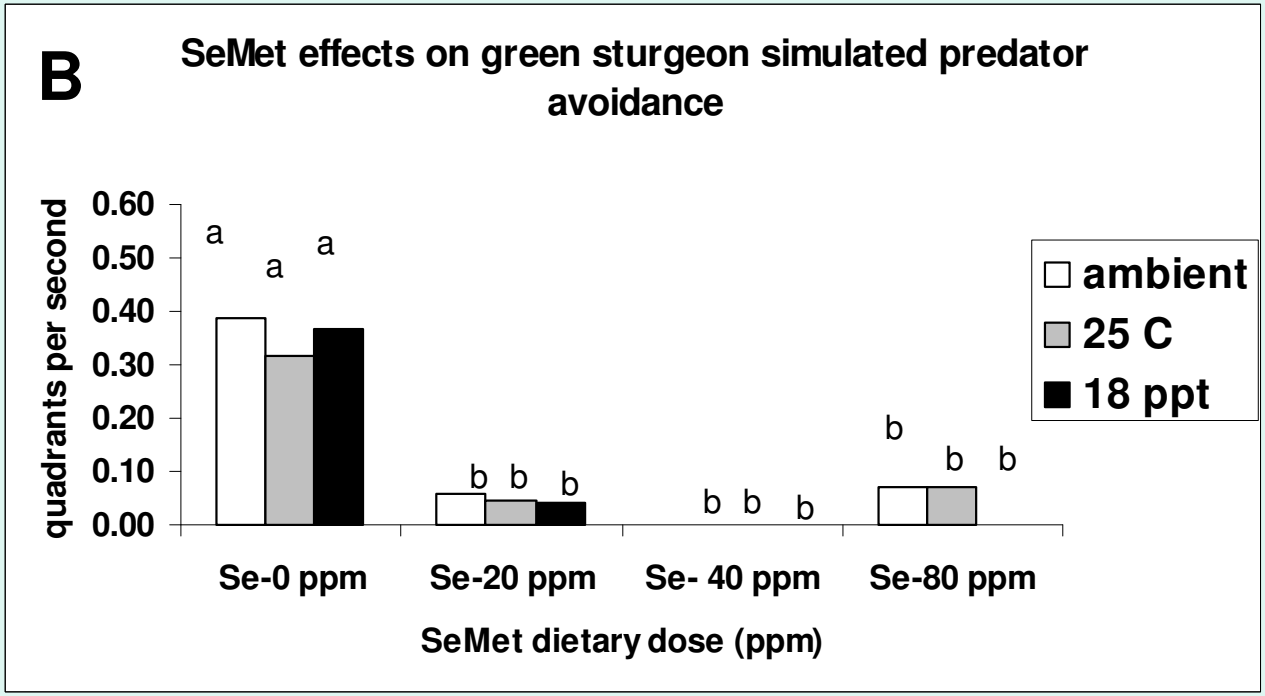
A

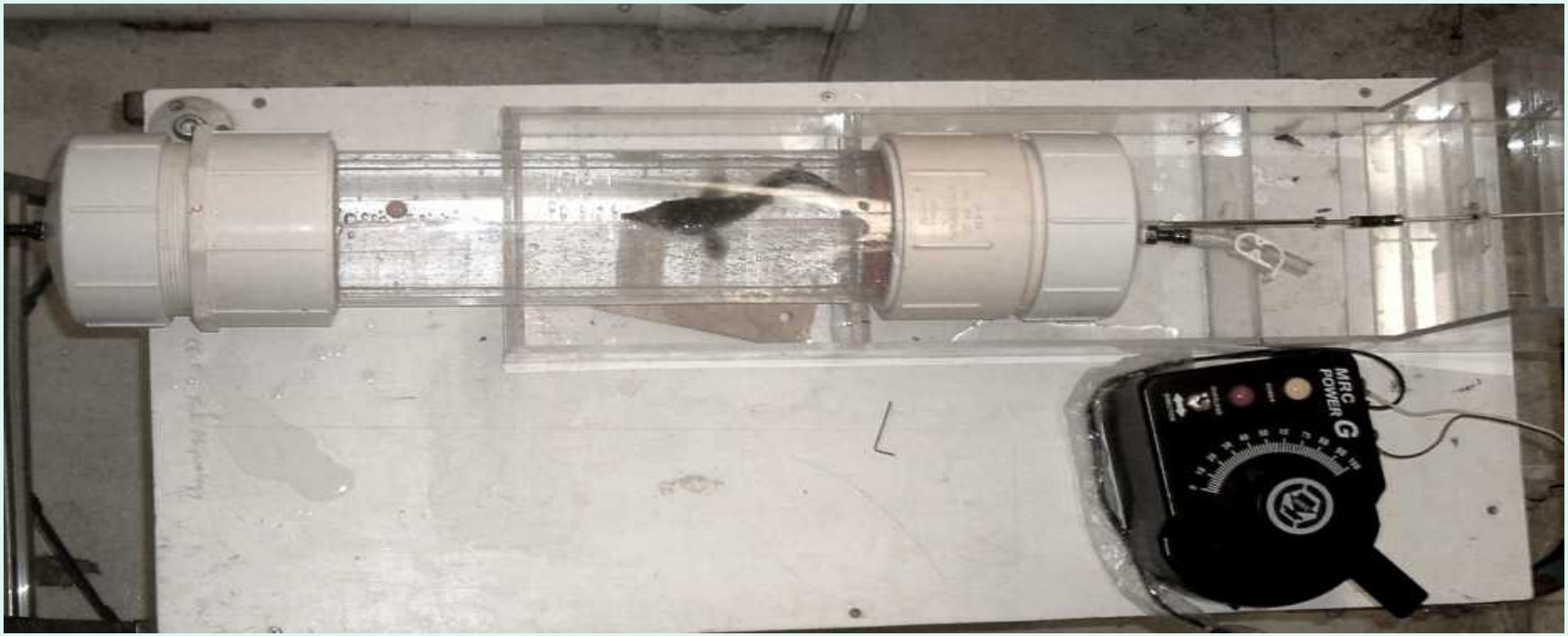

Dietary MeHg effects on white sturgeon predator avoidance
quadrants per second
0.6
0.5
0.4
0.3
0.2
0.1
0
a
a
b
MeHg-0 ppm
MeHg-25 ppm
MeHg-50 ppm
MeHg dose (ppm)
ambient
25 C
18 ppt

B

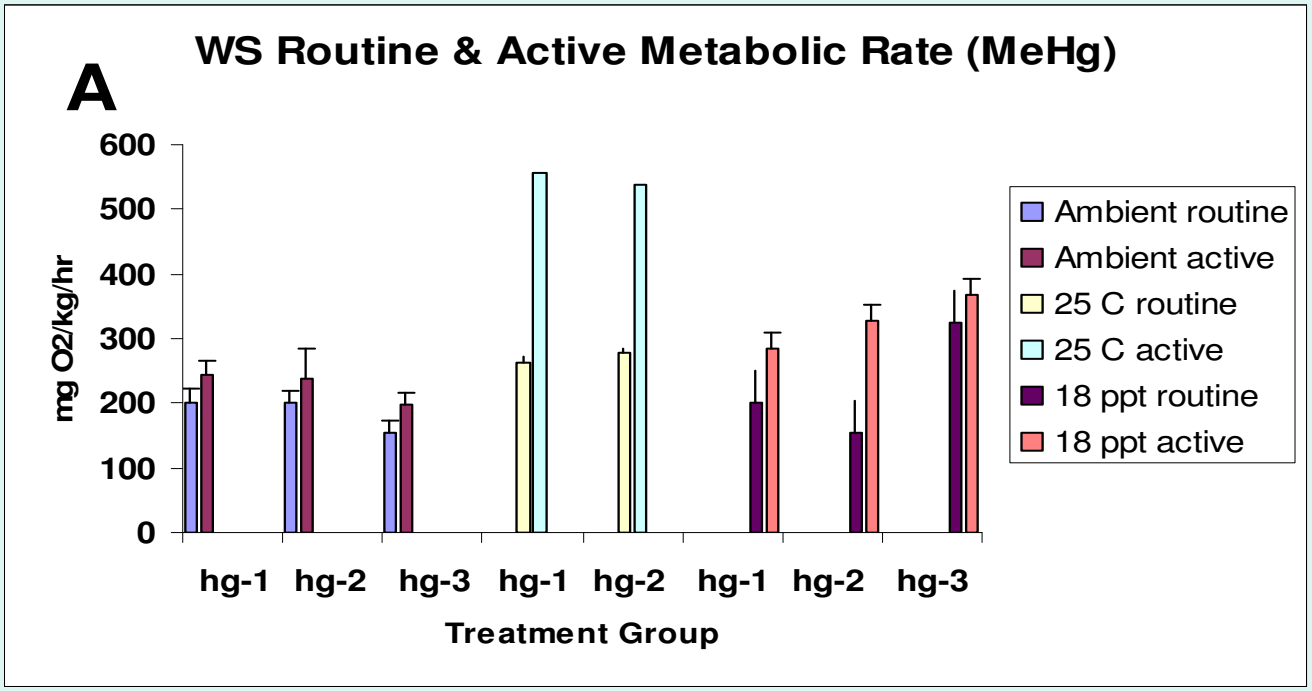

Dietary MeHg effects on green sturgeon predator avoidance.
quadrants per second
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
c
b
d
Hg-0 ppm
Hg-25 ppm
Hg dose (0 ppm)
ambient
25 C
18 ppt

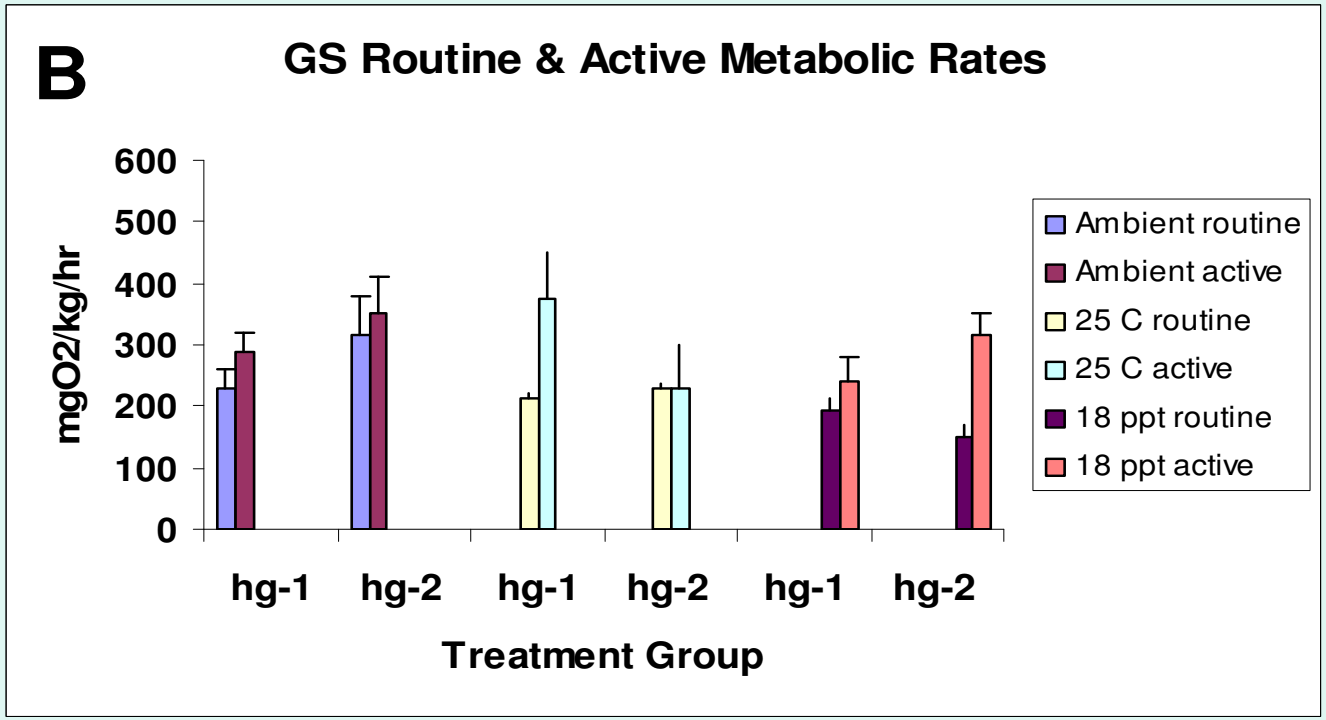

A
SeMet effects on simulated predator avoidance in white sturgeon
quadrants per second
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
a c e
a c e
a d f
b d f
ambient
25 C
18 ppt
Se-0 ppm
Se-20 ppm
Se-40 ppm
Se-80 ppm
Dietary dose SeMet (ppm)

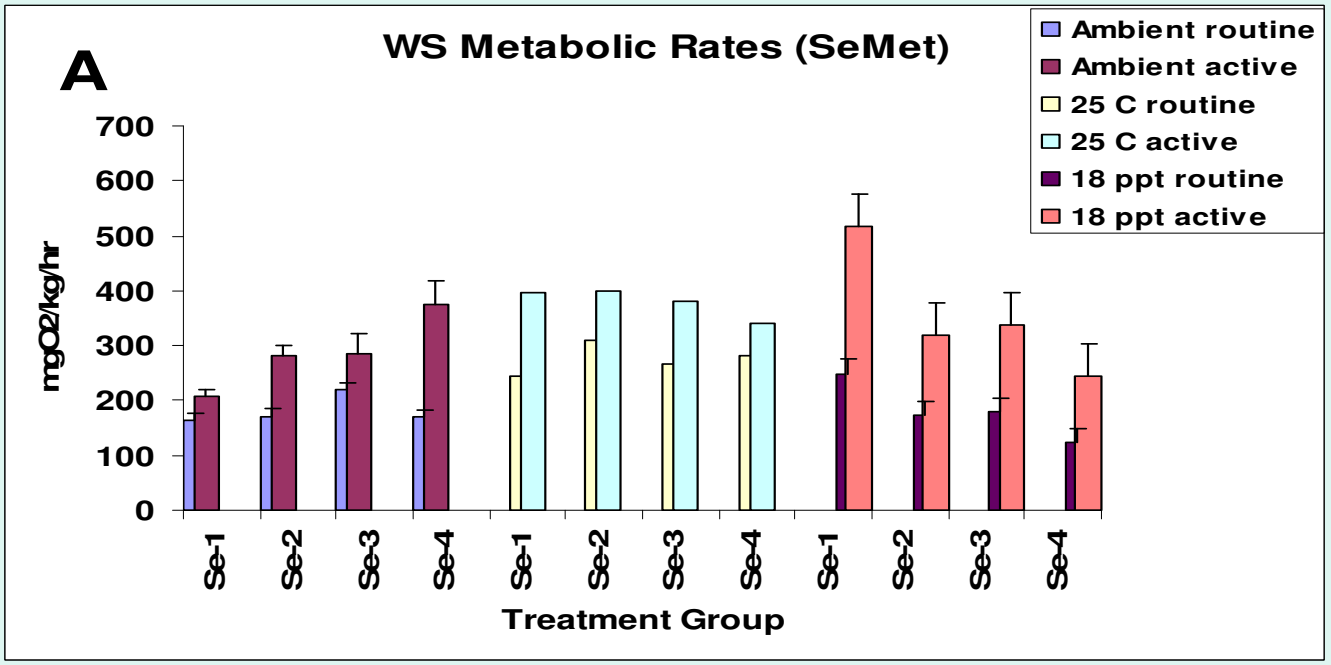

B
SeMet effects on green sturgeon simulated predator avoidance
quadrants per second
0.60
0.50
0.40
0.30
0.20
0.10
0.00
a a a
b b b
b b b
b b b
ambient
25 C
18 ppt
Se-0 ppm
Se-20 ppm
Se- 40 ppm
Se-80 ppm
SeMet dietary dose (ppm)

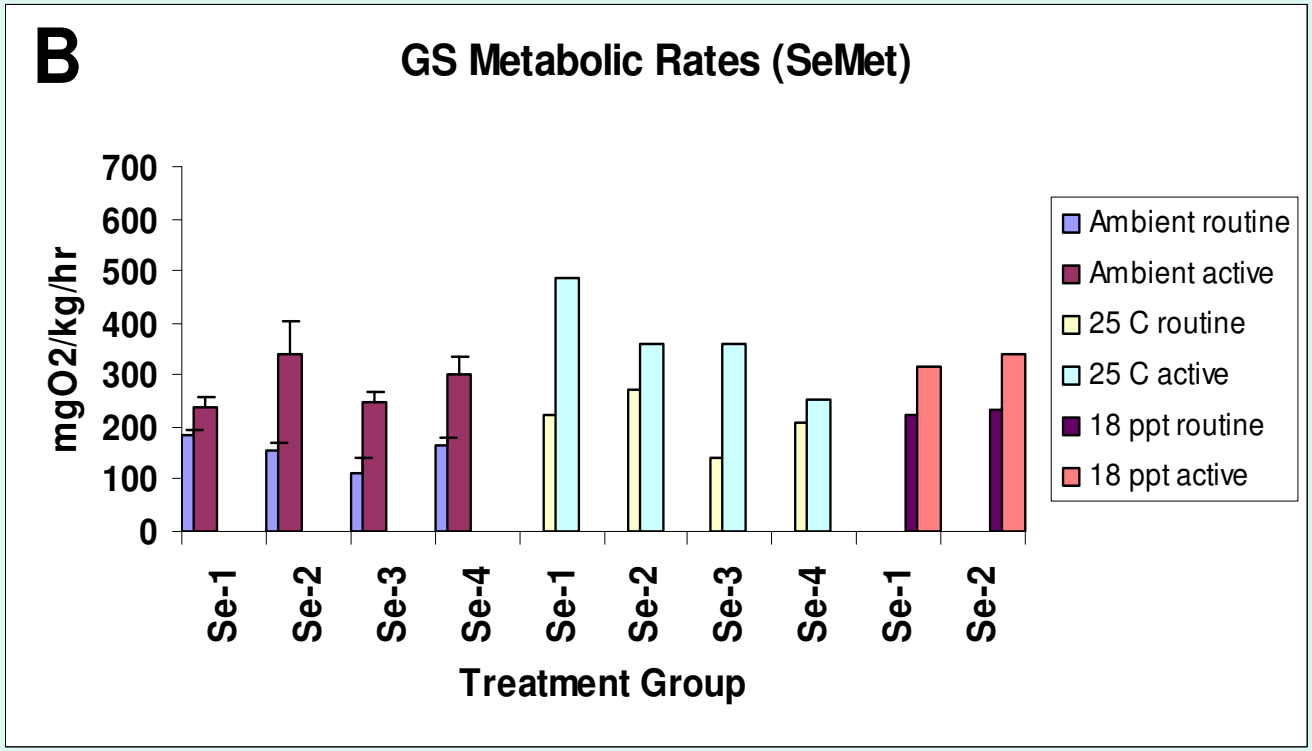


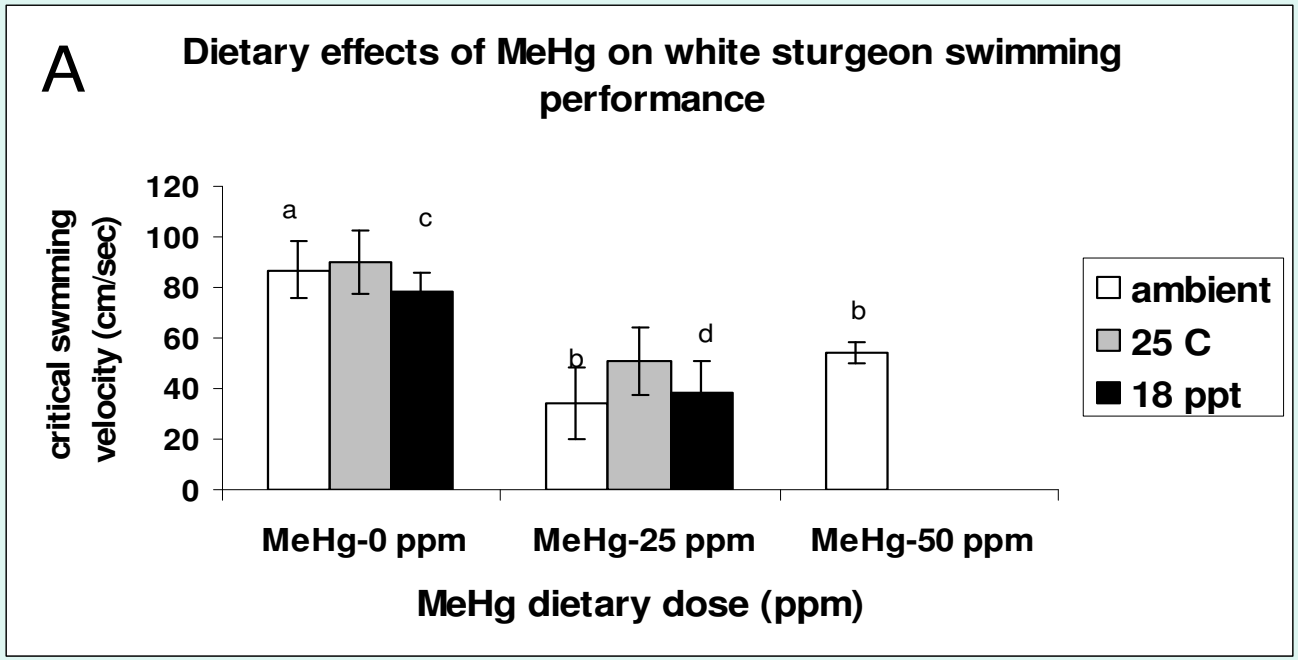
Routine and ‘active’ metabolic rates determined using Blazka-type respirometers after the growth expt.

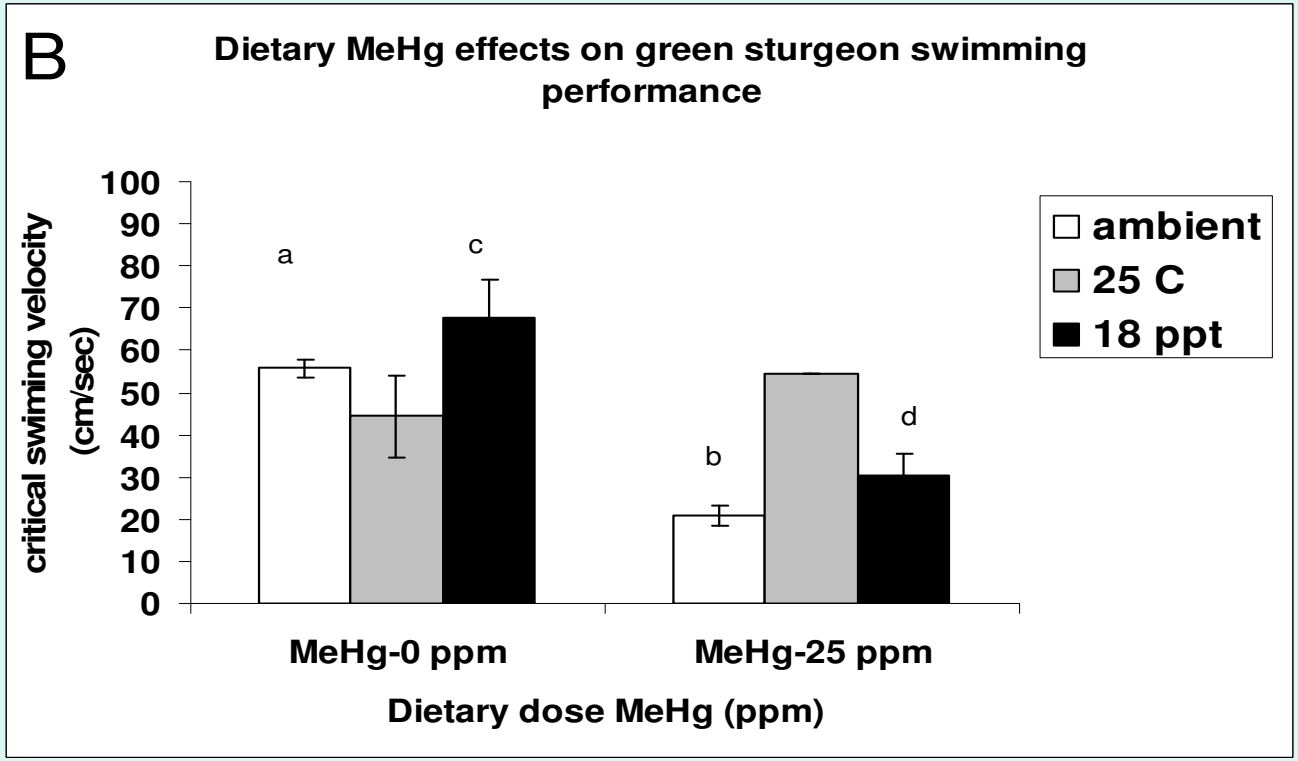

A
WS Routine & Active Metabolic Rate (MeHg)
mg O2/kg/hr
600
500
400
300
200
100
0
hg-1
hg-2
hg-3
hg-1
hg-2
hg-1
hg-2
hg-3
Treatment Group
Ambient routine
Ambient active
25 C routine
25 C active
18 ppt routine
18 ppt active

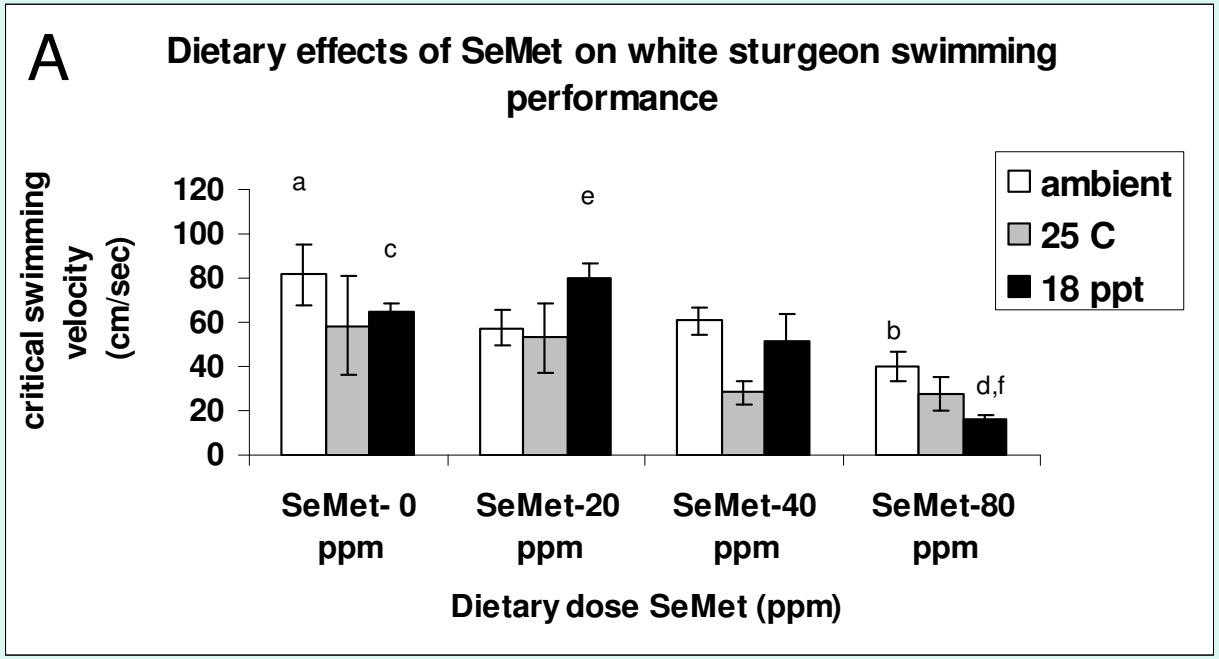

B
GS Routine & Active Metabolic Rates
mgO2/kg/hr
600
500
400
300
200
100
0
hg-1
hg-2
hg-1
hg-2
hg-1
hg-2
Treatment Group
Ambient routine
Ambient active
25 C routine
25 C active
18 ppt routine
18 ppt active

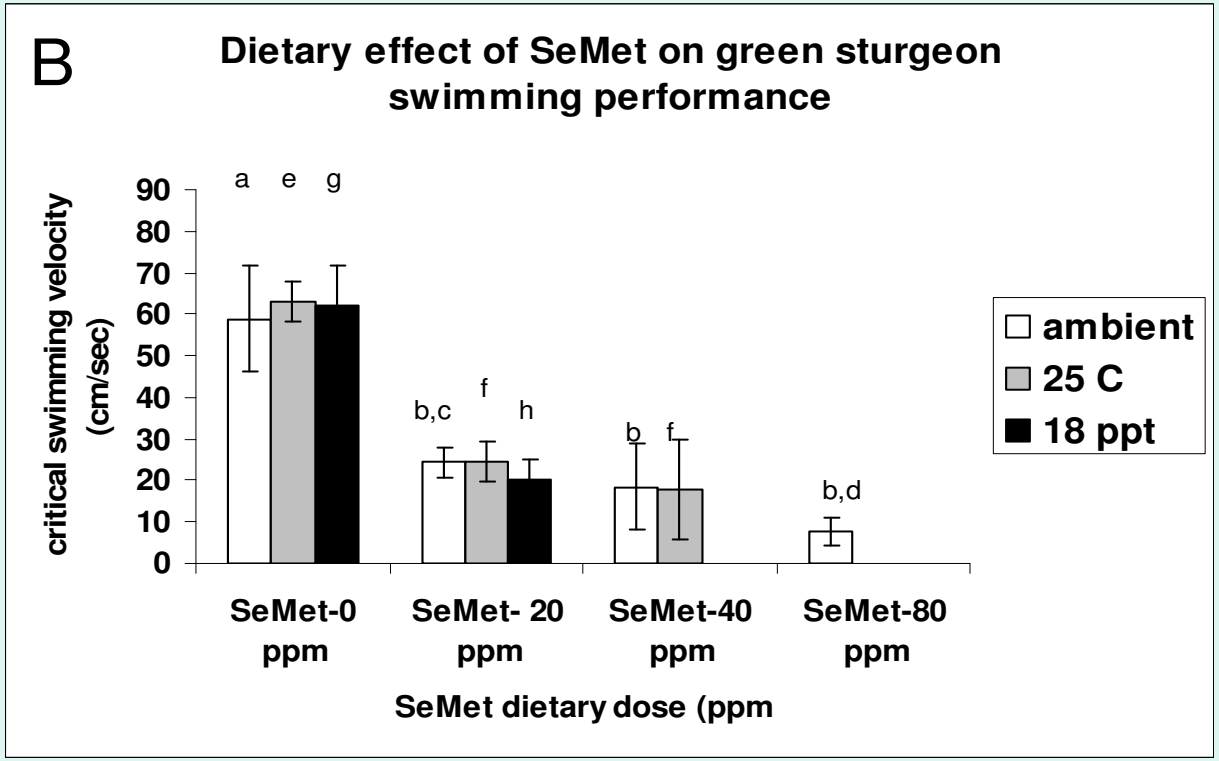

A
WS Metabolic Rates (SeMet)
Ambient routine
Ambient active
25 C routine
25 C active
18 ppt routine
18 ppt active
mgO2/kg/hr
700
600
500
400
300
200
100
0
Se-1
Se-2
Se-3
Se-4
Se-1
Se-2
Se-3
Se-4
Se-1
Se-2
Se-3
Se-4
Treatment Group


B
GS Metabolic Rates (SeMet)
mgO2/kg/hr
700
600
500
400
300
200
100
0
Ambient routine
Ambient active
25 C routine
25 C active
18 ppt routine
18 ppt active
Se-1
Se-2
Se-3
Se-4
Se-1
Se-2
Se-3
Se-4
Se-1
Se-2
Treatment Group


A
Dietary effects of MeHg on white sturgeon swimming performance
critical swmming velocity (cm/sec)
120
100
80
60
40
20
0
a
c
b
d
b
ambient
25 C
18 ppt
MeHg-0 ppm
MeHg-25 ppm
MeHg-50 ppm
MeHg dietary dose (ppm)


B
Dietary MeHg effects on green sturgeon swimming performance
critical swiming velocity (cm/sec)
100
90
80
70
60
50
40
30
20
10
0
a
c
b
d
ambient
25 C
18 ppt
MeHg-0 ppm
MeHg-25 ppm
Dietary dose MeHg (ppm)


A
Dietary effects of SeMet on white sturgeon swimming performance
critical swimming velocity (cm/sec)
120
100
80
60
40
20
0
a
c
e
b
d,f
ambient
25 C
18 ppt
SeMet- 0 ppm
SeMet-20 ppm
SeMet-40 ppm
SeMet-80 ppm
Dietary dose SeMet (ppm)


B
Dietary effect of SeMet on green sturgeon swimming performance
critical swimming velocity (cm/sec)
90
80
70
60
50
40
30
20
10
0
a e g
b,c
f
h
b f
b,d
ambient
25 C
18 ppt
SeMet-0 ppm
SeMet- 20 ppm
SeMet-40 ppm
SeMet-80 ppm
SeMet dietary dose (ppm

Conclusions

- Significant differences in ‘predator’ avoidance observed in both species with the most dramatic effect in GS
- Dietary SeMet treatments produced significant decreases in ‘active’ metabolism in WS at highest dose
- Dietary MeHg treatments produced significant decreases in bioenergetics albeit at very high doses
- Dietary SeMet resulted in significant declines in performance measures in both species with green sturgeon showing a greater sensitivity to this toxicant at all levels tested
- White sturgeon are not an appropriate surrogate for green sturgeon in determining the effects of these toxicants on sturgeon bioenergetics

Future Course

- Develop reliable source of green sturgeon larvae and juveniles for toxicity testing and tracking studies to determine habitat usage by green sturgeon juveniles, e.g., wild-caught broodstock
- Determine the NOEC of SeMet in green sturgeon juveniles

Related Posters

- Linares-Casenave, J., et al.....*360*
- Madison*, R.K., and D. Kueltz.....*368*
- Doroshov, S.I., et al. *312*

Acknowledgements

- We thank CALFED for providing the funding for this study as well as the efforts of Dr. Doroshov's laboratory in development of a captive green sturgeon broodstock program which provided the larvae and juveniles for this study.

Anthropogenic warming has increased drought risk in California

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Edited by Jane Lubchenco, Oregon State University, Corvallis, OR, and approved January 30, 2015 (received for review November 22, 2014)

California is currently in the midst of a record-setting drought. The drought began in 2012 and now includes the lowest calendar-year and 12-mo precipitation, the highest annual temperature, and the most extreme drought indicators on record. The extremely warm and dry conditions have led to acute water shortages, groundwater overdraft, critically low streamflow, and enhanced wildfire risk. Analyzing historical climate observations from California, we find that precipitation deficits in California were more than twice as likely to yield drought years if they occurred when conditions were warm. We find that although there has not been a substantial change in the probability of either negative or moderately negative precipitation anomalies in recent decades, the occurrence of drought years has been greater in the past two decades than in the preceding century. In addition, the probability that precipitation deficits co-occur with warm conditions and the probability that precipitation deficits produce drought have both increased. Climate model experiments with and without anthropogenic forcings reveal that human activities have increased the probability that dry precipitation years are also warm. Further, a large ensemble of climate model realizations reveals that additional global warming over the next few decades is very likely to create ∼100% probability that any annual-scale dry period is also extremely warm. We therefore conclude that anthropogenic warming is increasing the probability of co-occurring warm–dry conditions like those that have created the acute human and ecosystem impacts associated with the "exceptional" 2012–2014 drought in California.

drought | climate extremes | climate change detection | event attribution | CMIP5

The state of California is the largest contributor to the economic and agricultural activity of the United States, accounting for a greater share of population (12%) (1), gross domestic product (12%) (2), and cash farm receipts (11%) (3) than any other state. California also includes a diverse array of marine and terrestrial ecosystems that span a wide range of climatic tolerances and together encompass a global biodiversity "hotspot" (4). These human and natural systems face a complex web of competing demands for freshwater (5). The state's agricultural sector accounts for 77% of California water use (5), and hydroelectric power provides more than 9% of the state's electricity (6). Because the majority of California's precipitation occurs far from its urban centers and primary agricultural zones, California maintains a vast and complex water management, storage, and distribution/conveyance infrastructure that has been the focus of nearly constant legislative, legal, and political battles (5). As a result, many riverine ecosystems depend on mandated "environmental flows" released by upstream dams, which become a point of contention during critically dry periods (5).

California is currently in the midst of a multiyear drought (7). The event encompasses the lowest calendar-year and 12-mo precipitation on record (8), and almost every month between December 2011 and September 2014 exhibited multiple indicators of drought (Fig. S1). The proximal cause of the precipitation deficits was the recurring poleward deflection of the cool-season storm track by a region of persistently high atmospheric pressure, which steered Pacific storms away from California over consecutive seasons (8–11). Although the extremely persistent high pressure is at least a century-scale occurrence (8), anthropogenic global warming has very likely increased the probability of such conditions (8, 9).

Despite insights into the causes and historical context of precipitation deficits (8–11), the influence of historical temperature changes on the probability of individual droughts has—until recently—received less attention (12–14). Although precipitation deficits are a prerequisite for the moisture deficits that constitute "drought" (by any definition) (15), elevated temperatures can greatly amplify evaporative demand, thereby increasing overall drought intensity and impact (16, 17). Temperature is especially important in California, where water storage and distribution systems are critically dependent on winter/spring snowpack, and excess demand is typically met by groundwater withdrawal (18–20). The impacts of runoff and soil moisture deficits associated with warm temperatures can be acute, including enhanced wildfire risk (21), land subsidence from excessive groundwater withdrawals (22), decreased hydropower production (23), and damage to habitat of vulnerable riparian species (24).

Recent work suggests that the aggregate combination of extremely high temperatures and very low precipitation during the 2012–2014 event is the most severe in over a millennium (12). Given the known influence of temperature on drought, the fact that the 2012–2014 record drought severity has co-occurred with record statewide warmth (7) raises the question of whether long-term warming has altered the probability that precipitation deficits yield extreme drought in California.

Significance

California ranks first in the United States in population, economic activity, and agricultural value. The state is currently experiencing a record-setting drought, which has led to acute water shortages, groundwater overdraft, critically low streamflow, and enhanced wildfire risk. Our analyses show that California has historically been more likely to experience drought if precipitation deficits co-occur with warm conditions and that such confluences have increased in recent decades, leading to increases in the fraction of low-precipitation years that yield drought. In addition, we find that human emissions have increased the probability that low-precipitation years are also warm, suggesting that anthropogenic warming is increasing the probability of the co-occurring warm–dry conditions that have created the current California drought.

Author contributions: N.S.D., D.L.S., and D.T. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

See Commentary on page 3858.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1422385112/-/DCSupplemental.

Results

We analyze the "Palmer" drought metrics available from the US National Climatic Data Center (NCDC) (25). The NCDC Palmer metrics are based on the Palmer Drought Severity Index (PDSI), which uses monthly precipitation and temperature to calculate moisture balance using a simple "supply-and-demand" model (26) (*Materials and Methods*). We focus on the Palmer Modified Drought Index (PMDI), which moderates transitions between wet and dry periods (compared with the PDSI) (27). However, we note that the long-term time series of the PMDI is similar to that of other Palmer drought indicators, particularly at the annual scale (Figs. S1 and S2).

Because multiple drought indicators reached historic lows in July 2014 (Figs. S1–S3), we initially focus on statewide PMDI, temperature, and precipitation averaged over the August–July 12-mo period. We find that years with a negative PMDI anomaly exceeding –1.0 SDs (hereafter "1-SD drought") have occurred approximately twice as often in the past two decades as in the preceding century (six events in 1995–2014 = 30% of years; 14 events in 1896–1994 = 14% of years) (Fig. 1*A* and Fig. S4). This increase in the occurrence of 1-SD drought years has taken place without a substantial change in the probability of negative precipitation anomalies (53% in 1896–2014 and 55% in 1995–2014) (Figs. 1*B* and 2 *A* and *B*). Rather, the observed doubling of the occurrence of 1-SD drought years has coincided with a doubling of the frequency with which a negative precipitation year produces a 1-SD drought, with 55% of negative precipitation years in 1995–2014 co-occurring with a –1.0 SD PMDI anomaly, compared with 27% in 1896–1994 (Fig. 1 *A* and *B*).

Most 1-SD drought years have occurred when conditions were both dry (precipitation anomaly < 0) and warm (temperature anomaly > 0), including 15 of 20 1-SD drought years during 1896–2014 (Fig. 2*A* and Fig. S4) and 6 of 6 during 1995–2014 (Fig. 2*B* and Fig. S4). Similarly, negative precipitation anomalies are much more likely to produce 1-SD drought if they co-occur with a positive temperature anomaly. For example, of the 63 negative precipitation years during 1896–2014, 15 of the 32 warm–dry years (47%) produced 1-SD drought, compared with only 5 of the 31 cool–dry years (16%) (Fig. 2*A*). (During 1896–1994, 41% of warm–dry years produced 1-SD droughts, compared with 17% of cool–dry years.) The probability that a negative precipitation anomaly co-occurs with a positive temperature anomaly has increased recently, with warm–dry years occurring more than twice as often in the past two decades (91%) as in the preceding century (42%) (Fig. 1*B*).

All 20 August–July 12-mo periods that exhibited a –1.0 SD PMDI anomaly also exhibited a –0.5 SD precipitation anomaly (Fig. 1*B* and 2*E*), suggesting that moderately low precipitation is prerequisite for a 1-SD drought year. However, the occurrence of –0.5 SD precipitation anomalies has not increased in recent years (40% in 1896–2014 and 40% in 1995–2014) (Fig. 2 *A* and *B*). Rather, these moderate precipitation deficits have been far more likely to produce 1-SD drought when they occur in a warm year. For example, during 1896–2014, 1-SD drought occurred in 15 of the 28 years (54%) that exhibited both a –0.5 SD precipitation anomaly and a positive temperature anomaly, but in only 5 of the 20 years (25%) that exhibited a –0.5 SD precipitation anomaly and a negative temperature anomaly (Fig. 2*A*). During 1995–2014, 6 of the 8 moderately dry years produced 1-SD drought (Fig. 1*A*), with all 6 occurring in years in which the precipitation anomaly exceeded –0.5 SD and the temperature anomaly exceeded 0.5 SD (Fig. 1*C*).

Taken together, the observed record from California suggests that (*i*) precipitation deficits are more likely to yield 1-SD PMDI droughts if they occur when conditions are warm and (*ii*) the occurrence of 1-SD PMDI droughts, the probability of precipitation deficits producing 1-SD PMDI droughts, and the probability of precipitation deficits co-occurring with warm conditions have all been greater in the past two decades than in the preceding century.

These increases in drought risk have occurred despite a lack of substantial change in the occurrence of low or moderately low precipitation years (Figs. 1*B* and 2 *A* and *B*). In contrast, statewide warming (Fig. 1*C*) has led to a substantial increase in warm conditions, with 80% of years in 1995–2014 exhibiting a positive temperature anomaly (Fig. 2*B*), compared with 45% of years in 1896–2014 (Fig. 2*A*). As a result, whereas 58% of moderately dry years were warm during 1896–2014 (Fig. 2*A*) and 50% were warm during 1896–1994, 100% of the 8 moderately dry years in 1995–2014 co-occurred with a positive temperature anomaly (Fig. 2*B*). The observed statewide warming (Fig. 1*C*) has therefore substantially increased the probability that when moderate precipitation deficits occur, they occur during warm years.

The recent statewide warming clearly occurs in climate model simulations that include both natural and human forcings ("Historical" experiment), but not in simulations that include only natural forcings ("Natural" experiment) (Fig. 3*B*). In particular, the Historical and Natural temperatures are found to be different at the 0.001 significance level during the most recent 20-, 30-, and 40-y periods of the historical simulations (using the block bootstrap resampling applied in ref. 28). In contrast, although the Historical experiment exhibits a slightly higher mean annual precipitation (0.023 significance level), there is no statistically

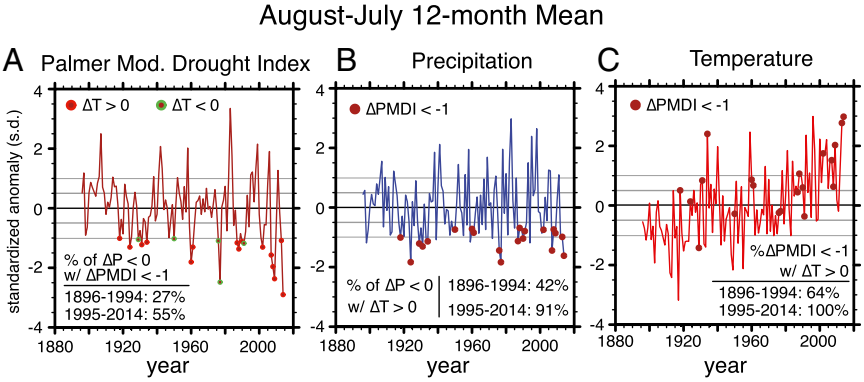

Fig. 1. Historical time series of drought (*A*), precipitation (*B*), and temperature (*C*) in California. Values are calculated for the August–July 12-mo mean in each year of the observed record, beginning in August 1895. In each year, the standardized anomaly is expressed as the magnitude of the anomaly from the long-term annual mean, divided by the SD of the detrended historical annual anomaly time series. The PMDI is used as the primary drought indicator, although the other Palmer indicators exhibit similar historical time series (Figs. S1 and S2). Circles show the years in which the PMDI exhibited a negative anomaly exceeding –1.0 SDs, which are referred to as 1-SD drought years in the text.

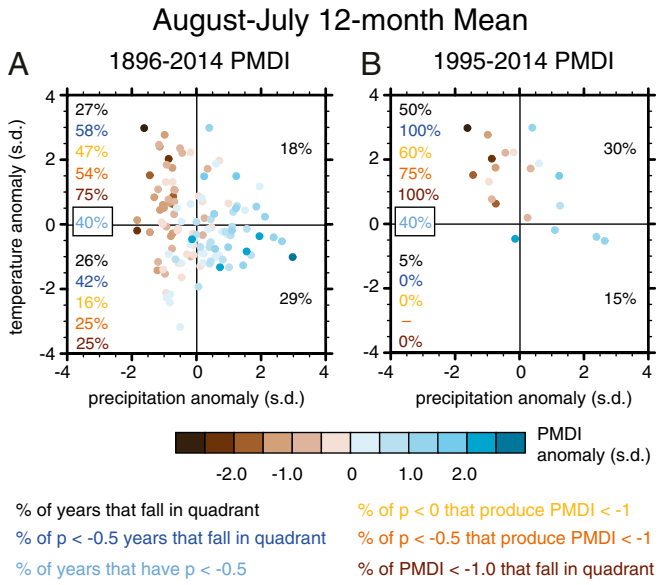

Fig. 2. Historical occurrence of drought, precipitation, and temperature in California. Standardized anomalies are shown for each August–July 12-mo period in the historical record (calculated as in Fig. 1). Anomalies are shown for the full historical record (*A*) and for the most recent two decades (*B*). Percentage values show the percentage of years meeting different precipitation and drought criteria that fall in each quadrant of the temperature–precipitation space. The respective criteria are identified by different colors of text.

significant difference in probability of a –0.5 SD precipitation anomaly (Fig. 3 *A* and *C*). However, the Historical experiment exhibits greater probability of a –0.5 SD precipitation anomaly co-occurring with a positive temperature anomaly (0.001 significance level) (Fig. 3*D*), suggesting that human forcing has caused the observed increase in probability that moderately dry precipitation years are also warm.

The fact that the occurrence of warm and moderately dry years approaches that of moderately dry years in the last decades of the Historical experiment (Fig. 3 *B* and *C*) and that 91% of negative precipitation years in 1995–2014 co-occurred with warm anomalies (Fig. 1*B*) suggests possible emergence of a regime in which nearly all dry years co-occur with warm conditions. We assess this possibility using an ensemble of 30 realizations of a single global climate model [the National Center for Atmospheric Research (NCAR) Community Earth System Model (CESM1) Large Ensemble experiment ("LENS")] (29) (*Materials and Methods*). Before ~1980, the simulated probability of a warm–dry year is approximately half that of a dry year (Fig. 4*B*), similar to observations (Figs. 1*B* and 2). However, the simulated probability of a warm–dry year becomes equal to that of a dry year by ~2030 of RCP8.5. Likewise, the probabilities of co-occurring 0.5, 1.0 and 1.5 SD warm–dry anomalies become approximately equal to those of 0.5, 1.0, and 1.5 SD dry anomalies (respectively) by ~2030 (Fig. 4*B*).

The probability of co-occurring extremely warm and extremely dry conditions (1.5 SD anomaly) remains greatly elevated throughout the 21st century (Fig. 4*B*). In addition, the number of multiyear periods in which a –0.5 SD precipitation anomaly co-occurs with a 0.5 SD temperature anomaly more than doubles between the Historical and RCP8.5 experiments (Fig. 4*A*). We find similar results using a 12-mo moving average (Fig. 4*C*). As with the August–July 12-mo mean (Fig. 4*B*), the probability of a dry year is approximately twice the probability of a warm–dry year for all 12-mo periods before ~1980 (Fig. 4*C*). However, the occurrence of warm years (including +1.5 SD temperature anomalies) increases after ~1980, reaching 1.0 by ~2030. This increase implies a transition to a permanent condition of ~100% risk that any negative—or extremely negative—12-mo precipitation anomaly is also extremely warm.

The overall occurrence of dry years declines after ~2040 (Fig. 4*C*). However, the occurrence of extreme 12-mo precipitation deficits (–1.5 SD) is greater in 2006–2080 than in 1920–2005 (<0.03 significance level). This detectable increase in extremely low-precipitation years adds to the effect of rising temperatures and contributes to the increasing occurrence of extremely warm–dry 12-mo periods during the 21st century.

All four 3-mo seasons likewise show higher probability of co-occurring 1.5 SD warm–dry anomalies after ~1980, with the probability of an extremely warm–dry season equaling that of an extremely dry season by ~2030 for spring, summer, and autumn, and by ~2060 for winter (Fig. 4*D*). In addition, the probability of a –1.5 SD precipitation anomaly increases in spring ($P < 0.001$) and autumn ($P = 0.01$) in 2006–2080 relative to 1920–2005, with spring occurrence increasing by ~75% and autumn occurrence increasing by ~44%—which represents a substantial and statistically significant increase in the risk of extremely low-precipitation events at both margins of California's wet season. In contrast, there is no statistically significant difference in the probability of a –1.5 SD precipitation anomaly for winter.

Discussion

A recent report by Seager et al. (30) found no significant long-term trend in cool-season precipitation in California during the 20th and early 21st centuries, which is consistent with our

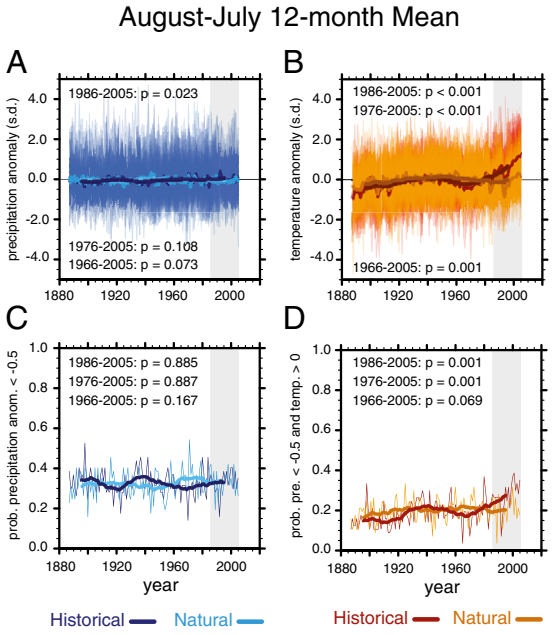

Fig. 3. Influence of anthropogenic forcing on the probability of warm–dry years in California. Temperature and precipitation values are calculated for the August–July 12-mo mean in each year of the CMIP5 Historical and Natural forcing experiments (*Materials and Methods*). The *Top* panels (*A* and *B*) show the time series of ensemble-mean standardized temperature and precipitation anomalies. The *Bottom* panels (*C* and *D*) show the unconditional probability (across the ensemble) that the annual precipitation anomaly is less than –0.5 SDs, and the conditional probability that both the annual precipitation anomaly is less than –0.5 SDs and the temperature anomaly is greater than 0. The bold curves show the 20-y running mean of each annual time series. The CMIP5 Historical and Natural forcing experiments were run until the year 2005. *P* values are shown for the difference between the Historical and Natural experiments for the most recent 20-y (1986–2005; gray band), 30-y (1976–2005), and 40-y (1966–2005) periods of the CMIP5 protocol. *P* values are calculated using the block bootstrap resampling approach of ref. 28 (*Materials and Methods*).

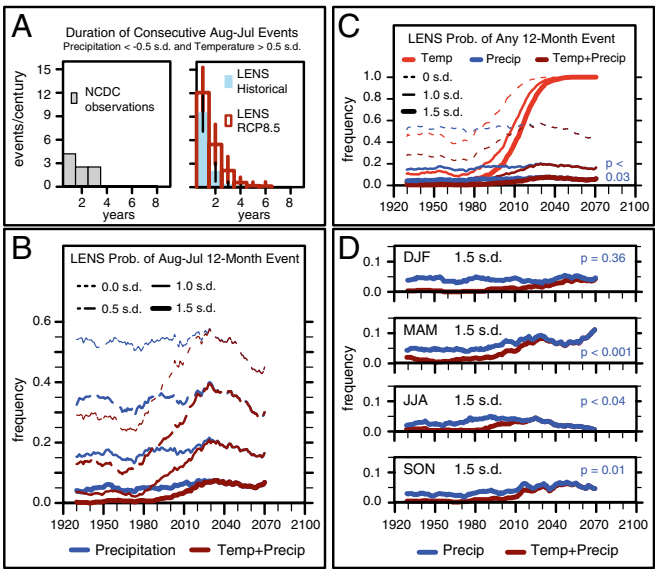

Fig. 4. Projected changes in the probability of co-occurring warm–dry conditions in the 21st century. (*A*) Histogram of the frequency of occurrence of consecutive August–July 12-mo periods in which the 12-mo precipitation anomaly is less than –0.5 SDs and the 12-mo temperature anomaly is at least 0.5 SDs, in historical observations and the LENS large ensemble experiment. (*B*) The probability that a negative 12-mo precipitation anomaly and a positive 12-mo temperature anomaly equal to or exceeding a given magnitude occur in the same August–July 12-mo period, for varying severity of anomalies. (*C*) The probability that a negative precipitation anomaly and a positive temperature anomaly equal to or exceeding a given magnitude occur in the same 12-mo period, for all possible 12-mo periods (using a 12-mo running mean; see *Materials and Methods*), for varying severity of anomalies. (*D*) The unconditional probability of a –1.5 SD seasonal precipitation anomaly (blue curve) and the conditional probability that a –1.5 SD seasonal precipitation anomaly occurs in conjunction with a 1.5 SD seasonal temperature anomaly (red curve), for each of the four 3-mo seasons. Time series show the 20-y running mean of each annual time series. *P* values are shown for the difference in occurrence of –1.5 SD precipitation anomalies between the Historical period (1920–2005) and the RCP8.5 period (2006–2080).

findings. Further, under a scenario of strongly elevated greenhouse forcing, Neelin et al. (31) found a modest increase in California mean December–January–February (DJF) precipitation associated with a local eastward extension of the mean subtropical jet stream west of California. However, considerable evidence (8–11, 31–33) simultaneously suggests that the response of northeastern Pacific atmospheric circulation to anthropogenic warming is likely to be complex and spatiotemporally inhomogeneous, and that changes in the atmospheric mean state may not be reflective of changes in the risk of extreme events (including atmospheric configurations conducive to precipitation extremes). Although there is clearly value in understanding possible changes in precipitation, our results highlight the fact that efforts to understand drought without examining the role of temperature miss a critical contributor to drought risk. Indeed, our results show that even in the absence of trends in mean precipitation—or trends in the occurrence of extremely low-precipitation events—the risk of severe drought in California has already increased due to extremely warm conditions induced by anthropogenic global warming.

We note that the interplay between the existence of a well-defined summer dry period and the historical prevalence of a substantial high-elevation snowpack may create particular susceptibility to temperature-driven increases in drought duration and/or intensity in California. In regions where precipitation exhibits a distinct seasonal cycle, recovery from preexisting drought conditions is unlikely during the characteristic yearly dry spell (34). Because California's dry season occurs during the warm summer months, soil moisture loss through evapotranspiration (ET) is typically high—meaning that soil moisture deficits that exist at the beginning of the dry season are exacerbated by the warm conditions that develop during the dry season, as occurred during the summers of 2013 and 2014 (7).

Further, California's seasonal snowpack (which resides almost entirely in the Sierra Nevada Mountains) provides a critical source of runoff during the low-precipitation spring and summer months. Trends toward earlier runoff in the Sierra Nevada have already been detected in observations (e.g., ref. 35), and continued global warming is likely to result in earlier snowmelt and increased rain-to-snow ratios (35, 36). As a result, the peaks in California's snowmelt and surface runoff are likely to be more pronounced and to occur earlier in the calendar year (35, 36), increasing the duration of the warm-season low-runoff period (36) and potentially reducing montane surface soil moisture (37). Although these hydrological changes could potentially increase soil water availability in previously snow-covered regions during the cool low-ET season (34), this effect would likely be outweighed by the influence of warming temperatures (and decreased runoff) during the warm high-ET season (36, 38), as well as by the increasing occurrence of consecutive years with low precipitation and high temperature (Fig. 4*A*).

The increasing risk of consecutive warm–dry years (Fig. 4*A*) raises the possibility of extended drought periods such as those found in the paleoclimate record (14, 39, 40). Recent work suggests that record warmth could have made the current event the most severe annual-scale drought of the past millennium (12). However, numerous paleoclimate records also suggest that the region has experienced multidecadal periods in which most years were in a drought state (14, 39, 41, 42), albeit less acute than the current California event (12, 39, 41). Although multidecadal ocean variability was a primary cause of the megadroughts of the last millenium (41), the emergence of a condition in which there is ∼100% probability of an extremely warm year (Fig. 4) substantially increases the risk of prolonged drought conditions in the region (14, 39, 40).

A number of caveats should be considered. For example, ours is an implicit approach that analyzes the temperature and precipitation conditions that have historically occurred with low PMDI years, but does not explicitly explore the physical processes that produce drought. The impact of increasing temperatures on the processes governing runoff, baseflow, groundwater, soil moisture, and land-atmosphere evaporative feedbacks over both the historical period and in response to further global warming remains a critical uncertainty (43). Likewise, our analyses of anthropogenic forcing rely on global climate models that do not resolve the topographic complexity that strongly influences California's precipitation and temperature. Further investigation using high-resolution modeling approaches that better resolve the boundary conditions and fine-scale physical processes (44–46) and/or using analyses that focus on the underlying large-scale climate dynamics of individual extreme events (8) could help to overcome the limitations of simulated precipitation and temperature in the current generation of global climate models.

Conclusions

Our results suggest that anthropogenic warming has increased the probability of the co-occurring temperature and precipitation conditions that have historically led to drought in California. In addition, continued global warming is likely to cause a transition to a regime in which essentially every seasonal, annual, and multiannual precipitation deficit co-occurs with historically warm conditions. The current warm–dry event in California—as well as historical observations of previous seasonal, annual, and multiannual warm–dry events—suggests such a regime would substantially increase the risk of severe impacts on human and natural systems. For example, the projected increase in extremely

low precipitation and extremely high temperature during spring and autumn has substantial implications for snowpack water storage, wildfire risk, and terrestrial ecosystems (47). Likewise, the projected increase in annual and multiannual warm–dry periods implies increasing risk of the acute water shortages, critical groundwater overdraft, and species extinction potential that have been experienced during the 2012–2014 drought (5, 20).

California's human population (38.33 million as of 2013) has increased by nearly 72% since the much-remembered 1976–1977 drought (1). Gains in urban and agricultural water use efficiency have offset this rapid increase in the number of water users to the extent that overall water demand is nearly the same in 2013 as it was in 1977 (5). As a result, California's per capita water use has declined in recent decades, meaning that additional short-term water conservation in response to acute shortages during drought conditions has become increasingly challenging. Although a variety of opportunities exist to manage drought risk through long-term changes in water policy, management, and infrastructure (5), our results strongly suggest that global warming is already increasing the probability of conditions that have historically created high-impact drought in California.

Materials and Methods

We use historical time series of observed California statewide temperature, precipitation, and drought data from the National Oceanic and Atmospheric Administration's NCDC (7). The data are from the NCDC "nClimDiv" divisional temperature–precipitation–drought database, available at monthly time resolution from January 1895 to the present (7, 25). The NCDC nClimDiv database includes temperature, precipitation, and multiple Palmer drought indicators, aggregated at statewide and substate climate division levels for the United States. The available Palmer drought indicators include PDSI, the Palmer Hydrological Drought Index (PHDI), and PMDI.

PMDI and PHDI are variants of PDSI (25–27, 48, 49). PDSI is an index that measures the severity of wet and dry anomalies (26). The NCDC nClimDiv PDSI calculation is reported at the monthly scale, based on monthly temperature and precipitation (49). Together, the monthly temperature and precipitation values are used to compute the net moisture balance, based on a simple supply-and-demand model that uses potential evapotranspiration (PET) calculated using the Thornthwaite method. Calculated PET values can be very different when using other methods (e.g., Penman–Monteith), with the Thornthwaite method's dependence on surface temperature creating the potential for overestimation of PET (e.g., ref. 43). However, it has been found that the choice of methods in the calculation of PET does not critically influence the outcome of historical PDSI estimates in the vicinity of California (15, 43, 50). In contrast, the sensitivity of the PET calculation to large increases in temperature could make the PDSI inappropriate for calculating the response of drought to high levels of greenhouse forcing (15). As a result, we analyze the NCDC Palmer indicators in conjunction with observed temperature and precipitation data for the historical period, but we do not calculate the Palmer indicators for the future (for future projections of the PDSI, refer to refs. 15 and 40).

Because the PDSI is based on recent temperature and precipitation conditions (and does not include human demand for water), it is considered an indicator of "meterological" drought (25). The PDSI calculates "wet," "dry," and "transition" indices, using the wet or dry index when the probability is 100% and the transition index when the probability is less than 100% (26). Because the PMDI always calculates a probability-weighted average of the wet and dry indices (27), the PDSI and PMDI will give equal values in periods that are clearly wet or dry, but the PMDI will yield smoother transitions between wet and dry periods (25). In this work, we use the PMDI as our primary drought indicator, although we note that the long-term time series of the PMDI is similar to that of the PDSI and PHDI, particularly at the annual scale considered here (Figs. S1 and S2).

We analyze global climate model simulations from phase 5 of the Coupled Model Intercomparison Project (CMIP5) (51). We compare two of the CMIP5 multimodel historical experiments (which were run through 2005): (*i*) the Historical experiment, in which the climate models are prescribed both anthropogenic and nonanthropogenic historical climate forcings, and (*ii*) the Natural experiment, in which the climate models are prescribed only the nonanthropogenic historical climate forcings. We analyze those realizations for which both temperature and precipitation were available from both experiments at the time of data acquisition. We calculate the temperature and precipitation values over the state of California at each model's native resolution using all grid points that overlap with the geographical borders of California, as defined by a high-resolution shapefile (vector digital data obtained from the US Geological Survey via the National Weather Service at www.nws.noaa.gov/geodata/catalog/national/html/us_state.htm).

We also analyze NCAR's large ensemble ("LENS") climate model experiment (29). The LENS experiment includes 30 realizations of the NCAR CESM1. This large single-model experiment enables quantification of the uncertainty arising from internal climate system variability. Although the calculation of this "irreducible" uncertainty likely varies between climate models, it exists independent of uncertainty arising from model structure, model parameter values, and climate forcing pathway. At the time of acquisition, LENS results were available for 1920–2005 in the Historical experiment and 2006–2080 in the RCP8.5 (Representative Concentration Pathway) experiment. The four RCPs are mostly indistinguishable over the first half of the 21st century (52). RCP8.5 has the highest forcing in the second half of the 21st century and reaches ∼4 °C of global warming by the year 2100 (52).

Given that the ongoing California drought encompasses the most extreme 12-mo precipitation deficit on record (8) and that both temperature and many drought indicators reached their most extreme historical values for California in July 2014 (7) (Fig. 1 and Figs. S1 and S2), we use the 12-mo August–July period as one period of analysis. However, because severe conditions can manifest at both multiannual and subannual timescales, we also analyze the probability of occurrence of co-occurring warm and dry conditions for multiannual periods, for all possible 12-mo periods, and for the winter (DJF), spring (March–April–May), summer (June–July–August), and autumn (September–October–November) seasons.

We use the monthly-mean time series from NCDC to calculate observed time series of statewide 12-mo values of temperature, precipitation, and PMDI. Likewise, we use the monthly-mean time series from CMIP5 and LENS to calculate simulated time series of statewide 12-mo and seasonal values of temperature and precipitation. From the time series of annual-mean values for each observed or simulated realization, we calculate (*i*) the baseline mean value over the length of the record, (*ii*) the annual anomaly from the baseline mean value, (*iii*) the SD of the detrended baseline annual anomaly time series, and (*iv*) the ratio of each individual annual anomaly value to the SD of the detrended baseline annual anomaly time series. (For the 21st-century simulations, we use the Historical simulation as the baseline.) Our time series of standardized values are thereby derived from the time series of 12-mo annual (or 3-mo seasonal) mean anomaly values that occur in each year.

For the multiannual analysis, we calculate consecutive occurrences of August–July 12-mo values. For the analysis of all possible 12-mo periods, we generate the annual time series of each 12-mo period (January–December, February–January, etc.) using a 12-mo running mean. For the seasonal analysis, we generate the time series by calculating the mean of the respective 3-mo season in each year.

We quantify the statistical significance of differences in the populations of different time periods using the block bootstrap resampling approach of ref. 28. For the CMIP5 Historical and Natural ensembles, we compare the populations of the August–July values in the two experiments for the 1986–2005, 1976–2005, and 1966–2005 periods. For the LENS seasonal analysis, we compare the respective populations of DJF, March–April–May, June–July–August, and September–October–November values in the 1920–2005 and 2006–2080 periods. For the LENS 12-mo analysis, we compare the populations of 12-mo values in the 1920–2005 and 2006–2080 periods, testing block lengths up to 16 to account for temporal autocorrelation out to 16 mo for the 12-mo running mean data. (Autocorrelations beyond 16 mo are found to be negligible.)

Throughout the text, we consider drought to be those years in which negative 12-mo PMDI anomalies exceed –1.0 SDs of the historical interannual PMDI variability. We stress that this value is indicative of the variability of the annual (12-mo) PMDI, rather than of the monthly values (compare Fig. 1 and Figs. S1 and S2). We consider "moderate" temperature and precipitation anomalies to be those that exceed 0.5 SDs ("0.5 SD") and "extreme" temperature and precipitation anomalies to be those that exceed 1.5 SDs ("1.5 SD").

ACKNOWLEDGMENTS. We thank the editor and two anonymous reviewers for insightful comments; Deepti Singh for assistance with the block bootstrap resampling; the National Oceanic and Atmospheric Administration's NCDC for access to the historical temperature, precipitation, and drought data; the World Climate Research Program and Department of Energy's Program for Climate Model Diagnosis and Intercomparison for access to the CMIP5 simulations; and NCAR for access to the LENS simulations. Our work was supported by National Science Foundation Award 0955283 and National Institutes of Health Award 1R01AI090159-01.

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MARCH 2013

SAN FRANCISCO ESTUARY & WATERSHED SCIENCE
Sponsored by the Delta Science Program and the UC Davis John Muir Institute of the Environment

Ecosystem-Scale Selenium Model for the San Francisco Bay-Delta Regional Ecosystem Restoration Implementation Plan (DRERIP)

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ABSTRACT

Environmental restoration, regulatory protections, and competing interests for water are changing the balance of selenium (Se) discharges to the San Francisco Bay–Delta Estuary (Bay–Delta). The model for Se described here as part of the Delta Regional Ecosystem Restoration Implementation Plan (DRERIP) draws both from the current state of knowledge of the Bay–Delta and of environmental Se science. It is an ecosystem-scale methodology that is a conceptual and quantitative tool to (1) evaluate implications of Se contamination; (2) better understand protection for fish and aquatic-dependent wildlife; and (3) help evaluate future restoration actions. The model builds from five basic principles that determine ecological risks from Se in aquatic environments: (1) dissolved Se transformation to particulate material Se, which is partly driven by the chemical species of dissolved Se, sets dynamics at the base of the food web; (2) diet drives bioavailability of Se to animals; (3) bioaccumulation differs widely among invertebrates, but not necessarily among fish; (4) ecological risks differ among food webs and predator species; and (5) risk for each predator is driven by a combination of exposures via their specific food web and the species' inherent sensitivity to Se toxicity. Spatially and temporally matched data sets across media (i.e., water, suspended particulate material, prey, and predator) are needed for initiating modeling and for providing ecologically consistent predictions. The methodology, applied site-specifically to the Bay–Delta, includes use of (1) salinity-specific partitioning factors based on empirical estuary data to quantify the effects of dissolved speciation and phase transformation; (2) species-specific dietary biodynamics to quantify foodweb bioaccumulation; and (3) habitat use and life-cycle data for Bay–Delta predator species to illustrate exposure. Model outcomes show that the north Bay–Delta functions as an efficient biomagnifier of Se in benthic food webs, with the greatest risks to predaceous benthivores occurring under low flow conditions. Improving the characterization of ecological risks from Se in the Bay–Delta will require modernization of the Se database and continuing integration of biogeochemical, ecological, and hydrological dynamics into the model.

KEY WORDS

Selenium, biodynamics, bioaccumulation, food webs, ecotoxicology, ecology.

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INTRODUCTION

The Delta Regional Ecosystem Restoration Implementation Plan (DRERIP) process focuses on construction of conceptual models that describe and define the relationships among the processes, habitats, species, and stressors for the Bay-Delta (DiGennaro and others 2012). The models use common elements and are designed to interconnect to achieve the goals of evaluating and informing Bay-Delta restoration actions. Selenium is recognized as an important stressor in aquatic environments because of its potency as a reproductive toxin and its ability to bioaccumulate through food webs (Chapman and others 2010; Presser and Luoma 2010a). Selenium's role is well documented in extirpation (i.e., local extinctions) of fish populations (Lemly 2002) and in occurrences of deformities of aquatic birds in affected habitats (Skorupa 1998). For Se, exposure is specific to a predator species' choice of food web and physiology, making some predators more vulnerable and, thus, more likely than others to disappear from moderately contaminated environments (Lemly 2002; Luoma and Presser 2009; Stewart and others 2004).

Concern about Se as a stressor in the Bay-Delta watershed originates from the damage to avian and fish populations that resulted when an agricultural drain to alleviate subsurface drainage conditions in the western San Joaquin Valley released Se into the Kesterson National Wildlife Refuge in the 1980s (Presser and Ohlendorf 1987). Later it was recognized that (1) some aquatic predators in the Bay-Delta were bioaccumulating sufficient Se to threaten their reproductive capabilities (SWRCB 1987, 1988, 1989, 1991) and; (2) primary Se sources included not only organic enriched sedimentary deposits in the San Joaquin Valley and elsewhere, but also their anthropogenic by-products such as oil (Cutter 1989; Presser 1994; Presser and others 2004). Proposals in 1978 and 2006 to extend an agricultural drain from the western San Joaquin Valley directly to the Bay-Delta as a way of removing Se from the valley were found both times to present substantial and broad ecological risks (e.g., USBR 1978, 2006; Presser and Luoma 2006).

Currently, Se contamination is spatially distributed from the Delta through the North Bay (Suisun Bay, Carquinez Strait, and San Pablo Bay) to the Pacific Ocean, mainly from oil-refining discharges internal to the estuary, and agricultural drainage discharges exported via the San Joaquin River. Regulatory and planning processes have intervened in the cases of both existing Se sources resulting in a decline in contamination since 1986-1992 when concentrations were maximal (SWRCB 1987, 1988, 1989, 1991; Presser and Luoma 2006; USBR 1995, 2001, 2009). However, the North Bay, the Delta, and segments of the San Joaquin River and some of its tributaries and marshes remain designated as impaired by Se (SWRCB 2011). More recently, the State initiated a Se Total Maximum Daily Load (TMDL) process to target both agricultural and oil refinery sources of Se (SFBRWQCB 2007, 2011) in coordination with development and implementation of site-specific water quality Se criteria for the protection of fish and wildlife by the U.S. Environmental Protection Agency (USEPA 2011a). The presence of a major oil-refining industry in the North Bay, and the substantial accumulated reservoir of Se in the soils and aquifers of the western San Joaquin Valley suggest that the potential for ecological risk from Se within the Bay-Delta watershed will continue into the foreseeable future as Se management and mitigation efforts take place (Presser and Luoma 2006; Presser and Schwarzbach 2008; USBR 2008; Appendix A.1).

Historic and more recent data show that certain predator species are considered most at risk from Se in the Bay-Delta (e.g., white and green sturgeon, scoter, scaup) because of high exposures obtained when they consume the estuary's dominant bivalve, *Corbula amurensis*, an efficient bioaccumulator of this metalloid (Stewart and others 2004; Presser and Luoma 2006). The latest available surveys of Se concentrations in *C. amurensis* and white sturgeon (*Acipenser transmontanus*) that were feeding (based upon isotopic evidence) in Carquinez Strait, Suisun Bay, and San Pablo Bay (Stewart and others 2004; Linares and others 2004; Kleckner and others 2010; Presser and Luoma 2010b; SFEI 2009) continue to show concentrations exceeding U.S. Fish and Wildlife Service (USFWS) dietary and tissue toxicity guide-

lines (Skorupa and others 2004; Presser and Luoma 2010b). Sturgeon contain higher concentrations of Se than any other fish species, reflecting their position as a top benthic predator (Stewart and others 2004). Surveys of surf scoter (*Melanitta perspicillata*) and greater scaup (*Aythya marila*) that feed voraciously on *C. amurensis* as they overwinter in Suisun Bay (SFEI 2005; De La Cruz and others 2008; De La Cruz 2010; Presser and Luoma 2010b) show Se has bioaccumulated to levels in muscle and liver tissue that may affect their ability to successfully migrate and breed (Heinz 1996; USDOI 1998; Ohlendorf and Heinz 2011).

Endangered Species Act requirements led to a number of species being determined as jeopardized by Se in the Bay-Delta under a proposed chronic aquatic life Se criterion of 5 µg L^{-1} (USFWS and NOAA Fisheries 2000), including delta smelt (*Hypomesus transpacificus*); longfin smelt (*Spirinchus thaleichthys*); Sacramento splittail (*Pogonichthys macrolepidotus*); Sacramento perch (*Archoplites interruptus*); tidewater goby (*Eucyclogobius newberryi*); green sturgeon (*Acipenser medirostris*) and its surrogate white sturgeon (*Acipenser transmontanus*); steelhead trout (*Oncorhynchus mykiss*); Chinook salmon (*Oncorhynchus tshawytscha*); California clapper rail (*Rallus longirostris obsoletus*); California least tern (*Sterna antillarum browni*); bald eagle (*Haliaeetus leucocephalus*); California brown pelican (*Pelecanus occidentalis californicus*); marbled murrelet (*Brachyramphus marmoratus*); and giant garter snake (*Thamnophis gigas*). Recent analysis by the USFWS (2008a) of 45 species assumed the species most at risk depended on benthic food webs: greater scaup; lesser scaup (*Aythya affinis*); white-winged scoter (*Melanitta fusca*); surf scoter; black scoter (*Melanitta nigra*); California clapper rail; Sacramento splittail; green sturgeon; and white sturgeon. Not enough species-specific information is currently available for consideration of Se exposures for the giant garter snake, an endangered aquatic predator (USFWS 2006, 2009); the Dungeness crab (*Cancer magister*), an invertebrate that consumes *C. amurensis* (Stewart and others 2004); or for species that are within the Dungeness-crab food webs.

Human health advisories currently are posted for the Bay-Delta for the consumption of scoter, greater scaup, and lesser scaup based on elevated Se concentrations in their muscle and liver tissue (CDFG 2012, 2013). Selenium was found to be below the level of human health concern for consumption of edible tissue in certain species of fish, including white sturgeon, from the estuary (OEHHA 2011). White sturgeon contained the highest levels of Se among species of fish surveyed. Some individual white sturgeon sampled from North Bay locations had Se concentrations that exceeded Se advisory levels, based on specific consumption rates (see later detailed discussion under "Human Health" on page 23). Additionally, white sturgeon recreational fishing is limited, based on a decreasing species population (CDFG 2012).

It was recently suggested that the traditional regulatory approach to managing Se contamination is deeply flawed (Reiley and others 2003; Luoma and Presser 2009; Chapman and others 2010), and that a new conceptual model of the processes that control its toxicity is needed for regulatory purposes, especially in estuarine environments like the Bay-Delta. In recognition of the issues with the traditional approach to deriving a criterion for Se, the USEPA is leading a cooperative effort to develop site-specific fish and wildlife Se criteria for habitats affected by Se in California. Specifically for the Bay-Delta, the effort includes protection of Federally listed species and designated critical habitat (USFWS and NOAA Fisheries 2000; USEPA 2011a). Development of Se criteria for the Bay-Delta is proceeding first in this effort because the estuary is considered a sensitive hydrologic system and habitat in terms of Se and it was thought that protection here would elicit regulatory compliance upstream (USEPA 2011a). On the broader scale, Se is considered a general stressor of the estuary, and a constituent that should be analyzed as part of management and restoration planning and implementation (USEPA 2011b; NRC 2010, 2011, 2012).

The cooperative regulatory effort specifically recognizes that the new conceptual model must consider (1) the inaccuracies of deriving toxicity from waterborne Se concentrations; (2) the bioaccumulative nature of Se in aquatic systems; (3) Se's long-term

persistence in aquatic sediments and food webs; and (4) the importance of dietary pathways in determining toxicity (USEPA 1992, 2000a; USFWS and NOAA Fisheries 2000; Luoma and Presser 2009; Presser and Luoma 2006, 2010a, 2010b). Revisions by USEPA also are occurring at the national level to incorporate into the basis for regulation recent advances in the environmental science of Se. For example, a fish tissue Se criterion and implementation plan are being proposed to better integrate dietary exposure pathways into regulatory frameworks, and ensure an adequate link to toxicity (USEPA 2004, 2011b). During this transitional period when species may be jeopardized and while Se criteria are being revised, USEPA has applied the national chronic freshwater Se criterion of 5 µg L^{-1} to the estuary (USEPA 1992, 2000a).

We present here an ecosystem-scale Se conceptual model for the Bay-Delta that addresses the needs of both the DRERIP process and the USEPA. Quantitative applications of the model are also possible. Quantification provides an opportunity to evaluate site-specific Se risks under different circumstances, using field data combined with a systematic quantification of each of the influential processes that link source inputs of Se to toxicity. The methodology is presented in terms of specified DRERIP components (i.e., drivers, linkages, and outcomes). As an example of how quantitative applications can be used, we calculate the dissolved ambient Se concentrations that would result in compliance with a chosen fish or bird tissue guideline under different assumptions or environmental conditions. Uncertainties and model sensitivities are illustrated by comparing outcomes of different exposure scenarios. The scenario approach could facilitate the model's use by decision-makers for quantitative evaluation of restoration alternatives for ecosystem management and protection.

MODEL OVERVIEW

The DRERIP Ecosystem-Scale Selenium Model for the Bay-Delta (Figure 1) has five interconnected modules that depict drivers (sources and hydrology), linkages (ecosystem-scale processes), concentration outcomes (Se concentrations in water, particulates, and organisms), and food web exposure outcomes (effects on fish, wildlife, and human health). Model outcomes in Figure 1 are further refined to critical choices for modeling and species-specific risk scenarios for the Bay-Delta. Together the five modules consider the essential aspects of environmental Se exposure: biogeochemistry, food web transfer, and effects. They also take into account the estuary's ecology and hydrology as well as the functional ecology, physiology and ecotoxicology of the most vulnerable predator species. The modules define relationships that are important to conceptualizing and quantifying how Se is processed from water through diet to prey and predators, and the resulting effect on components of the food web. Thus, the DRERIP Ecosystem-Scale Selenium Model combines fundamental knowledge of Se behavior in ecosystems (Se drivers, linkages, and outcomes) with site-specific knowledge of the Bay-Delta (Bay-Delta drivers, linkages, and outcomes) to define site-specific Se risk (Figure 1).

The DRERIP Se submodels provide details for

- Sources and Hydrology (submodel A, Figure 2);
- Ecosystem-Scale Se Modeling (submodel B, Figure 3);
- Exposure: Food Webs, Seasonal Cycles, Habitat Use (submodels C, D; Figures 4, 5);
- Fish and Wildlife Health: Ecotoxicology and Effects (submodels E, F; Figures 6, 7); and
- Human Health (submodel G, Figure 8).

A human health pathway is designated, but emphasis here is on Se pathways to fish and wildlife health. The North Bay and the Delta are emphasized because the important Se sources have the potential to most affect those habitats and ecosystems (submodel A, Figure 2).

The quantitative DRERIP Ecosystem-Scale Selenium Model is based upon concepts and parameters developed elsewhere for a wide variety of aquatic systems and their food webs (submodel B, Figure 3; submodel E, Figure 6) (Luoma and Rainbow 2005; Luoma and Presser 2009; Chapman and others 2010; Presser and Luoma 2010a). To quantitatively apply the rela-

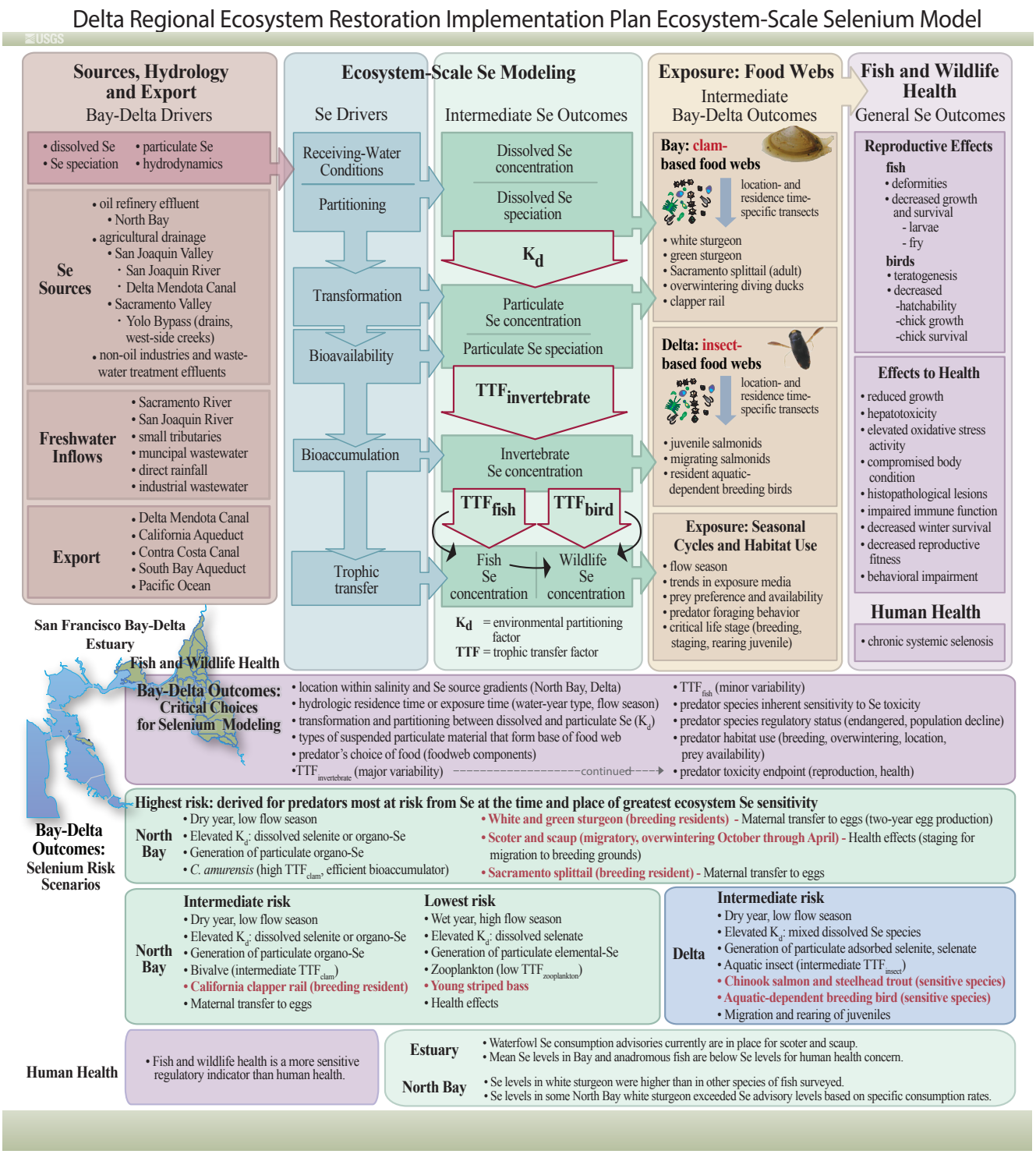

Figure 1 The DRERIP Ecosystem-Scale Selenium Model illustrates five interconnected modules that depict essential aspects of the Bay-Delta's hydrology, biochemistry, and ecology and of the exposure and ecotoxicology of predators at risk from selenium. These modules, and the detailed sub-models that follow, conceptualize (1) how selenium is processed from water through diet to predators and (2) its effects on ecosystems. Critical choices for modeling are summarized, and a quantitative application of the model for the estuary is derived for predators most at risk from Se at the time and place of greatest ecosystem Se sensitivity.

tionships in the conceptual model, we use empirical data from the Bay-Delta (e.g., Cutter and Cutter 2004; Presser and Luoma 2006, 2010b) to (1) help define environmental partitioning factors (K_ds) that quantify transformation of dissolved Se into particulate forms; and (2) help define biodynamic trophic transfer factors (TTFs) that quantify uptake by consumer species and their predators (submodel C, Figure 4; submodel D, Figure 5; submodel F, Figure 7). The broader, ecosystem-scale Se modeling approach was validated by comparing model forecasts with field data, across both a range of common food webs and hydrologic environments (Luoma and Rainbow 2005; Presser and Luoma 2010a) and specifically for the Bay-Delta and Newport Bay (Presser and Luoma 2006, 2009, 2010b).

The organizing principle for quantification is the progressive solution of a set of simple equations, each of which quantifies a process important in Se exposure (submodel B, Figure 3). The interaction of Se loading from different sources, hydrology, and hydrodynamics determine dissolved Se concentrations in the Bay-Delta. Transformation of Se from its dissolved form to a particulate form (represented here operationally as K_d) ultimately determines bioavailability to the food web. In a given environment, Se is taken up much faster from food than from solution by animals. Thus, the entry of Se into the food web can be estimated by a TTF for each trophic level. $TTF_{invertebrate}$ defines dietary uptake by a consumer species, which occurs when invertebrates (or herbivorous fish), feed on primary producers, detritus, microbes, or other types of particulate materials. Selenium bioaccumulation differs widely among invertebrate species because of different physiologies (Luoma and Rainbow 2005). These differences are captured by employing species-specific TTFs (Luoma and Presser 2009). Species-specific TTFs for predaceous fish and birds ($TTF_{predator}$) also are applied to the transfer of Se from invertebrate prey species to their predators (Presser and Luoma 2010a).

For the Bay-Delta, Stewart and others (2004) showed that Se concentrations differ widely among predators that live in the same environment. The main reason for those differences lies in the prey preferences of predators. For example, bass eating from the water-column food web consume invertebrates with much lower Se concentrations than sturgeon eating benthic invertebrates, especially bivalves (Stewart and others 2004). The differences in Se uptake among predator species ($C_{predator}$) can be captured only if the correct prey species (or class of prey species) is included in the equation (submodel B, Figure. 3) and the conceptualization (submodel C, Figure 4). This also means that the choice of predator species is critical in assessing risks from Se contamination.

Selenium concentrations in predators can be predicted with surprisingly strong correlation to observations from nature if particulate Se concentrations are known and an appropriate food web is used for the predator (Luoma and Presser 2009; Presser and Luoma 2010a). One use of these calculations might be to quantify the degree to which different species of birds and fish might be threatened by Se in a specified environment, for example. The correspondence between observed $C_{predator}$ and predictions of $C_{predator}$ from the series of equations that begins with dissolved concentrations (submodel B, Figure 3) depends upon how closely the partitioning between dissolved and particulate Se used in the model matches that occurring in the ecosystem of interest. One use of quantification in this instance is to run the model in the reverse direction to determine the dissolved Se concentration in a specific type of hydrologic environment and food web that would result in a specified Se concentration in the predator. Later, we present a detailed example of how the latter might be applied to real-world issues.

In the final step, effects on the reproduction and health of predaceous fish and birds are determined from bioaccumulated Se concentrations. Selenium is one of the few trace elements for which tissue concentrations have been correlated to these adverse effects in both dietary toxicity tests and field studies. The toxicity data for some of the key species in the Bay-Delta are limited or non-existent. The necessity of establishing effects thresholds from surrogate species adds some uncertainty to assessments of risk. Therefore, in our examples, we use different possible choices for such thresholds.

Additionally, modeling here is within a specified location and flow condition to provide context for

exposure and to help narrow the uncertainties in quantifying the ecological and physiological potential for bioaccumulation (Presser and Luoma 2010b).

MODULES

Sources, Hydrology, and Export

Estuary Mass Balance

The major portion of the estuary from the rivers to the Golden Gate Bridge is termed the Northern Reach, with Suisun Bay near the head of the estuary (submodel A, Figure 2). Selenium sources and their hydraulic connections within that reach have been documented in a number of publications (Cutter 1989; Cutter and San Diego–McGlone 1990; Cutter and Cutter 2004; Meseck and Cutter 2006; Presser and Luoma 2006, 2010b; SFBRQWCB 2011) (Figure 1; submodel A, Figure 2). In brief, the most important regulated estuarine sources of Se are (1) internal inputs of oil refinery wastewaters from processing of crude oils at North Bay refineries; and (2) external inputs of irrigation drainage from agricultural lands of the western San Joaquin Valley conveyed mainly through the San Joaquin River. (submodel A, Figure 2). These and other potential Se sources are described in detail in Appendix A.1. These details reflect the depth of history for Se management within the Bay-Delta watershed and the continuing tradeoffs that accompany their presence.

The Sacramento and San Joaquin rivers are the main sources of freshwater inflow to the Bay-Delta, with the Sacramento River being the dominant inflow under most conditions (Conomos and others 1979; Peterson and others 1985). The rivers provide 92% of the freshwater inflows to the Bay-Delta, with small tributaries and municipal wastewater providing approximately 3% each (McKee and others 2008).

In general, Se concentrations in the Sacramento River (above tidal influence, e.g., at Freeport) are low and relatively constant (1998 to 1999 average: 0.07 µg L^{-1}; range 0.05 to 0.11 µg L^{-1}) (Cutter and Cutter 2004). Dissolved Se concentrations in the San Joaquin River (above tidal influence, e.g., at Vernalis) were about an order-of-magnitude higher than those in the Sacramento River in 1999 (1998 to 1999 average: 0.71 µg L^{-1}; range 0.4 to 1.07 µg L^{-1}) (Cutter and Cutter 2004) and are much more variable. In the late 1980s and early 1990s concentrations above 5 µg L^{-1} were observed occasionally in the San Joaquin River (Presser and Luoma 2006), but in-valley source control efforts have reduced Se loads and concentrations (Appendix A.1).

In the present configuration of the Bay-Delta, the San Joaquin River is predominantly re-routed and exported back to the San Joaquin Valley (submodel A, Figure 2; Appendix A.1). Hence, for the purposes of evaluating Se contamination sources, the simplest assumption is that the "baseline" Se concentrations (undisturbed by human activities) in the Delta would be close to the Se concentrations in the Sacramento River. The pre-disturbance baseline Se concentrations in the Bay or tidal reaches of the rivers would be concentrations in the Sacramento River mixed with concentrations in coastal waters, as reflected by the salinity of the sampling location. Deviations from that baseline reflect inputs of Se internal to the Bay (industrial or local streams) (Cutter and San Diego-McGlone 1990; Cutter and Cutter 2004) or input of Se to the Bay from the San Joaquin River.

The current San Joaquin River contributions to the Bay, thought to be minimal during most flow conditions, are especially difficult to measure (Appendix A.1). However, that could change. Under some proposals for modifications in water infrastructure, increased diversion of the Sacramento River through tunnels or canals would be accompanied by greater inflows from the San Joaquin River to the Delta and the Bay. In simulations available of the implications of such a change, Meseck and Cutter (2006) found that Se concentrations doubled in particulate material in the Bay.

The conceptual model described above suggests that parameters critical in determining the mass balance model for Se inputs for the Bay-Delta are (1) total river discharge (Sacramento River and San Joaquin River); (2) water diversions or exports (i.e., pumping at Tracy and Clifton Court Forebay south to the Delta–Mendota Canal and the California Aqueduct); (3) proportion of the San Joaquin River directly

Submodel A

Sources and Hydrology

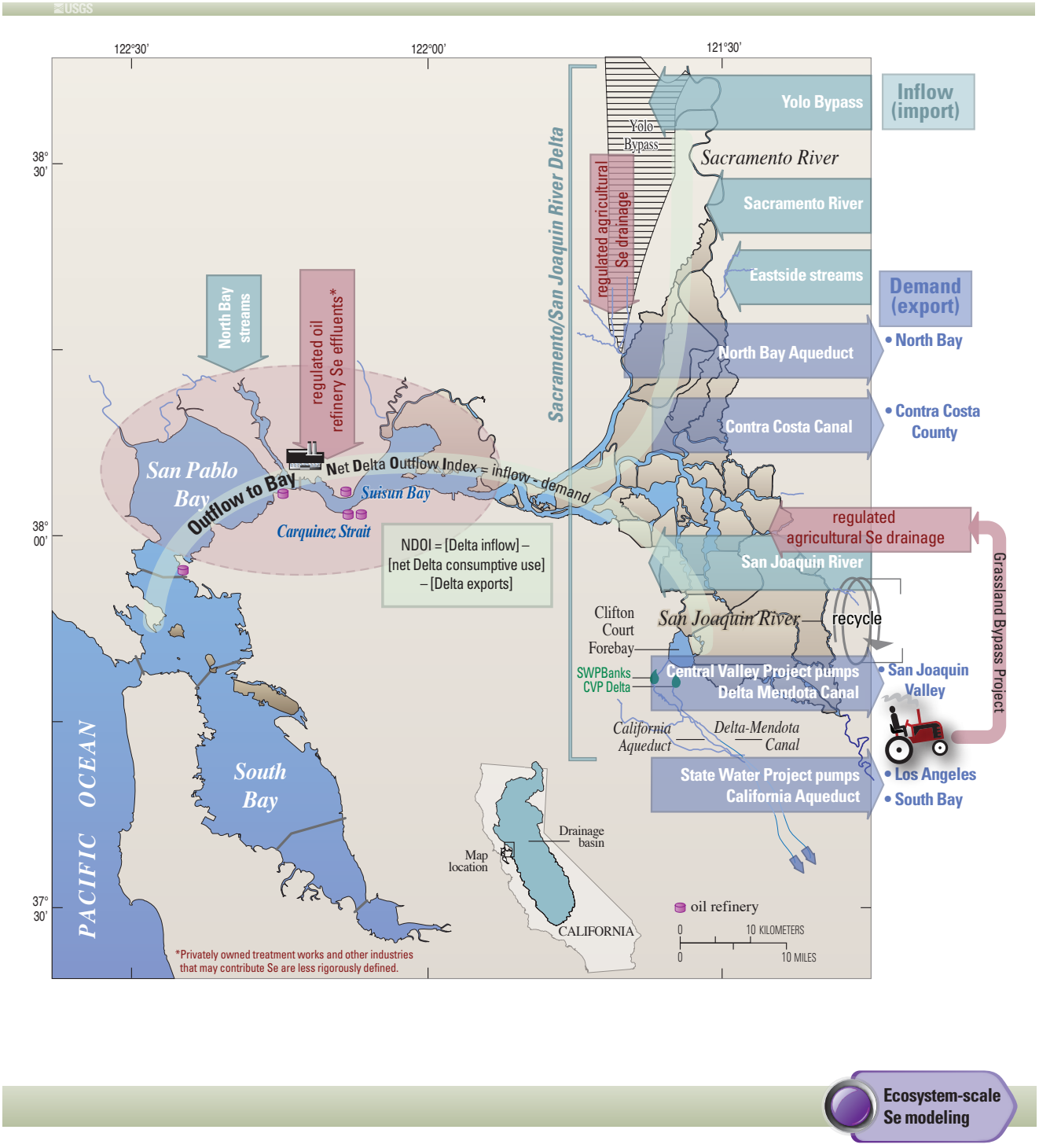

Figure 2 Submodel A. Sources and Hydrology

recycled south before it enters the Bay; 4) Se concentrations in each of the internal and external sources; and 5) total outflow of the rivers to the Bay or Net Delta Outflow Index (NDOI).

There are several uncertainties in quantification of the Se mass balance. One is the difficulty of precisely defining the contribution of the San Joaquin River to the NDOI, and hence the agricultural component of Se inputs to the Bay. Diversions and Delta hydrodynamics are sufficiently complex that every method available to determine that contribution has serious uncertainties (e.g., subtracting Sacramento River flow at Rio Vista from NDOI). Simple water accounting suggests minimal potential for flow from the San Joaquin River to enter the Bay (i.e., as measured by the percent by which river flow at Vernalis exceeds total export) during many months of the year (USBR 2012). Inputs are possible during spring months (April and May), wet and above normal years, and times of low capture efficiency (e.g., when river barriers are in-place) or when the ratio of the Sacramento River and San Joaquin River discharges is lowest in the fall.

A second uncertainty is that the strong tidal circulation in the Bay and the Delta mixes dissolved and particulate Se through the entire tidal reach, distorting spatial patterns that might otherwise help identify important sources of Se input (Ganju and others 2004). The three-dimensional nature of tidally driven hydrodynamics dissociates distributions of dissolved and particulate Se as well, adding complexity. One important outcome of this is that particulates contaminated with Se from industrial sources in Suisun Bay could feasibly be found throughout the full tidal range in both rivers, including otherwise uncontaminated segments of the Sacramento River. Riverine endmember concentrations of particulate Se, therefore, must be defined from landward of the reach of the tides, although river discharge at those locations does not necessarily represent riverine outflow to the Bay. Collecting an adequate mass of suspended particulate material for Se analysis in non-tidal freshwaters is challenging; therefore, few such data exist for the Sacramento River and even for some of the areas possibly affected by agricultural drainage. Hydrodynamic models of varying complexity are available that can approximate water movements in this complex situation (e.g., Delta Simulation Model II). But modeling the distribution of particulate material (crucial for understanding implications of Se) is much more difficult (Ganju and others 2004).

Links Between Source Inputs and Water Inflows

Both Sacramento River and San Joaquin River discharges vary dramatically during the year depending on runoff, water management, and diversions. Residence (or retention) time is affected by river discharges (e.g., Cutter and Cutter 2004), but the strong tidal influences make that difficult to precisely define. Nevertheless, even a coarse differentiation of seasonal periods (low flow and high flow) and classification by water year (critically dry, dry, below normal, normal, above normal and wet) can be useful in evaluating influences on processes important to the fate and bioavailability of Se (Presser and Luoma 2006). Empirical data suggest processes such as dilution of local inputs and phase transformations that incorporate Se into organic particulate material appear to be affected by changes in retention time in the estuary, at least to some extent (Cutter and Cutter 2004; Doblin and others 2006; Presser and Luoma 2006, 2010a, 2010b). For example, Cutter and San Diego-McGlone (1990) found that a peak in selenite concentrations was centered around the area of inputs from oil refineries during low riverine inflows to the Bay in the 1980s; but that peak disappeared during periods of high riverine discharge. They used a one-dimensional model of the water and a Se mass balance to show that the mass of Se discharged by the refineries was the dominant source of selenite during low flows, but that it was insignificant compared to the mass of Se input from the Sacramento River during high flows. The selenite peak was reduced and replaced by a different pattern of dissolved Se speciation when Se discharges from the refineries were reduced by about half in 1999 (Cutter and Cutter 2004). Similarly, high Se concentrations in the southernmost Delta (Stockton) reflect San Joaquin River inputs, but concentrations seaward of this location decline as they are diluted by the large volumes of Se-poor Sacramento River water channeled into the Delta for export (Lucas and Stewart 2007). Local

tributaries could be an internal source of Se to the Bay, but these inputs occur almost entirely during high riverine inflow periods when their Se loads are insignificant compared to the large mass of Se carried into the Bay by high discharge from the Se-poor Sacramento River.

The NDOI, essentially inflow minus demand, is often used to indicate hydrologic influences on Se concentrations, including differences in retention time of a parcel of water in the Bay and Delta (Cutter and Cutter 2004). Increased exposure time (i.e., the cumulative amount of time a particle spends within a domain, taking into consideration repeated visits over multiple tidal cycles; L. Doyle, W. Fleenor, and J. Lund, University of California, Davis, pers. comms.; 2012) at the lowest inflows may explain why NDOI is a relevant indicator of the effect of flow on processes such as conversion of Se from dissolved to particulate forms.

Exports

The Delta–Mendota Canal, California Aqueduct, Contra Costa Canal, and South Bay Aqueduct all export water from the Delta. Thus, all are secondary recipients of the Se sources considered here (submodel A, Figure 2). The Delta–Mendota Canal also receives agricultural drainage directly, with that source proposed to be under regulatory control (USFWS 2009; USBR 2011). In general, however, few data are available to assess a mass balance for Se through the State Water Project, Central Valley Project, and other water-delivery systems.

In terms of export of Se to the Pacific Ocean from the Bay, some data are available for seaward locations in the Bay. Dissolved concentrations at these locations are among the lowest observed in the system when not under flood flows (Cutter 1989; Cutter and San Diego–McGlone 1990; Cutter and Cutter 2004); particulate concentrations are occasionally high, however. Under shorter residence times during high flows, increased dissolved concentrations near the Golden Gate Bridge (Cutter and Cutter 2004) suggest sources internal to the Bay affect ocean-dissolved Se concentrations. Outflows to the sea have been estimated in simple mass balance models (Cutter and San Diego-McGlone 1990) although there are some uncertainties in such estimates. Ocean disposal was considered as one of the alternatives for comprehensive agricultural drainage management from the western San Joaquin Valley (USBR 2006). However, efficient Se recycling within productive ocean ecosystems and the opportunities for Se biomagnification in complex marine food webs suggest serious risks are likely (Cutter and Bruland 1984); hence, there are reasons for careful study before such options are considered.

Ecosystem-Scale Selenium Modeling

Dissolved Selenium Concentrations, Speciation, and Transformation

Total dissolved Se concentrations within the Bay range from 0.070 to 0.303 μg L^{-1}, with a mean of 0.128 ± 0.035 μg L^{-1} and a median of 0.125 μg L^{-1} across 128 samples collected since 1997 (Doblin and others 2006; Lucas and Stewart 2007). The mean concentration is only approximately two times higher than Se concentrations in the dominant freshwater endmember (the Sacramento River). In all surveys since the 1980s, Se concentrations in the tidal Bay and Delta are highest in Suisun Bay, with a downward spatial trend from Carquinez Strait toward the ocean. The latter suggests that dissolved concentrations in the ocean endmember are about the same as those in the Sacramento River.

The dissolved gradients of Se concentration are not necessarily the best indicators of the distribution of Se effects. Ecological implications depend upon the biogeochemical transformation from dissolved to particulate Se. Phase transformation of Se is of toxicological significance because particulate Se is the primary form by which Se enters food webs (Figures 1, 3 and 4) (Luoma and others 1992). Speciation of dissolved Se into its three dominant oxidation states is an important component in many conceptual models. In the Bay-Delta, speciation of dissolved Se is important because it influences the type and rate of phase transformation reaction that creates particulate Se. Examples of phase transformation reactions include (1) uptake by plants and phytoplankton of selenate, selenite, or dissolved organo-Se and transformation to particulate organo-Se by

Submodel B

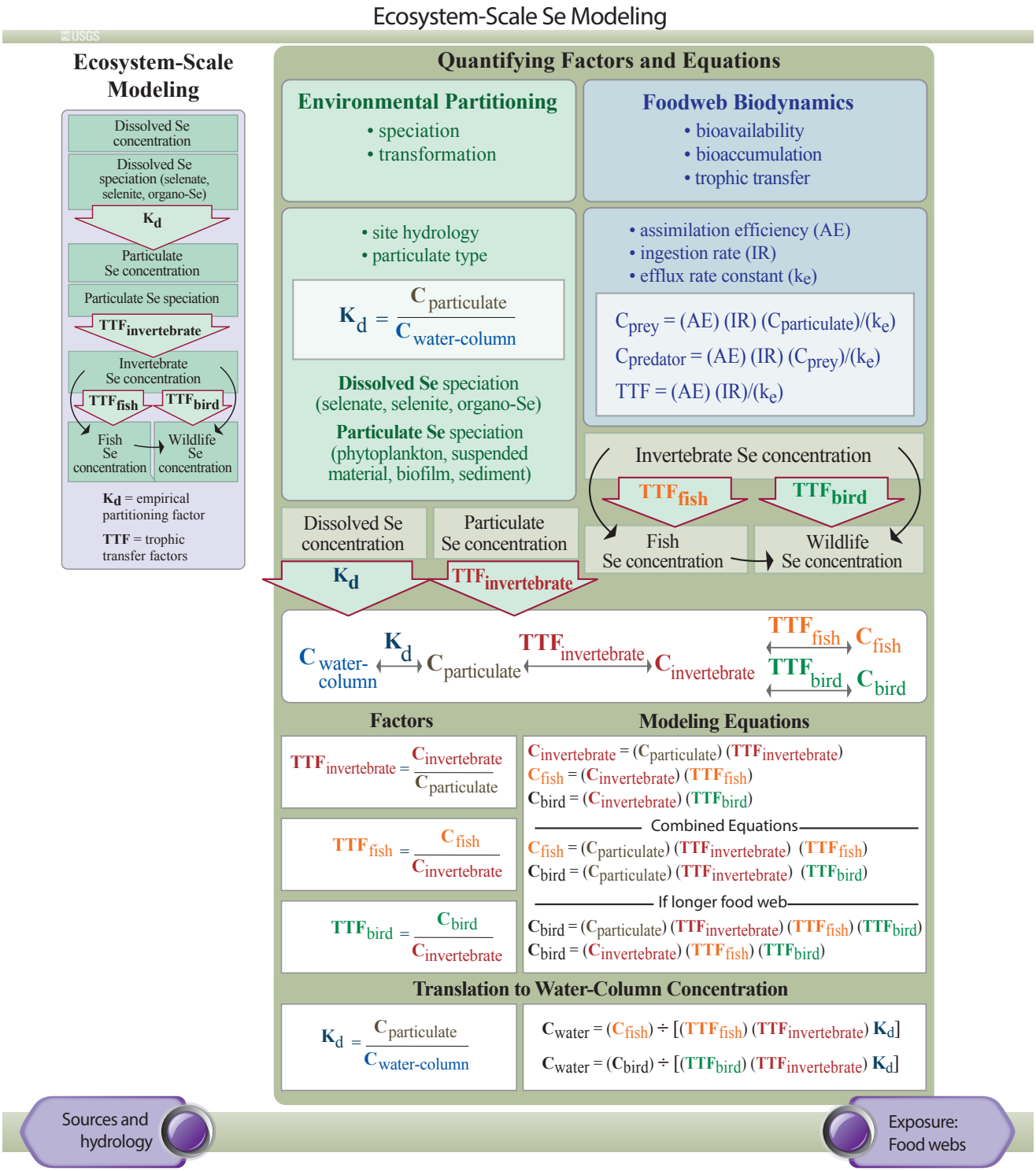

Figure 3 Submodel B. Ecosystem-Scale Se Modeling

assimilatory reduction, where uptake of selenate is considerably slower than uptake of the other two forms (e.g., Sandholm and others 1973; Riedel and others 1996; Wang and Dei 1999; Fournier and others 2006); (2) sequestration of selenate into sediments as particulate elemental Se by dissimilatory biogeochemical reduction (e.g., Oremland and others 1989); (3) adsorption as co-precipitated selenite through reactions with particle surfaces; and (4) recycling of particulate phases back into water as detritus or as dissolved organo-Se, after organisms die and decay (e.g., Velinsky and Cutter 1991; Reinfelder and Fisher 1991; Zhang and Moore 1996).

These different biogeochemical transformation reactions result in different forms of Se in particulate material: organo-Se, adsorbed Se, or elemental Se. Although only a few studies have determined speciation of particulate Se (e.g., Doblin and others 2006), such data can greatly aid in understanding bioavailability. Experimental studies show that particulate organo-Se is the most bioavailable form when it is eaten by a consumer species (Luoma and others 1992). Detrital or adsorbed Se is also bioavailable when ingested by animals, although to a lesser extent than organo-Se (Wang and others 1996). Non-particle associated elemental Se is not bioavailable (Schlekat and others 2000).

Concentrations of Se in particulate materials (per unit mass material) within the Bay and tidal freshwaters range widely from 0.1 to 2.2 $\mu g\ g^{-1}$ dry weight (dw), with a mean of $0.56 \pm 0.32\ \mu g\ g^{-1}$ dw and a median of $0.45\ \mu g\ g^{-1}$ dw ($n = 128$) since 1997 (Doblin and others 2006; Lucas and Stewart 2007). The 15-fold range in particulate concentrations contrasts sharply with the 4-fold range in dissolved concentrations, as do the contrasts in standard deviations. Not only are particulate concentrations much more dynamic than dissolved concentrations, but they also are about four times higher if expressed in common units. Both reflect biogeochemical transformation processes and, perhaps, inorganic adsorption. The latter is probably more important in soils than in the aquatic environment. Given the different dynamics and the variability of dissolved and particulate Se, it is not surprising that the ratio of the two also is quite variable.

Geochemical models that attempt to capture phase transformations of Se under different conditions are problematic. In fact, no models are available that can predict particulate Se concentrations based solely upon dissolved concentrations and biogeochemical conditions. One reason is that conventional thermodynamic equilibrium-partitioning models are inadequate for Se. Critical Se transformation processes are biological, and not predictable from thermodynamics. Some model approaches predict the particulate Se added on to a pre-existing particulate concentration, using a combination of phytoplankton productivity and re-suspension (Meseck and Cutter 2006; SWRCB 2011; Tetra Tech, Inc. 2010). While such models provide interesting estimates of temporal and spatial distributions of particulate Se, their major limitations lie in the basis upon which the pre-existing concentration is chosen and their inability to comprehensively account for all the processes involved in transformation.

The choice of the (pre-existing) baseline particulate Se concentration is critical to the questions models can address. Local data can be used for choosing pre-existing Se concentrations at the seaward and landward boundaries in the Bay-Delta. But the data used to date are from tidally affected reaches of the river, and are likely to be biased by redistribution of already contaminated particles from tidal pumping. As noted above, few data exist for particulate Se concentrations above the tidal reach of the Sacramento River; nor are there adequate determinations of Se concentrations on particulates from the coastal zone. In such a case, answers to questions about changing the internal Se inputs to the Bay are biased in that the boundary condition already includes such inputs (SWRCB 2011; Tetra Tech, Inc. 2010). On the other hand, this modeling approach appears to be well suited to test the influence of changing inputs from one boundary or from primary production alone (Meseck and Cutter 2006; Tetra Tech, Inc. 2010).

Observations of environmental partitioning of Se between dissolved and particulate phases can be employed to estimate transformation efficiencies in lieu of a comprehensive approach to modeling biogeochemical phase transformation for Se. Presser and Luoma (2006) first used field observations to

quantify partitioning, which they described by the somewhat controversial term K_d. Luoma and Presser (2009) were careful to emphasize that their K_ds represented conditional observations from the Bay-Delta at a specific time and place; and were not meant to be equilibrium constants. Thermodynamic equilibrium constants would be inappropriate to describe an inorganic to organic transformation. They pointed out that no single constant could be expected to apply to all environmental conditions either in the Bay-Delta or elsewhere. Site hydrology, dissolved speciation, and the type of particulate material are all influential, although specific influences were not necessarily predictable in quantitative terms. An operational approach was therefore chosen to try to estimate influences of such processes.

They defined K_d as the ratio of particulate material Se concentration (in dw) to the dissolved Se concentration observed at any instant in simultaneously collected samples. The specific equation is

$$K_d = (C_{\text{particulate material}}, \mu g\ kg^{-1}\ dw) \div (C_{water}, \mu g\ L^{-1}) \quad (1)$$

Of interest here is the particulate matter at the base of the food web. As sampled in the environment that can include suspended particulate Se (which is a physically inseparable mix of phytoplankton, periphyton, detritus and inorganic suspended material), biofilm, sediment and/or attached vascular plants. Feeding characteristics of the organisms in question and data availability dictate the best choice among these. For example, for a filter-feeding bivalve in the Bay-Delta, Se concentrations determined in suspended particulate material (in $\mu g\ g^{-1}$ dw) are the preferred parameter for modeling because these animals filter their food from the water-column.

Some broad generalizations are possible about K_ds for Se (Presser and Luoma 2010a). For example, if all other conditions are the same, K_d will increase as selenite and dissolved organo-Se concentrations increase relative to selenate. Calculations using data from laboratory microcosms and experimental ponds show speciation-specific K_ds of 140 to 493 where selenate is the dominant form; 720 to 2,800 when an elevated proportion of selenite exists; and 12,197 to 36,300 for 100% dissolved seleno-methionine uptake into algae or periphyton (Besser and others 1989; Graham and others 1992; Kiffney and Knight 1990). Compilations of K_ds also show different general ranges for rivers, streams, lakes, ponds, wetlands, and estuaries that are affected by Se inputs (Presser and Luoma 2010a), although with some overlap. Exposure time for phase transformation is probably an important factor driving differences among such systems. Estuaries are among the sites with the highest values (range of medians from 4,000 to 21,500) indicating efficient conversion of dissolved Se to particulate Se. Finally, although the influence of exposure time for a particle within an estuary is challenging to understand precisely, especially in the Bay-Delta because of the dominance of tidally driven circulation, K_ds seem to be higher during conditions where more time is available for transformation reactions to occur (Presser and Luoma 2010b).

The most recent transects of the Bay that provide spatially and temporally matched data for derivation of K_ds from dissolved and particulate Se concentrations were from June 1998 to November 1999 (Cutter and Cutter 2004; Doblin and others 2006). In these studies, samples were collected at 1 meter below the surface, and included dissolved Se concentrations, suspended particulate material Se concentrations, dissolved Se speciation, suspended particulate Se speciation, salinity, and total suspended material. These data were collected in four different transects across the salinity gradient in the Northern Reach under a variety of river discharge and presumed residence time conditions. The full range of dissolved Se concentrations in these transects was 0.070 to 0.303 $\mu g\ L^{-1}$. The suspended particulate material Se concentrations were more variable: 0.15 to 2.2 $\mu g\ g^{-1}$ dw. Calculated K_ds ranged from 712 to 26,912. The degree of variability across this whole data set is large. However, the largest part of the variability was driven by very high values in the landward-most and seaward-most samples, where dissolved concentrations were very low. Such ratios can be artificially inflated when values become very low in the denominator, if the numerator does not decline as rapidly. Tidal pumping of contaminated particles from the Bay upstream into the less contaminated Sacramento River water is a possible cause of such an effect.

Downstream transport of highly contaminated particles from the San Joaquin River into Bay or Delta water could also be a cause. Finally, seaward, where residence times are elevated in Central and San Pablo bays, biological transformation could enrich Se in particles while depleting it from the water column. If the goal is to find conditions where there is sufficient linkage between dissolved and particulate Se to be useful in forecasts of one from the other, none of these conditions would apply. Presser and Luoma (2010b) avoided such biases and thereby constrained variability by restricting K_ds geographically to the middle range of the salinity zone in Suisun Bay. This also focused the modeling on the most contaminated segment of the estuary.

If location is restricted to Carquinez Strait–Suisun Bay—eliminating freshwater and ocean interfaces—then the range of dissolved Se concentrations is narrowed to 0.076 to 0.215 µg L^{-1} and the range of suspended particulate material Se concentrations is narrowed to 0.15 to 1.0 µg g^{-1} dw. The variation of K_d is narrowed to a range of means of 1,180 to 5,986 (or of individual measurements, 712 to 7,725). Because this data set is still large, median or mean concentrations, or a given percentile, can be used as viable indicators of partitioning in modeling scenarios.

Seasonality also is important, and restrictions to specific flow regimes also can be used to constrain variability. For example, the highest mean K_ds occur during periods of the lowest river inflows (and highest residence times). Constrained to Suisun Bay, the mean K_d was 1,180 ± 936 in June 1998. This was a high flow season wherein Cutter and Cutter (2004) estimated a residence time of 11 days. The mean K_d was 5,986 ± 1,353 in November 1999. This was a low flow season with an estimated residence time of 70 days. The mean K_d among all constrained samples was 3,317, and the mean for low flow seasons was 4,710.

Transects in the Delta were also conducted between 1998 and 2004 in different flow regimes (Doblin and others 2006; Lucas and Stewart 2007). Dissolved Se concentrations among all these samplings ranged from 0.083 to 1.0 µg L^{-1}, with a mean of 0.25 ± 0.24 (n = 72). Particulate concentrations ranged from 0.27 to 6.3 µg g^{-1} dw, with a mean of 0.98 ± 0.94 (n = 71). As in the Bay transects, the range in particulate concentrations (23-fold) exceeds the range in dissolved concentrations (12-fold). Concentrations and variability, thus, were even greater in the Delta, overall, than in the Bay. In the Delta, K_ds ranged from 554 to 38,194, with the range of means from 1,886 ± 1,081 in January 2003 (a high flow season) to 7,712 ± 3,282 in July 2000 (a low flow season). Sets of dissolved and particulate Se concentrations determined as part of focused research for the Delta in September 2001, the low flow season of a dry year, yielded some especially elevated K_ds (>10,000) (Lucas and Stewart 2007). In general, these elevated K_ds may reflect tidal pumping, or represent times and areas where Se is concentrating in particulate material because of differing hydrologic environments (e.g., slow-moving backwaters with high productivity). Constraining variability is more difficult in the Delta, hence, quantifying phase transformation from empirical data is more uncertain in this system.

Given the degree of variability in both the Bay and the Delta, modeling that requires linking dissolved Se to particulate Se should include several scenarios using different K_ds that are within a range of values constrained, as described above.

Uptake Into Food Webs

Kinetic bioaccumulation models (i.e., biodynamic models, Luoma and Fisher 1997; Luoma and Rainbow 2005, 2008) account for the now well-established principle that Se bioaccumulates in food webs principally through dietary exposure. Uptake attributable to dissolved exposure makes up less than 5% of bioaccumulated Se in almost all circumstances (Fowler and Benayoun 1976; Luoma and others 1992; Roditi and Fisher 1999; Wang and Fisher 1999; Wang 2002; Schlekat and others 2004; Lee and others 2006). Biodynamic modeling (submodels B and C, Figures 3 and 4) shows that Se bioaccumulation (the concentration achieved by the organism) is driven by physiological processes specific to each species (Reinfelder and others 1998; Wang 2002; Baines and others 2002; Stewart and others 2004). Biodynamic models have the further advantage of providing a basis for

deriving a simplified measure of the linkage between trophic levels: TTFs. For each species, a TTF can be derived from either experimental studies or field observations.

Experimental derivation of TTFs is based on the capability of a species to accumulate Se from dietary exposure as expressed in the biodynamic equation (Luoma and Rainbow 2005):

$$dC_{species}/dt = (AE)\ (IR)\ (C_{food}) - (k_e + k_g)\ (C_{species}) \quad (2)$$

where $C_{species}$ is the contaminant concentration in the animals (µg g^{-1} dw), t is the time of exposure in days (d), AE is the assimilation efficiency from ingested particles (%), IR is the ingestion rate of particles (g g^{-1} d^{-1}), C_{food} is the contaminant concentration in ingested particles (µg g^{-1} dw), k_e is the efflux rate constant (d^{-1}) that describes Se excretion or loss from the animal, and k_g is the growth rate constant (d^{-1}). Key determinants of Se bioaccumulation are the ingestion rate of the animal, the efficiency with which Se is assimilated from food, and the rate constant that describe Se turnover or loss from the tissues of the animal (Luoma and Rainbow 2005; Presser and Luoma 2010a). Experimental protocols for measuring such parameters as AE, IR, and k_e are now well developed for aquatic animals (Luoma and others 1992; Wang and others 1996; Luoma and Rainbow 2005). The rate constant of growth is significant only when it is comparable in magnitude to the rate constant of Se loss from the organism. Consideration of the complications of growth can usually be eliminated if the model is restricted to a long-term, averaged accumulation in adult animals (Wang and others 1996).

In the absence of rapid growth, a simplified, resolved biodynamic exposure equation for calculating a Se concentration in an invertebrate (submodel B, Figure 3) is

$$C_{invertebrate} = [(AE)(IR)(C_{particulate})] \div [k_e] \quad (3)$$

For modeling, these physiological parameters can be combined to calculate a $TTF_{invertebrate}$, which characterizes the potential for each invertebrate species to bioaccumulate Se. $TTF_{invertebrate}$ is defined as

$$TTF_{invertebrate} = [(AE)(IR)] \div k_e \quad (4)$$

Similarly, foodweb biodynamic equations for fish or birds are

$$C_{fish\ or\ bird} = [(AE)\ (IR)\ (C_{invertebrate})] \div k_e \quad (5)$$

and

$$TTF_{fish\ or\ bird} = [(AE)\ (IR)] \div k_e \quad (6)$$

Where laboratory data are not available, TTFs can be defined from field data, where the TTF defines the relationship between Se concentrations in an animal and in its food in dw. The field $TTF_{invertebrate}$ must be defined from spatially and temporally matched data sets (in dw or converted to dw) of particulate and invertebrate Se concentrations (submodel B, Figure 3) as

$$TTF_{invertebrate} = C_{invertebrate} \div C_{particulate} \quad (7)$$

A field derived species-specific TTF_{fish} is defined as

$$TTF_{fish} = C_{fish} \div C_{invertebrate} \quad (8)$$

where $C_{invertebrate}$ is for a known prey species, C_{fish} is reported as muscle or whole-body tissue, and both Se concentrations are reported in µg g^{-1} dw (submodel B, Figure 3).

Whether the TTFs are determined from the laboratory or the field, the modeling approach is sufficiently flexible to represent complexities such as mixed diets. For example, a diet that includes a mixed proportion of prey in the diet can be addressed using the equation

$$C_{fish} = (TTF_{fish})\ [(C_{invertebrate\ a})\ (\text{prey fraction}) + (C_{invertebrate\ b})\ (\text{prey fraction}) + (C_{invertebrate\ c})\ (\text{prey fraction})] \quad (9)$$

Equations are combined to represent step-wise bioaccumulation from particulate material through invertebrates to fish (submodel B, Figure 3) as

$$C_{fish} = (TTF_{invertebrate})\ (C_{particulate})\ (TTF_{fish}) \quad (10)$$

Similarly, for birds, the combined equation is

$$C_{bird} = (TTF_{invertebrate})\ (C_{particulate})\ (TTF_{bird}) \quad (11)$$

Modeling can accommodate longer food webs that contain more than one higher trophic level consumer (e.g., forage fish being eaten by predatory fish) by

incorporating additional TTFs. One equation for this type of example (submodel B, Figure 3) is

$$C_{predator\ fish} = (TTF_{invertebrate})\ (C_{particulate})\ (TTF_{forage\ fish})\ (TTF_{predator\ fish}) \quad (12)$$

Modeling for bird tissue also can represent Se transfer through longer or more complex food webs (e.g., TTFs for invertebrate to fish and fish to birds) as

$$C_{bird} = (TTF_{invertebrate})\ (C_{particulate})\ (TTF_{fish})\ (TTF_{bird}) \quad (13)$$

Variability or uncertainty in processes that determine AEs or IRs can be directly accounted for in sensitivity analysis (Wang and others 1996). This is accomplished by considering the range in the experimental observations for the specific animal in the model. Field-derived factors require some knowledge of feeding habits, and depend on available data for that species. Laboratory and field factors for a species can be compared and refined to reduce uncertainties in modeling (Presser and Luoma 2010a).

A substantial number of species-specific TTFs are available (Luoma and Presser 2009; Presser and Luoma 2010a). These are enough data at least to begin to model important food webs. Across invertebrate species, TTFs range from 0.6 to 23. Of the 29 species studied, 27 species have TTFs > 1. Thus, most invertebrate species bioaccumulate as much as or more Se than concentrated in the trophic level below them. In other words, the concentration of Se biogeochemically transformed into algae, microbes, seston, or sediments is preserved and/or (bio)magnified as Se passes up food webs. In general, TTFs for bivalves (clams, mussels, oysters) and for barnacles are the highest among species of invertebrates (i.e., an experimentally determined TTF range of approximately 4 to 23) (Presser and Luoma 2010a).

Trophic transfer factors from the available data for fish have a median of approximately one, and vary much less than among invertebrates: from 0.5 to 1.8 (Presser and Luoma 2010a). Compilations show that TTFs derived from laboratory biodynamic experiments range from 0.51 to 1.8; TTFs for different fish species derived from field studies are similar, ranging from 0.6 to 1.7.

Trophic transfer factors for aquatic birds (diet to bird egg) are less well developed, and laboratory data are limited (Presser and Luoma 2010a). The most robust data from the laboratory relate Se concentrations in the diet (as seleno-methionine) to egg Se concentrations from controlled feeding of captive mallards (*Anas platyrhynchos*). The range of $TTF_{bird\ egg}$ calculated from the compilation of nominal experimental diet Se concentrations and mean egg Se data given in Ohlendorf (2003) for mallards is 1.5 to 4.5. Using the detailed data from Heinz and others (1989) narrows this range to 2.0 to 3.9, with a mean of 2.6. Field data could be used to refine $TTF_{bird\ egg}$ on a site-specific basis, but variability in food sources and habitat use may add uncertainty to such data, and limits applications among habitats.

Exposure: Food Webs, Seasonal Cycles, and Habitat Use

Selenium is at least conserved and usually biomagnified at every step in a food web (Presser and Luoma 2010a). Selenium toxicity is generally assumed to be observed first in specific predator species as differences in food web exposure are propagated up trophic pathways (Luoma and Rainbow 2005; Stewart and others 2004). Some invertebrate species also may be susceptible to environmentally relevant Se concentrations (Conley and others 2009, 2011). Selenium is usually not detoxified in animal tissues by conjugation with metal-specific proteins or association with non-toxic inclusions (Luoma and Rainbow 2008). Hence, general mechanisms that semi-permanently sequester metals in non-toxic forms and lead to progressive accumulation with size or age probably are less applicable to the metalloid Se than to metals in general (Luoma and Presser 2009).

Predator population distribution, feeding preference, prey availability, life stage, gender, physiology, and species sensitivity are all variables that influence how a predator is affected by Se. Field factors such as varying weather, water depth, human disturbance, and food dispersion also affect foraging energetics, and accessibility of contaminants in foods on a localized level. Despite these complexities, some generalizations are possible at the present state of

understanding. Predator species for the Bay-Delta, their food webs, and potential exposure are shown in submodels C and D (Figures 4 and 5), with further supporting information compiled in Appendix A.2 and A.3.

Based upon studies of invertebrate bioaccumulation the greatest exposures to Se will occur in predators that ingest bivalves in the Bay-Delta (Stewart and others 2004; Presser and Luoma 2006, 2010b). The estimated maximum percentages of diet that are clam-based for various benthic predators were estimated by the USFWS (2008a) (submodel C, Figure 4): lesser scaup 96%; surf scoter 86%; greater scaup 81%; black scoter 80%; white-winged scoter 75%; California clapper rail 64%; bald eagle 23%; white sturgeon (and assumed for green sturgeon) 41%; and Sacramento splittail (2-year olds) 34%. Dietary estimates are not specific to *C. amurensis*, but a bivalve component to diet in general. Bald eagles are an example of a predator with a diet wherein 23% are those waterfowl (scaups and scoters) that primarily feed on benthic mollusks (USFWS 2008a). Clapper rails feed on benthic food webs, but are littoral feeders that usually do not eat *C. amurensis*, which is mostly subtidal. Figure 4 (submodel C) also shows potential food webs for Dungeness crab. Diet component data and kinetic loss rates are not documented for life stages of this crustacean, but isotopic data indicate that clams such as *C. amurensis* would be expected to be an important food for this species (Stewart and others 2004). Selenium concentration data, in turn, indicate that predators of this crab would be subjected to elevated dietary Se concentrations (submodel C, Figure 4).

Food webs illustrated for Delta inhabitants include aquatic insects to salmonids (submodel C, Figure 4). The diets of salmon and steelhead trout are dominated by species with TTFs lower than bivalves. These species thereby incur less dietary Se exposure than molluscivores. Field data for Se concentrations are limited to 1986 to 1987 for Chinook salmon (Saiki and others 1991) and absent for steelhead trout that inhabit the estuary and migration corridors. Although their exposures are not exceptionally high, these species may be vulnerable because of their toxicological sensitivity to Se (USFWS 2008a, 2008b; Janz 2012). Delta smelt are endemic to the estuary and are included here because population numbers for the Delta smelt are alarmingly low. Thus, the USFWS (2008a) concluded that this species is particularly vulnerable to any adverse effect. It should be noted, however, that the feeding habits of Delta smelt would not suggest high exposures compared to other species, and sensitivity or bioaccumulation data are not available.

Not all predators reside in the estuary throughout their lives. When a predator is present across flow seasons and during critical life stages may influence Se exposure and effects. Predator seasonal cycle diagrams are shown for migratory birds (scoter and scaup); breeding birds (California clapper rail, bald eagle); migrating/rearing juveniles (Chinook salmon, steelhead trout); and breeding fish (green sturgeon, white sturgeon, and Sacramento splittail) (submodel D, Figure 5). The North Bay is part of the migration corridor and feeding ground for anadromous fish such as white sturgeon, Chinook salmon, and striped bass. The estuary also serves seasonally as a nursery area for species that spawn either in freshwater (e.g., Sacramento splittail) or in the ocean (e.g., Dungeness crab). Migrating diving ducks on the Pacific flyway winter and feed in the estuary as they stage for breeding in the freshwater ecosystems of the boreal forests of Canada and Alaska (De La Cruz and others 2009). As migratory waterfowl move north to breed in the spring, there is the potential for depuration of Se (USFWS 2008a; Appendix A.2 and A.3).

Some of the highest *C. amurensis* Se concentrations of the annual cycle occur when overwintering scoter and scaup actively feed in Suisun Bay and San Pablo Bay during the fall and early winter, (Linville and others 2002; Kleckner and others 2010) (submodel D, Figure 5). Long-lived white sturgeon feed predominantly on *C. amurensis* and have a two-year internal egg maturation that makes them particularly vulnerable to loading of Se in eggs and reproductive effects (Linville 2006). As an indication of this potential, Linares and others (2004) found Se concentrations as high as 47 $\mu g\ g^{-1}$ dw in immature gonads of 39 white sturgeon captured in the estuary. In earlier studies, Kroll and Doroshov (1991) reported that Se concentrations in developing ovaries

Submodel C

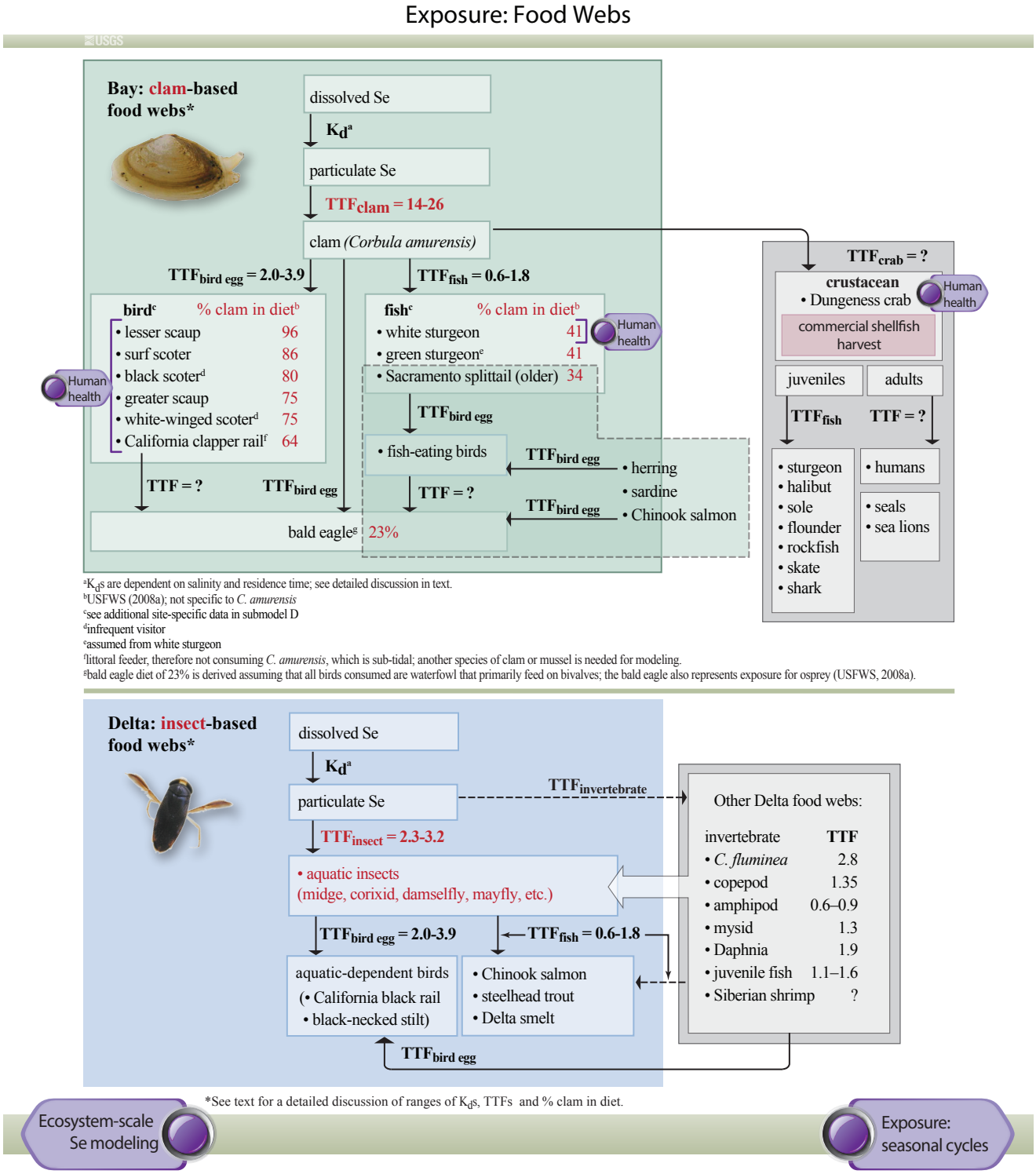

Figure 4 Submodel C. Exposure: Food Webs

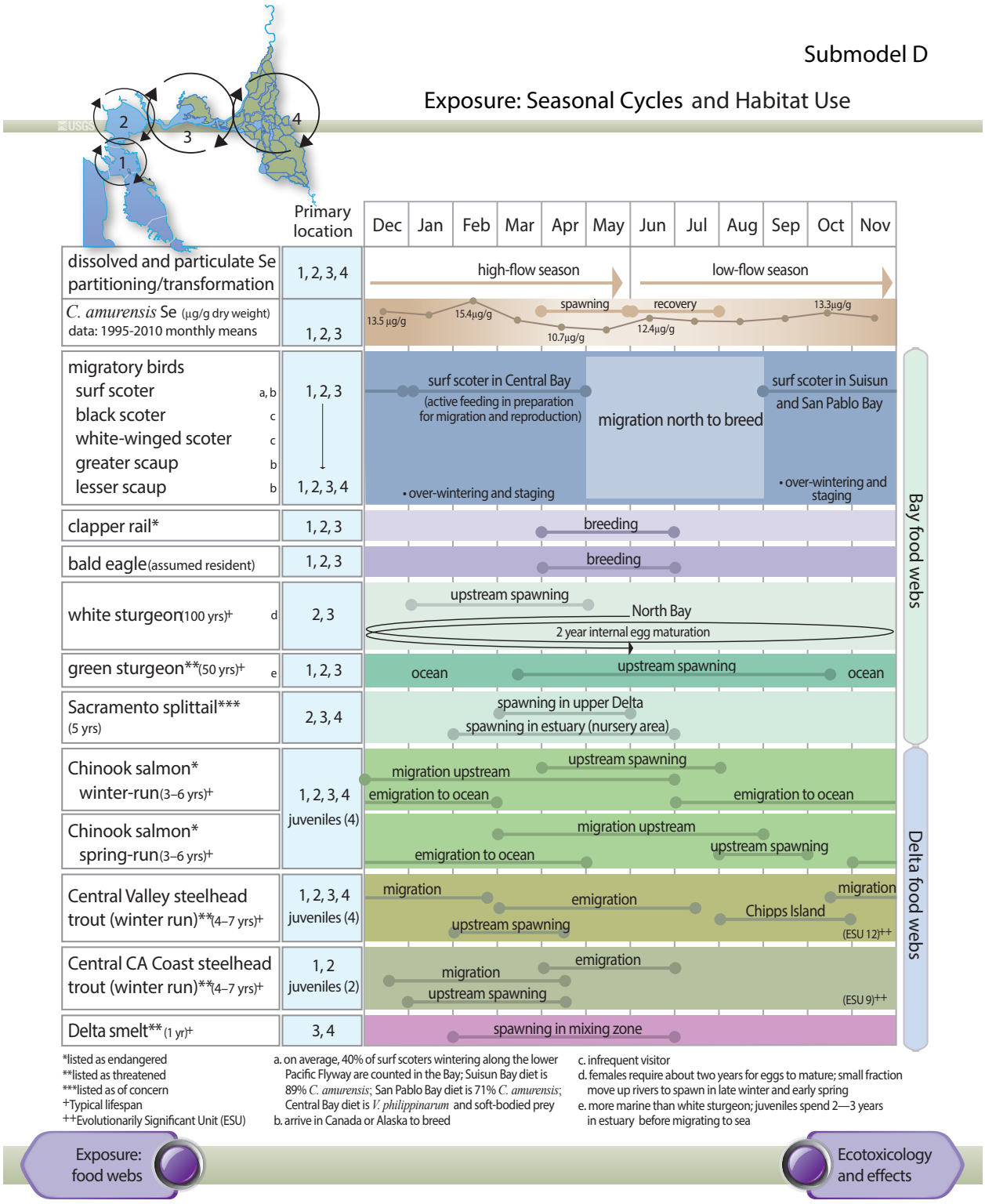

Figure 5 Submodel D. Exposure: Seasonal Cycles and Habitat Use

of white sturgeon from the Bay contained maxima of 72 µg $g^{-1}$and 29 µg g^{-1}. This range of wild white sturgeon reproductive tissue Se concentrations approach or exceed levels that cause severe deformities and mortalities in newly hatched larvae (Lemly 2002; Linville 2006). Larger, older Sacramento splittail also feed on *C. amurensis* and they are known to spawn both in the upper Delta and estuary (Stewart and others 2004). Modeling for species such as clapper rail would need specifics of diet composition (i.e., which species of clam, mussel, or crab is consumed), and whether prey species are efficient bioaccumulators of Se. Formalized, detailed knowledge such as this (submodel D, Figure 5), in turn, helps set choices in comparative modeling scenarios.

Fish and Wildlife Health: Ecotoxicology and Effects

Toxicity arises when dissolved Se is transformed to organic-Se by bacteria, algae, fungi, and plants (i.e., synthesis of Se-containing amino acids *de novo*) and then passed through food webs. It is generally thought that animals are unable to biochemically distinguish Se from sulfur, and therefore excess Se is substituted into proteins and alters their structure and function (Stadtman 1974). Other biochemical reactions also can determine and mediate toxicity (Chapman and others 2010). The effect of these reactions is recorded, most importantly in birds and fish, as failures in hatching or proper development (teratogenesis or larval deformities) (submodel E, Figure 6). Other toxicity endpoints include growth, winter survival, maintenance of body condition, reproductive fitness, and susceptibility to disease (submodel E, Figure 6; Appendix A.3). Specifically, Se can alter hepatic glutathione metabolism to cause oxidative stress (Hoffman and others 1998, 2002; Hoffman 2002) and diminished immune system function (Hoffman 2002).

Details of general ecotoxicological pathways of Se for fish and birds and effects of concern for Se are shown in submodel E (Figure 6). As represented here, birds and fish differ in how Se taken up from diet distributes among tissues (submodel E, Figure 6). Physiological pathways shown here for birds emphasize an exogenous dietary pathway and for fish an endogenous liver pathway. Species-specific Se effect models for the Bay-Delta are shown for breeding clapper rail; migratory scoter and scaup; white sturgeon; downstream-migrating juvenile salmonids; and upstream-migrating adult salmonids (submodel F, Figure 7). Details of Se-specific toxicological information for predator species considered here are compiled in Appendix A.3.

Such health effects are important to the overall ability of birds and fish to thrive and reproduce. But the consequences of Se transfer from the mother to her progeny via each reproductive stage are the most direct and sensitive predictors of the effects on birds and fish (Heinz 1996; Lemly 2002; Chapman and others 2010). Ultimately, it would be expected that effects on reproduction, especially in slowly reproducing, demographically vulnerable species (e.g., sturgeon), could lead to effects on populations and community changes.

To translate exposure into toxicity, effects levels are needed for predator species. Traditionally, guidelines relate Se concentrations in water to effects. But it is increasingly recognized that the concentrations of Se bioaccumulated in fish and bird tissues are more strongly related to signs of toxicity in nature, and would provide less ambiguous guidelines (Chapman and others 2010). The best correlations occur between Se in reproductive tissue and effects on reproductive processes. To assess implications of Se contamination in water from such relationships a bioaccumulation model is, then, necessary.

Experimental determination of tissue Se concentrations at which adverse effects occur is influenced by choice of endpoint, life-stage, dietary form, route of transfer, and choice of effect concentration. Another consideration in determining the guideline is the steepness of the Se dose-response curves and the choice of mathematical models to describe the curve (Skorupa 1998; Ohlendorf 2003; Lemly 2002; Environment Canada 2005; Beckon and others 2008; Chapman and others 2010). Effect guidelines that focus on a combination of the most sensitive assessment measures might include, for example, a selenomethionine diet, parental exposure, and embryonic or larval life-stage effect (Presser and Luoma 2006).

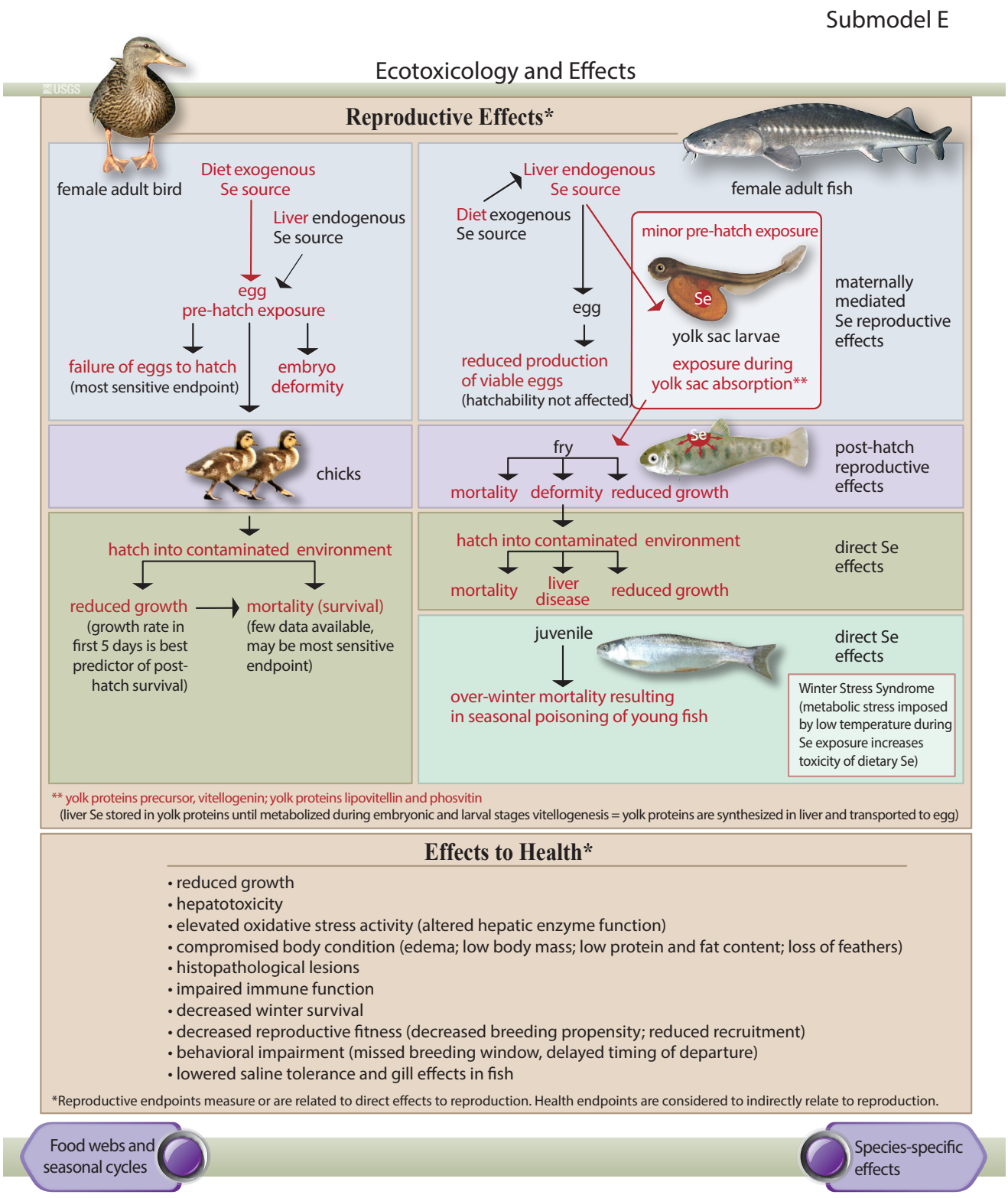

Figure 6 Submodel E. Ecotoxicology and Effects

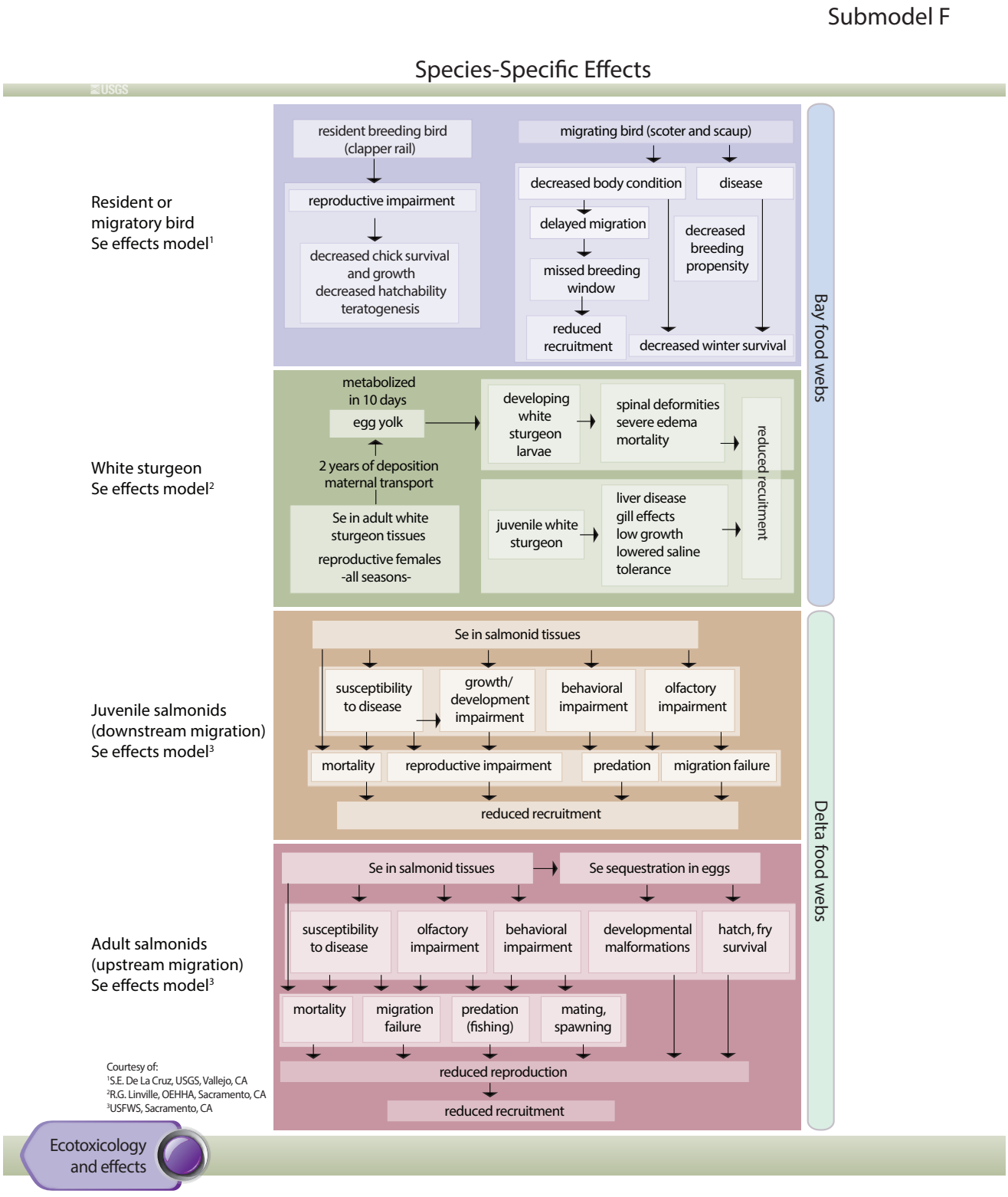

Figure 7 Submodel F. Species-Specific Effects

Even then the choice of statistical analysis and effect level can lead to disagreement about effect guidelines.

Human Health

A number of species from the Bay-Delta are consumed by humans (submodel G, Figure 8). Human health advisories against consumption of greater scaup, lesser scaup, and scoter because of elevated Se levels have been in effect since 1986 (Presser and Luoma 2006) for Suisun Bay, San Pablo Bay, Central Bay, and South Bay (CDFG 2012, 2013). The health warning states that no one should eat more than four ounces of scaup meat per week or more than four ounces of scoter meat in any two week period. Further, no one should eat the livers of ducks from these areas.

Fish consumption advisories, including for white sturgeon, exist for the Bay because of the effect of mercury and PCBs (OEHHA 2011, 2012). Pesticides, flame retardants, and Se also were tested, but a mean concentration calculated for each fish species collected from locations throughout the Bay-Delta over a range of years was found to be below that chemical's advisory tissue level (OEHHA 2011, 2012). Specifically for Se, concentrations in white sturgeon (n = 56 during 1997 to 2009, or 4.3 fish per year) were higher than other species of fish tested; and some Se concentrations for white sturgeon collected in North Bay locations (maximum 18.1 µg g^{-1} dw) exceeded Se advisory levels (e.g., 10.4 µg g^{-1} dw or 2.5 µg g^{-1} wet weight based on consumption of three 8-ounce meals per week (OEHHA 2011, 2012). Length restrictions (117 to 168 cm) and a bag limit of one fish per day are in effect for legal fishing of white sturgeon in the Bay, with a mean of 134 cm measured in fish collected for advisories.

A median per angler consumption rate of 16 g d^{-1} was determined specifically for Bay fish during 1998 and 1999 (SFEI 2000). This site-specific rate can be compared to a national recreational fisher consumption rate of 17.5 g d^{-1} and a national per capita rate of 7.5 g d^{-1} (USEPA 2000b).

Nutritional guidelines, toxicity symptoms, and national guidance concerning human health risk for consumption of fish are shown in submodel G (Figure 8). The details of how guidelines shown in Figure 8 were determined and how they might be linked to regulation of Se in wildlife and to fish health are presented in Appendix A.4.

QUANTITATIVE MODELING

This section presents an example of an application of the quantitative DRERIP Ecosystem-Scale Selenium Model. The questions addressed in this example are: What are the implications for ecosystem concentrations of Se if a fish tissue and/or wildlife Se guideline is implemented (a guideline based upon Se concentrations in a predator)? More specifically, what changes in dissolved or particulate Se concentration in the Bay-Delta would be necessary to achieve the selected tissue concentrations in predators? Agencies have traditionally regulated contaminants on the basis of dissolved concentrations, and managed inputs from different sources based upon their implications for dissolved concentrations (e.g., total mass daily loadings). This example shows a methodology that ties the new concept of tissue guidelines to the traditional concept of dissolved-concentration-based management. Inherent in every regulatory guideline are assumptions about the environment being regulated. The model allows an explicit evaluation of the implications of different assumptions.

The generalized equations for prediction of a dissolved Se concentration from a tissue Se concentration are given in submodel B (Figure 3). Table 1 gives the specific combinations of choices for food web, guideline, location, hydrologic condition, K_d, and TTFs used for the Bay-Delta application. In this example, several alternatives for a tissue guideline were chosen from among those that have been discussed in the regulatory context. Then, the invertebrate, particulate, and dissolved Se concentrations were calculated that would be expected if the tissue concentrations were in compliance with each choice of a guideline. Calculations also were conducted under different assumptions about K_d, food web, and TTFs. Finally, the calculated dissolved, particulate,

Submodel G

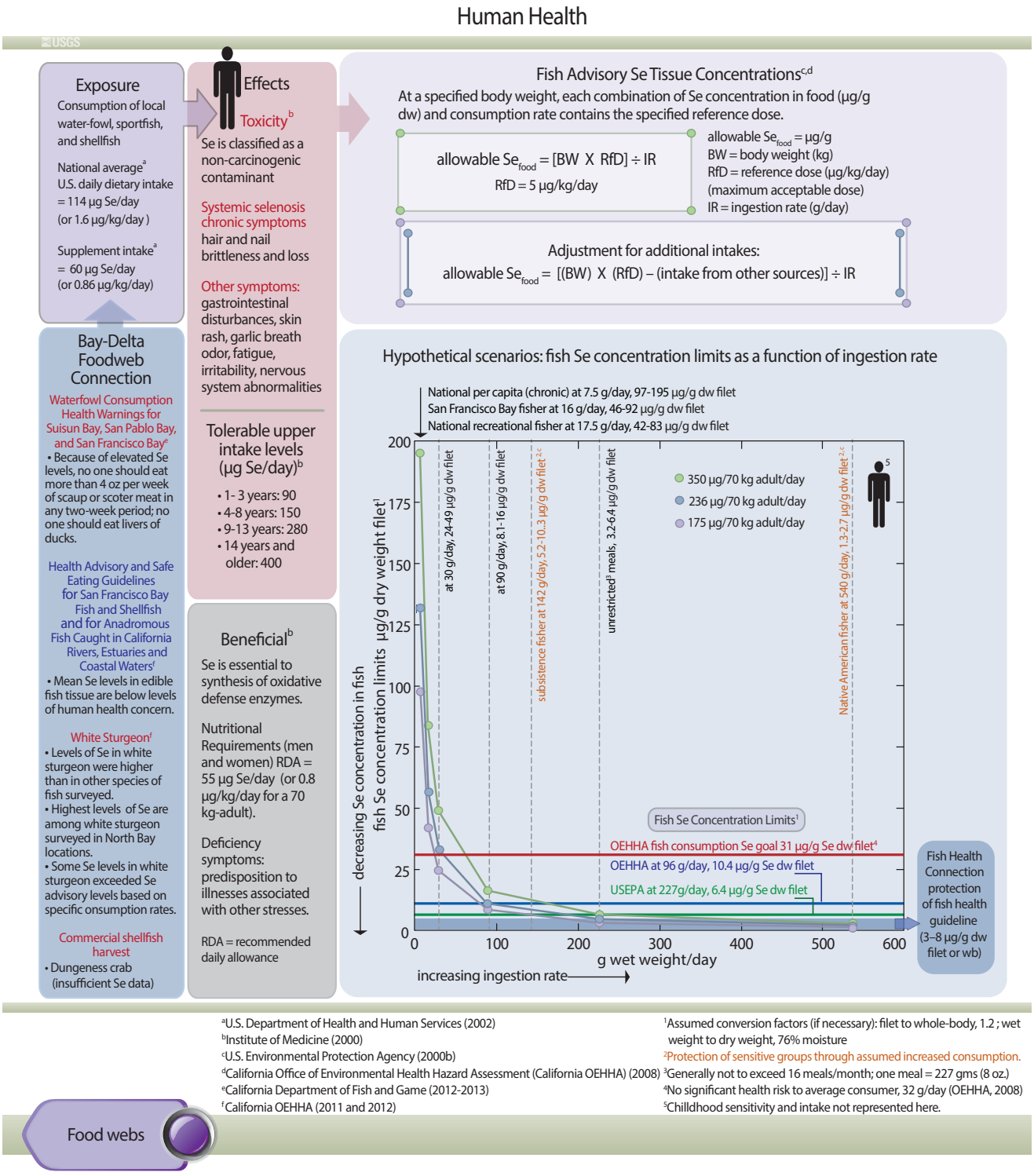

Figure 8 Submodel G. Human Health. See additional explanation in Appendix A.4.

Table 1 Locations, food webs, and model parameters for quantitative modeling examples

Location	Predator	Food web	Predator tissue target (µg g^{-1} Se, dw)	$TTF_{predator}$	Prey	TTF_{prey}	Particulate phase as base of food web	K_d	Flow condition
San Francisco Bay (Carquinez Strait – Suisun Bay)	sturgeon	clam-based	5 or 8 whole-body	1.3	50% *C. amurensis* 50% [amphipods plus other crustaceans]	9.2	suspended particulate material	5,986	low flow (Nov 1999)
	sturgeon	clam-based	5 or 8 whole-body	1.3	50% *C. amurensis* 50% [amphipods plus other crustaceans]	9.2	suspended particulate material	3,317	average condition
	young striped bass	zooplankton-based	8 whole-body	1.1	zooplankton	2.4	suspended particulate material	3,317	average condition
	bird	clam-based	7.7, 12.5, or 16.5 egg	2	50% *C. amurensis* 50% [amphipods plus other crustaceans]	9.2	suspended particulate material	5,986	low flow (Nov 1999)
	bird	clam-based	7.7, 12.5, or 16.5 egg	2	50% *C. amurensis* 50% [amphipods plus other crustaceans]	9.2	suspended particulate material	3,317	average condition
Sacramento–San Joaquin Delta	fish	insect-based	5 or 8 whole-body	1.1	aquatic insects	2.8	suspended particulate material	3,680	average condition
	bird	insect-based	7.7, 12.5, or 16.5 egg	2	aquatic insects	2.8	suspended particulate material	3,680	average condition
San Joaquin River (main stem at Vernalis)	fish	insect-based	5 or 8 whole-body	1.1	aquatic insects	2.8	suspended particulate material	1,212	generalized (July 2000)

and invertebrate Se concentrations were compared with observations of those values from the Bay-Delta to assess how much existing conditions would be need to change to achieve compliance with the chosen guidelines (Table 2). Implicitly, comparisons of outcomes with data from nature tests how well model predictions match reality (Luoma and Rainbow 2005). Comparisons under different assumed conditions test the sensitivity of the model to changes within a few critical parameters.

The method, as indicated in the conceptual model (Figures 3 and 4, especially) includes the following steps: (1) selection of tissue guidelines to test; (2) selection of places and times of interest; (3) derivation of K_d using spatially and temporally matched dissolved and particulate Se concentrations constrained within the selected place and time; (4) selection of a food web of interest to each locality; (5) determination of species-specific TTFs for invertebrates and their specific predators that are relevant to the place and food web; (6) prediction of invertebrate, particulate and dissolved Se concentrations; (7) comparison of predicted values to field observations of Se concentrations in these media in the Bay-Delta; and (8) conclusions about implications for compliance.

Modeling Parameters and Variables

Guidelines

The effect guidelines chosen for evaluation were 5 and 8 µg g^{-1} dw fish whole-body; as well as 7.7, 12.5, and 16.5 µg g^{-1} dw for bird eggs (Presser and Luoma 2010b) (Table 1). The regulatory community is debating appropriate critical tissue values that relate bioaccumulated Se concentrations and toxicity in predators (see previous discussion). We are not

Table 2 Predicted dissolved and particulate Se concentrations and percent exceedances for example scenarios

Location	Flow condition and tissue guideline (µg g^{-1} Se, dw fish whole-body or bird egg)	Predicted invertebrate concentration (µg g^{-1} Se, dw)	Predicted particulate concentration (µg g^{-1} Se, dw)	Percent particulate Se exceedance in ecosystem	Predicted dissolved concentration (µg L^{-1} Se)	Percent dissolved Se exceedance in ecosystem
San Francisco Bay: Carquinez Strait – Suisun Bay						
Bay sturgeon	low flow – 5.0	3.8	0.42	59	0.070	100%
	average – 5.0	3.8	0.42	59	0.126	47%
	low flow – 8.0	6.2	0.67	27	0.112	66%
	average – 8.0	6.2	0.67	27	0.202	3%
Bay striped bass	average – 8.0	7.3	3.0	0	0.914	0%
Bay birds	low flow – 7.7	3.9	0.42	59	0.070	100%
	average – 7.7	3.9	0.42	59	0.126	47%
	low flow – 12.5	6.3	0.68	25	0.113	64%
	average – 12.5	6.3	0.68	25	0.205	2%
	low flow – 16.5	8.3	0.90	11	0.150	23%
	average – 16.5	8.3	0.90	11	0.270	1%
Sacramento–San Joaquin Delta						
Delta fish	average – 5.0	4.5	1.6	7	0.441	19%
	average – 8.0	7.3	2.6	3	0.706	10%
Delta birds	average – 7.7	3.9	1.4	16	0.374	19%
	average – 12.5	6.3	2.2	3	0.607	11%
	average – 16.5	8.3	2.9	3	0.801	6%
San Joaquin River (main stem at Vernalis)						
River fish	July 2000 – 5.0	4.5	1.6	No data	1.3	16%
	July 2000 – 8.0	7.3	2.6	No data	2.1	3%

suggesting these are the best choices for guidelines; but they are within the range of those that are being discussed. In particular, the fish whole-body target of 5 µg g^{-1} and a bird egg target of 7.7 µg g^{-1} have been derived to provide additional protection for endangered species (Skorupa and others 2004; Skorupa 2008). The illustrated scenarios also considered the differences in the changes required if a bird egg-based guideline were used instead of a whole-body fish-based guideline.

Place and Time

The modeling scenarios compared two locations: a brackish-water Bay environment and a tidal freshwater Delta environment. For the Bay, we constrained consideration to the geographic area of Carquinez Strait and Suisun Bay (Presser and Luoma 2010b) (Table 1). In terms of drivers, this location is affected by oil-refinery effluents that contain Se, and also could be influenced by inputs from the San Joaquin Valley. As noted previously, Se concentrations in at least some predators (sturgeon and diving ducks) at this location now exceed USFWS Se guidelines (Presser and Luoma 2010b). For the Delta, the area considered was from Stockton westward through the Delta, and was constrained to the freshwater environment. We also compared scenarios for average conditions across the year(s) in the Bay, to a specific example of conditions for one low flow season

(November 1999). An average condition for the Delta was modeled.

Partitioning and K_ds

The approach of Presser and Luoma (2006, 2010b) was used to select two K_ds for the scenarios from the Bay and one for the Delta (Table 1). The data for the Bay were narrowed to a Carquinez Strait–Suisun Bay location (Cutter and Cutter 2004; Doblin and others 2006; Presser and Luoma 2010b) to focus on the most contaminated area in the estuary, and to exclude the extreme K_ds at the ocean and freshwater interfaces. We selected the mean of co-collected dissolved and particulate Se concentrations from a transect for November 1999 (K_d = 5,986) to represent low flow conditions. Average conditions in the Bay across all seasons and several years were represented by the grand mean of all transects through the Carquinez Strait–Suisun Bay area during 1998-1999 (K_d =3,317) and the freshwater Delta during 2003-2004 (K_d = 3,680). For comparison, the Delta grand mean K_d for low flow transects was 2,613 and for high flow transects 5,283. As discussed earlier, the value that describes transformation, even when constrained, is the most variable of any of the model parameters. The uncertainty associated with the choice of this value could be avoided if environmental guideline were based upon empirically determined particulate Se, but cannot be avoided if it is necessary to relate tissue Se to dissolved Se.

Food Webs and TTFs

For the Bay, the food web used was for suspended particulate material to *C. amurensis* to clam-eating fish or aquatic-dependent clam-eating bird (submodel C, Figure 4 and Table 1). The diet for both predators was assumed to be 50% clam and 50% benthic crustaceans. The bivalve food web is the most efficient at accumulating Se in the system, in both the field and in the quantitative model; therefore, it is the most environmentally protective to use in evaluating a tissue guideline. Different assumptions, of course, could be used for the percentage of diet that is clam-based (e.g., 75% to 96% for scoter and scaup, submodel C, Figure 4). Data on variability of benthic assemblages with time, Bay location, and hydrologic condition also can be used to adjust dietary considerations (Peterson and Vayssieres 2010). If migrating scoter and scaup were modeled, a guideline based on body-condition endpoint, rather than a direct reproductive guideline, would be appropriate. To test the sensitivity of the choice of predator, one comparative simulation was calculated for a pelagic food web in the Bay: suspended material to zooplankton to young striped bass. The food web for the Delta was suspended particulate material to aquatic insects to juvenile salmon or steelhead trout.

Only a few recent data sets from the Bay-Delta are available that analyze Se concentrations across a reasonably complete food web (e.g., Stewart and others 2004). Some important food webs have not been assessed at all (e.g., aquatic insects and Chinook salmon or steelhead trout) (Presser and Luoma 2010b). However, studies of Se concentrations in enough individual predator and prey species are available to assess the predictions from the model and to derive, in a few instances, some critical trophic transfer relationships (e.g., Linville and others 2002; Stewart and others 2004; Schwarzbach and others 2006; Lucas and Stewart 2007; De La Cruz and others 2008; De La Cruz 2010). For the Bay, the dominant bivalve in the Carquinez Strait–Suisun Bay area is *C. amurensis*. This species strongly bioaccumulates Se (Linville and others 2002). A species-specific $TTF_{C.\ amurensis}$ of 17 (a range of 14 to 26 over different estuary conditions) was used here based on the field calibration that Presser and Luoma (2010b) describe. Benthic crustaceans, like amphipods and isopods, are much less efficient than clams in bioaccumulating Se; TTFs can range from 0.8 for amphipods to 2.0 for other crustaceans (Presser and Luoma 2010a). Under the assumption of a mixed diet of *C. amurensis* ($TTF_{C.\ amurensis}$ = 17) and benthic crustaceans ($TTF_{benthic\ crustacean}$ = 0.8 and 2.0), the combined diet TTF used here is 9.2.

An important benthic predator, white sturgeon, was chosen for the example, because the Se biomagnifier *C. amurensis* is an important food source for this species in the Bay. White sturgeon accumulate higher concentrations of Se than any other fish in the Bay (Stewart and others 2004; OEHHA 2011), making it

the environmentally conservative choice for evaluating a guideline. From studies in the late 1980s, field TTFs derived specifically for white sturgeon from the Bay that used bivalves as prey, showed a range from 0.6 to 1.7, with a mean of 1.3 (Presser and Luoma 2006); similar to the value of 1.1, which is the mean among all fish species studied. Calculations from more recent data sets for *C. amurensis* at Carquinez Strait, and seaward white sturgeon, showed a somewhat lower TTF of 0.8 (Presser and Luoma 2010b).

For the Delta food web, Se TTFs for freshwater aquatic insects were selected from data from literature sources (submodel C, Figure 4). For example, Presser and Luoma (2010a) derived a mean Se TTF_{insect} of 2.8 (range 2.3 to 3.2) based on matched field data sets for particulate and insect Se concentrations in freshwater environments for several species of aquatic insect larvae including mayfly, caddisfly, dragonfly, midge, and waterboatman. These values generally compare well to laboratory-derived TTFs for aquatic insect larvae (Conley and others 2009). TTFs for other potential invertebrates in Delta food webs (range 0.6 to 2.8) also are shown in submodel C, Figure 4 (Presser and Luoma 2010a).

Much less data are available to evaluate bioaccumulation in avian food webs. Data from the study of toxicity in mallards (Heinz and others 1989, 1990) are the most comprehensive studies available to use for modeling dietary exposure. From these studies, the laboratory-derived $TTF_{bird\ egg}$ of 2.6 was assumed for transfer of Se from prey to bird eggs (which correlate best with toxicity). For the model, this choice of TTF for bird species was lowered to 2.0 to illustrate the possible effect of field variables on exposure factors that encompass habitat use and feeding behavior. A diet of 50% clams and 50% crustaceans was assumed for a clam-eating bird.

Implications of Model Choices and Estuary Conditions

Details of the calculations to evaluate implications of different guidelines, under different conditions, are summarized in Table 2. To compare the implications of these choices, we determined the percentage Se concentrations in dissolved and particulate form that exceeded the value predicted to be necessary to meet the tissue guideline. All published dissolved (n = 168) and particulate Se (n = 168) data from the Bay and from the Delta, collected after 1997, are employed in this estimate. Together, the scenarios depict a Bay for which there is ecological risk from Se contamination, but the degree of risk, judged by the degree of compliance with the guidelines, depends heavily upon assumptions about toxicity (the guideline), transformation, and choice of food web.

The occurrence of 8 $\mu g\ g^{-1}$ dw Se in sturgeon muscle from the contaminated area of San Francisco Bay (Linares and others 2004) is one of several lines of evidence that ecological risks from Se are occurring in the Bay. When this concentration was used for a predator guideline (Table 2), the model predicted Se concentrations in invertebrates and suspended particulate material and a dissolved Se concentration that were within the range typical of the Bay-Delta (Table 2). Thus, the model results appear to successfully capture the links between Se concentrations in different ecosystem components of the Bay, in general [also see Presser and Luoma (2010b) for further validation details]. This also suggests that the use of calibrated mean K_ds to reduce uncertainties about transformation adequately captures and constrains the variability in these processes. The agreement between ecosystem observations and the predicted Se concentrations in invertebrates and predators similarly points to the validity of the TTFs.

The most remarkable conclusion from the calculations is that fish tissue Se concentrations typical of risks to reproductive toxicity (the selected guideline examples) occur in the Bay at dissolved Se concentrations more than ten times less than the traditional water quality regulatory guideline of 5 $\mu g\ L^{-1}$ (Table 2). At least some food webs in the Bay and the Delta are particularly vulnerable to small changes in bioavailable Se concentrations. The very high K_ds consistently observed in both the Bay and the Delta, compared to many other ecosystems (Presser and Luoma 2010a), may be one reason for this sensitivity. Also influential is the strong ability of invertebrates such as *C. amurensis* to bioaccumulate Se when compared to other prey species. It appears that ecosys-

tems wherein dissolved Se is efficiently transformed to particulate Se, and in which particulate Se is propagated up a food web to predators, will amplify relatively small changes in concentrations of dissolved Se concentrations to levels that could affect predators.

Under low flow conditions, 23 to 66% of dissolved Se determinations in the Bay exceeded the value predicted to be necessary to meet the higher sturgeon-based guideline or the higher bird-based guidelines (Table 2). Under guidelines chosen to protect endangered species, 100% exceedance occurs at low flow conditions. Clearly, low flow conditions, like those in November 1999, are the time of greatest ecosystem sensitivity to Se inputs (as suggested by Presser and Luoma 2006). It is notable that the example presented here does not represent the most extreme condition of a low flow season of a dry year or critically dry year.

If annual average conditions are assumed (the mean of spatially constrained K_ds), compliance is much more sensitive to the choice of guideline. Few if any exceedances (1 to 3%) are observed if the higher fish or bird egg guidelines are implemented under that assumption. For endangered species protection under an average condition, exceedance is approximately 47% for both the fish and bird guidelines. Of course, regulations based upon average conditions run the risk of under-protecting species sensitive to Se exposure during the protracted time in every year (especially drier years) when Se is most bioavailable.

Considering the choice of different guidelines, if a 5 $\mu g\ g^{-1}$ guideline is implemented that uses sturgeon as the target organism, the entire Bay would be out of compliance. The model calculation suggests nearly all anthropogenic Se would have to be removed to drive sturgeon tissues to concentrations as low as 5 $\mu g\ g^{-1}$, especially during a low flow condition. The projected dissolved Se concentration necessary to reach that level in sturgeon tissue is approximately the value for the Sacramento River, and hence the pre-disturbance baseline condition for the Bay. The modeling results suggest that if it is assumed that 5 $\mu g\ g^{-1}$ represents the toxicity threshold for sturgeon, and if it were applied using concentrations in sturgeon from the field, then there is no room for any deviation from concentrations in the Sacramento River without risk to the species. It is important to remember, however, that this toxicity guideline was derived for the most sensitive fish species. So, the use of the most sensitive surrogate in the toxicity guideline combined with field determinations from the fish with the greatest exposure results in an ultra-sensitive outcome.

These model results also illustrate how sensitive the implementation of a tissue guideline can be to the choice of predator. For example, many of the differences between sturgeon-based guidelines and bird egg-based guidelines are relatively small. Both appear to be sensitive indicators of ecological risks. However, the outcomes of guidance based upon striped bass, a water-column predator, are quite different from outcomes based upon bird eggs or sturgeon. The model showed that while aquatic birds and sturgeon are at risk under most assumptions, few or no exceedances of Se concentrations occur if the choice of regulatory indicator is based upon striped bass tissues. The differences are the result of the different invertebrate prey of the two species. Sturgeon eat a diet that includes strong Se bioaccumulator species (bivalves); striped bass eat from prey that live in the water-column and do not strongly bioaccumulate Se.

Selenium concentrations in the water column or particulate material of the Delta are higher and more variable than in the Bay. Average K_ds are similar between the Delta and the Bay. Nevertheless, few exceedances of dissolved and particulate Se concentrations (3% to 19%) are predicted in the Delta, even when the most sensitive fish guideline is used. This is consistent with the observation of low Se concentrations in the few fish that have been sampled from the Delta (e.g., Foe 2010). Use of the local food web is extremely influential in this outcome. Bioaccumulation of Se in the aquatic insect larvae (and other arthropods) that are the primary prey species of most Delta fish and birds is much lower than bioaccumulation by bivalves. As a result, it appears that the Delta food webs are easier to protect from adverse effects of Se than benthic food webs in the Bay, even if it is assumed that the most sensitive fish guideline applies. Nevertheless, the actual concentrations of dissolved Se predicted to be

necessary to meet the tissue guidelines range from 0.37 to 0.80 µg L^{-1}, far below the Se concentrations typical of most existing dissolved guidelines for Se (Luoma and Presser 2009). This reflects the unusually high K_ds consistently observed in this freshwater environment.

Few determinations of Se concentrations in particulate material in the incoming rivers to the Bay are available outside the tidal range. Lucas and Stewart (2007) reported matched dissolved and particulate Se concentrations from which one K_d could be calculated (a value of 1,212) for the San Joaquin River during transect sampling in 2000. The example in Table 2 shows that if that were typical of the river, and the food web was mainly based upon arthropods, then compliance with a tissue guideline could occur at dissolved Se concentrations ten times higher than would be the case in the Bay. This river simulation is based on very limited data; it is given here for comparative purposes to show the sensitivity of the model to the choice of hydrologic setting. Comprehensive modeling of the San Joaquin River system would require data collection and analysis specific to the river's settings, predator species, food webs, and habitats. Percentage exceedance (Table 2) is based on weekly sampling of total Se for the river at Vernalis from water year 1995 through water year 2010 (SWRCB 2012)

CONCLUSIONS

The DRERIP Ecosystem-Scale Selenium Model outcomes for the Bay-Delta show critical choices for Se modeling, and derived risk scenarios that illustrate varying degrees of risk, depending on those choices (Figure 1; Tables 1 and 2). In general, the conceptual model for Se shows that the focus of concern for this contaminant is the top of the food web. Quantitative model calculations show that enough is known to adequately characterize the distribution of Se through the Bay-Delta ecosystem, although the available data from which to validate the outcomes is dated and does not include conditions within a low flow season of a dry year or critically dry year. Presser and Luoma (2010b) give additional specifics for updated data collection and model refinements.

Selenium concentrations in fish or bird tissues alone appear to be good indicators of ecological risks from Se. Key invertebrates (e.g., the bivalve *C. amurensis* in the Bay) may be a more pragmatic indictor for frequent monitoring. Given that (1) suspended particulate material Se concentrations are key to accurate prediction of prey and predator Se concentrations; and (2) dissolved Se concentrations are constrained to a narrow dynamic range within the estuary, a suspended particulate material Se concentration also may be a sensitive parameter on which to assess change. Dissolved Se concentrations appear to be the variable of choice for regulatory agencies, however, because of links to total maximum daily loads.

The ability to quantitatively characterize distributions among all these ecosystem components from field determination of only one component allows great flexibility in future monitoring whatever the choice of indicator. The detailed site-specific conceptual model, and the ability to quantitatively apply that model, also provide perspective on the processes that are most influential in determining Se contamination in the predators of this Se-sensitive environment (Figure 1).

The quantitative example (Tables 1 and 2) provides some lessons for implementing regulations to manage Se in this system. First, it is notable that extremely small changes in dissolved Se concentrations, in absolute terms, have strong implications for compliance with the tissue guidelines. A regulatory program that focuses on dissolved Se would require an extremely rich data set to reliably detect the differences between compliance and non-compliance, based upon the translation from tissue to dissolved Se. This is another reason why regulation of suspended particulate material Se concentration may be a more sensitive parameter on which to assess change.

Second, if compliance is determined from tissue concentrations in a predator, the choice of that predator is crucial. Predators of bivalves in benthic food webs are much more at risk than predators from pelagic food webs. The former should be the basis of tissue monitoring in the Bay.

Third, any decision as to whether reductions in ambient concentrations of Se would be required to comply with the tissue guidelines depends upon the choice

of guideline and assumed environmental conditions. For example, the modeling suggests that a fish tissue guideline of 5 μg g^{-1} would ultimately require essentially all enriched Se inputs to the Bay to be eliminated if the guideline were applied using Se concentrations in sturgeon. According to the calculations, dissolved Se concentrations in the Bay would have to decline to nearly those in the Sacramento River to comply with such a guideline. If a guideline of 8 μg g^{-1} was used, the Bay would be near compliance under average conditions; but 66% out of compliance in a situation like November 1999 (i.e., low flow). Calculating in the opposite direction from a traditional dissolved Se concentration guideline, allowing dissolved concentrations of Se in the Bay to reach 5 μg L^{-1} (the current regulatory guideline) or even 2 μg L^{-1} would result in tissue concentrations (potentially greater than 100 μg g^{-1} in *C. amurensis*) that could threaten many of the predators in the Bay, if other conditions stay as they are.

Fourth, the current food webs in the Delta are less at risk from Se than the benthic food webs of the Bay, because of the differences in food webs. The differences between the Delta and the Bay are not the result of the freshwater versus brackish water nature of the systems of interest because, on average, transformation efficiencies are similar in the two. Where transformation processes are greatly different between two ecosystems, then a different outcome from implementing the same tissue guideline might be expected. The San Joaquin River example shows how a less efficient transformation of dissolved Se to particulate Se in the river can result in less sensitivity of the ecosystem to changes in Se concentrations.

Finally, the more specificity added to the model, the less uncertainty in predictions. If, for example, the geographic range is narrowed by using data only from Carquinez Strait–Suisun Bay, then freshwater and ocean interfaces are avoided. If the temporal range is narrowed to low flow seasons of dry years (i.e., high residence time or high exposure time), then focus can be on times when the transformative nature of the estuary is elevated. Juxtaposition of times when suspended particulate material or prey species achieve maximum Se concentrations with critical life stages of species at risk being present allows regulatory considerations to focus on times that govern Se's ecological effects (i.e., ecological bottlenecks) (Figure 1).

The greatest strength of the analytical and modeling processes is that it is an orderly, ecologically consistent approach for assessing different aspects of the fate and effects of Se. Assessments such as the examples shown here can represent a starting point for initiating management decisions. Application of the DRERIP Ecosystem-Scale Selenium Model shows that management of Se requires incorporation of the complexity of dietary exposures and the systematic consideration of critical aspects of hydrology, biogeochemistry, physiology, ecology, and ecotoxicology to define ecosystem protection. Although this is complex, scenarios can be developed from specific questions that arise in the planning and implementation of restoration actions for the Bay-Delta. Quantitative evaluation of those scenarios is feasible. However, the Se database and monitoring program need to be modernized (e.g., refocused and expanded). Specifically, monitoring should include (1) representation of conditions in dry and critically dry years; and (2) collection of spatially and temporally matched data sets across media (i.e., water, suspended particulate material, prey, and predator) to ensure that derived site-specific factors are current for the ecological and hydrological dynamics of the Bay-Delta. Only then will predictions from the model remain relevant and realistic to a constantly evolving estuary.

ACKNOWLEDGEMENTS

This work was supported by CALFED (in the project's earlier phases), the U.S. Geological Survey National Research and Toxics Substances Hydrology Programs, and the U.S. Environmental Protection Agency (San Francisco, California, Region 9). Jeanne DiLeo (U.S. Geological Survey, Menlo Park Publishing Service Center) prepared the outstanding graphics. We thank William Beckon and Thomas Maurer (U.S. Fish and Wildlife Service, Sacramento, California) for provision of species at risk for the Bay-Delta. We also thank William Beckon, Susan De La Cruz (U.S. Geological Survey, Vallejo, California) and Regina Linville (Office of Environmental Health Hazard Assessment,

Sacramento, California) for assistance with species-specific effects models and human health considerations. Thanks also go to Mark Huebner (U.S Geological Survey, Menlo Park, California) for exceptional assistance with manuscript preparation.

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U.S. Geological Survey

U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

U.S. Geological Survey, Reston, Virginia: 2010

Suggested citation:
Kleckner, A.E., Stewart, A.R., Elrick, K., and Luoma, S.N., 2010, Selenium concentrations and stable isotopic compositions of carbon and nitrogen in the benthic clam *Corbula amurensis* from Northern San Francisco Bay, California: May 1995–February 2010: U.S. Geological Survey Open-File Report 2010-1252, 34 p.

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Conversion Factors, Abbreviations, and Acronyms

Conversion Factors

Multiply	By	To obtain
foot (ft)	0.3048	meter
gallon (gal)	3.785	liter (L)
inch (in.)	2.54	centimeter
inch (in.)	25,400	micrometer (µm)
micromolar (µM)	molecular weight	micrograms per liter
micron (µm)	1,000,000	meter
mile (mi)	1.609	kilometer
ounce (oz)	28.35	gram (g)
part per million	1	microgram per gram (µg/g)

Concentrations of chemical constituents in solids are given in micrograms per gram (µg/g, dry weight).

Isotopic values (∂) are expressed in parts per thousand, or per mil.

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83)

Abbreviations and Acronyms

Abbreviations and Acronym	Meaning
HGAAS	Hydride Generation Atomic Absorption Spectroscopy
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
IAEA	International Atomic Energy Agency
NIST	National Institute of Science and Technology
NRCC	National Research Council Canada
QA/QC	Quality Assurance/Quality Control
USGS	U.S. Geological Survey

Selenium Concentrations and Stable Isotopic Compositions of Carbon and Nitrogen in the Benthic Clam *Corbula amurensis* from Northern San Francisco Bay, California: May 1995–February 2010

By Amy E. Kleckner, A. Robin Stewart, Kent Elrick, and Samuel N. Luoma

Abstract

The clam-based food webs of San Francisco Bay, California efficiently bioaccumlate selenium and thus provide pathways for exposure to predators important to the estuary. This study documents changes in monthly selenium concentrations for the clam *Corbula amurensis*, a keystone species of the estuary, at five locations in northern San Francisco Bay from 1995 through 2010. Samples were collected from designated U.S. Geological Survey stations and prepared and analyzed by U.S. Geological Survey methods. Stable isotopes of carbon and nitrogen in soft tissues of clams also were measured as an indicator of sources of selenium for the clams. These monitoring data indicate that clam selenium concentrations ranged from a low of 2 to a high of 22 micrograms per gram dry weight with strong spatial and seasonal variation over the period of study.

Introduction

Contaminants that bioaccumulate, such as selenium (Se), have the potential to threaten fish and birds and thus to impede ecosystem restoration efforts. Selenium is a contaminant of concern and a challenge for resource managers in the San Francisco Bay because of oil refinery and agricultural sources of Se. The resident clam *Corbula amurensis* is an ecologically significant species in terms of critical food webs of the Bay. This estuarine clam invaded the estuary in 1986 and was established in the northern reaches of the estuary by autumn 1987. The invasion of *C. amurensis* has been linked to significant shifts in food web structure (Feyrer and others, 2003), loss of native pelagic invertebrates (Kimmerer and others, 1994; Kimmerer and Orsi, 1996; Kimmerer, 2002), and declines in pelagic organisms (Sommer and others, 2007).

C. amurensis is an efficient accumulator of Se when compared to other invertebrates (Presser and Luoma, 2010). This species of clam also is an efficient accumulator when compared to other bivalve species because of its high Se assimilation efficiency and slow Se loss rates from its tissues (Schlekat and others, 2000; Stewart and others, 2004; Lee and others, 2006). Stewart and others (2004) showed that a combination of food-web structure and the physiology of invertebrate species explain how Se is propagated up different food webs and which predators are therefore at risk.

The purpose of this study is to provide data that are representative of the spatial (five benthic stations in northern San Francisco Bay) and temporal (seasonal and inter-annual) variation in Se concentration in clams. These data document 15 years of Se concentrations, stable isotope (C and N) values and element compositions (% C and N, molar C:N) in the soft tissues of *C. amurensis*. Clam shell lengths and dry weights are also provided.

Methods

Sites and Dates of Collection

Samples of *C. amurensis* were collected from five locations in northern San Francisco Bay (fig. 1, table 1). The sites in San Pablo Bay, Carquinez Strait, and Suisun Bay are near the head of the estuary, seaward of the confluence of the Sacramento-San Joaquin River system. USGS benthic stations 4.1, 6.1, 8.1, and 12.5 are located along the main channel extending from Chipps Island through the North Bay (fig. 1, table 1). These stations are sampled monthly for chlorophyll-*a*, salinity, and suspended-sediment concentrations as part of a larger water-quality program that has been ongoing since 1968 (U.S. Geological Survey, 2010, *http://sfbay.wr.usgs.gov/access/wqdata/index.html*). USGS benthic station 415.1 is located near where Montezuma Slough enters Grizzly Bay. USGS benthic stations 405.1 and 411.1 are located in Suisun Bay between stations 8.1 and 415.1 (table 1). USGS benthic stations 4.1, 6.1, and 8.1 have depths of 11.6, 10.1, and 14.3 m respectively (table 1). USGS benthic stations 12.5, 405.1, 411.1, and 415.1 have shallower depths of 6.7, 7.9, 4.9, and 3.0 m respectively (table 1).

During the sampling period May 1995 – February 2010, the collection of *C. amurensis* for stations 4.1 and 8.1 was nearly monthly and is on-going (tables 3 and 5). The dates of sample collection for stations 6.1, 12.5, 405.1, 411.1, and 415.1 were more limited (tables 4, 6–9). Besides sampling logistics, collection depended on availability of clam populations.

Table 1. Locations and depths for USGS benthic stations, northern San Francisco Bay, California.

[Abbreviations: m, meters; N, north; W, west]

USGS station name	Latitude	Longitude	Depth (m)
Benthic 4.1	38º 03.427' N	121° 56.691' W	11.6
Benthic 6.1	38º 04.042' N	122º 02.933' W	10.1
Benthic 8.1	38º 01.900' N	122º 08.416' W	14.3
Benthic 12.5	38º 02.425' N	122º 18.850' W	6.7
Benthic 405.1	38º 02.885' N	122º 07.353' W	7.9
Benthic 411.1	38º 05.811' N	122º 03.491' W	4.9
Benthic 415.1	38º 07.743' N	122º 03.405' W	3.0

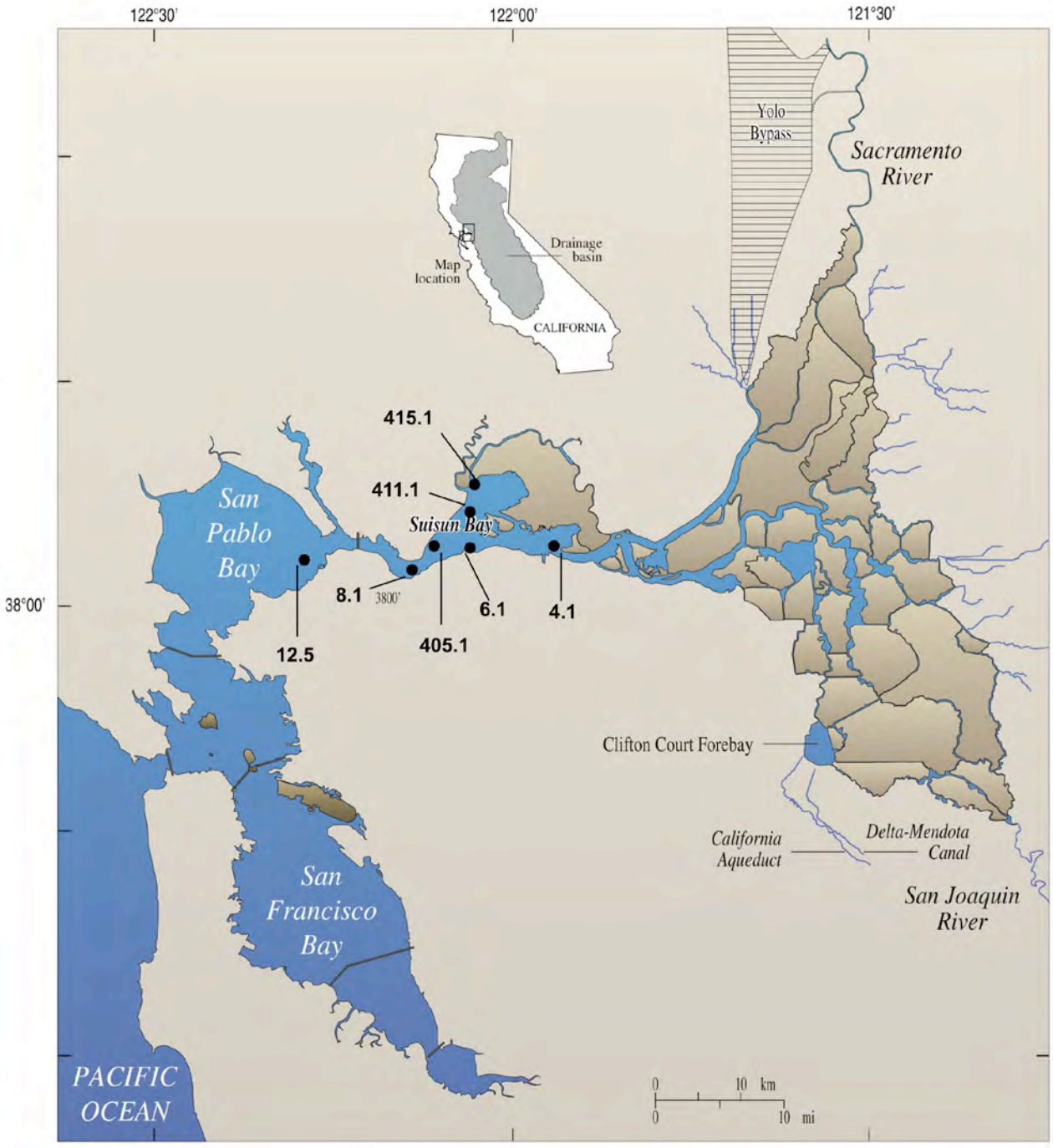

Figure 1. Study area with USGS benthic station locations in northern San Francisco Bay, California.

Sample Preparation and Selenium and Stable Isotope Analysis

Samples of *C. amurensis* were collected from the USGS ship, the R/V Polaris, using a benthic grab sampler. At each station, multiple benthic grab samples were taken until approximately 80 individual clams ranging in size from 9 to 18 mm were collected. The clams were placed in bottom water drained from the surface of the grab and depurated for 48 h (Brown and Luoma, 1995). Samples were processed as described in Linville and others (2002). Clams were measured to the nearest millimeter using electronic calipers and pooled by size to create three composite samples of varying mean length. Each composite was bagged separately and frozen at -80 °C until dissected. The numbers of individuals per sample are listed in tables 3-9. Upon dissection, soft tissues were removed from shells, pooled by size, weighed, refrozen, and then freeze-dried (VirTis Freezemobile 12ES). Freeze-dried samples were further ground into a coarse powder using a ball-mill (SPEX CertiPrep 5100).

For Se analysis, approximately 100–200 mg of ground tissue was weighed out into an open Teflon® beaker and then digested using a modification of the procedure described in Elrick and Horowitz (1985). Specifically, Lefort aqua regia was substituted for nitric acid in the first step of the digestion, and nitric acid was substituted for hydrofluoric acid in the second addition of $HF\text{-}HClO_4$. Samples were then brought up to volume in 0.5% HNO_3. A 5-mL aliquot was taken and mixed with 5 mL 12M HCl to reduce the Se to the most favorable valence for hydride generation. The Se digestates were analyzed by hydride generation atomic absorption spectroscopy (HGAAS) during the period 1995 through mid-2001 and more recently by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Selenium concentrations are expressed as micrograms per gram (µg/g) on a dry weight (dw) basis.

Stable isotopes of carbon (C) and nitrogen (N) in soft tissues of *C. amurensis* were measured beginning in summer 1999 (Canuel and others, 1995). Stable isotope ratios of nitrogen ($\delta^{15}N$) provide a spatially and temporally integrated measure of trophic relationships in a food web (that is, primary producers → invertebrates → fish) because $\delta^{15}N$ becomes enriched by 2.5–5 per mil (‰) between prey and predator (Peterson and Fry, 1987). Stable isotope ratios of carbon ($\delta^{13}C$) show little or no enrichment (<1‰) with each trophic level, but can help identify contributions of different Se sources that affect clam tissues (France, 1995).

A subsample of freeze-dried clam soft tissues was analyzed for carbon and nitrogen isotope ratios and masses at the Stable Isotope Facility, University of California, Davis, using a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer and Europa ANCA-SL elemental analyzer. Results are presented as deviations from standards, expressed as $\delta^{13}C$ and $\delta^{15}N$:

$$\delta X = [R_{sample}/R_{standard} - 1] \times 10^3 \text{ ‰}$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon is Peedee Belemnite, and for nitrogen, it is atmospheric diatomic nitrogen. Instrument precision was 0.1‰ for carbon and 0.3‰ for nitrogen based on replicate analyses of standard reference materials (Cloern and others, 2002).

Quality Assurance

All glassware and plasticware used for sample collection, preparation, and analysis were first cleaned to remove contamination. The cleaning process included several sequential steps including a detergent wash, a rinse in deionized water, a 15-percent nitric acid wash, and a thorough rinse in double-deionized water (approximately 18 MΩ resistivity). Materials were dried in a dust-free positive pressure environment, sealed, and stored in a dust-free cabinet.

Quality assurance/quality control (QA/QC) for the determination of Se was through the co-digestion and analysis of various standard reference materials (SRMs) from several sources, including the National Institute of Science and Technology (NIST), the National Research Council Canada (NRCC), and the International Atomic Energy Agency (IAEA). SRM samples accounted for 20 percent of each assay. Ten percent of the clam tissue samples in each assay were analyzed in duplicate. Reagent blanks were processed to ensure the purity of the acids and other reagents. Observed concentrations fell within the range of certified values for these materials (table 2).

Results

Tables 3-9 give Se concentrations, stable isotope (C and N) values, element compositions (% C and N, molar C:N), clam shell lengths, and dry weights for clam sample composites from USGS benthic stations listed in numerical sequence of stations (that is, station 4.1, table 3; station 6.1, table 4; station 8.1,table 5; station 12.5, table 6; station 405.1, table 7; station 411.1, station 8; and station 415.1, table 9.

Clam Se concentrations ranged from a low of 2 to a high of 22 micrograms per gram dry weight with strong spatial and seasonal variation over the period of study.

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Table 2. Observed and certified concentrations of selenium (µg/g dw) in standard reference materials (SRM) analyzed.

[The certified concentration as reported by the National Institute of Standards and Technology (NIST), the International Atomic Energy Agency (IAEA), and the National Research Council Canada (NRCC) are the mean and 95-percent confidence interval. The observed concentrations are the mean and 1 standard deviation (n=1-7; --, no data]

Analysis date	NIST 1566A	NIST 1566B	NIST 2709	NIST 2976	IAEA MA-A-1/TM	IAEA MA-A-2/TM	IAEA MA-B-3/TM	NRCC TORT-1	NRCC TORT-2	NRCC DORM-1	NRCC DORM-2	NRCC DOLT-2	NRCC DOLT-3	NRCC MESS-2
	Certified Se Concentration													
	2.2 ± 0.2	2.1 ± 0.2	1.6 ± 0.1	1.8 ± 0.2	2.9 ± 0.5	1.7 ± 0.3	1.5 ± 0.2	6.9 ± 0.5	5.6 ± 0.7	1.6 ± 0.1	1.4 ± 0.1	6.1 ± 0.5	7.1 ± 0.5	0.7 ± 0.1
	Observed Se Concentration													
Oct-99	2.1 ± 0.1	--	--	1.6 ± 0.04	2.9 ± 0.07	--	1.5 ± 0.07	--	5.1 ± 0.3	--	1.3 ± 0.0	5.7 ± 0.07	--	--
Dec-99	2.3 ± 0.2	--	1.6	--	3.0	--	1.4 ± 0.1	--	4.7	--	1.2 ± 0.3	6.2 ± 0.3	--	--
June-00	2.2 ± 0.1	--	1.4	1.8 ± 0.2	2.7 ± 0.3	--	1.4 ± 0.03	--	5.2 ± 0.3	--	1.4 ± 0.1	5.8 ± 0.3	--	0.8
Aug-00	--	--	--	1.8 ± 0.1	--	--	1.4	--	4.9 + 1.0	--	1.4 ± 0.1	5.9 ± 0.2	--	--
Feb-01	--	--	--	2.1 ± 0.2	3.1 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	6.8 ± 0.3	6.3 ± 0.3	--	1.5 ± 0.1	5.9 ± 0.4	--	--
Jan-02	--	--	--	1.9 ± 0.3	--	--	1.7 ± 0.2	--	5.8 ± 0.0	--	1.4 ± 0.1	6.4 ± 0.1	--	--
May-02	--	--	--	1.8 ± 0.1	3.1 ± 0.3	--	1.5 ± 0.2	--	5.3 ± 0.6	--	1.6 ± 0.1	5.9 ± 0.4	--	--
Nov-02	--	--	--	1.8 ± 0.2	3.1 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	--	5.4 ± 0.4	--	1.3 ± 0.1	5.7 ± 0.3	--	--
Sept-03	--	2.0 ± 0.1	--	1.9 ± 0.1	3.0 ± 0.1	--	1.4 ± 0.1	--	5.8 ± 0.1	--	1.4 ± 0.1	6.0 ± 0.2	--	--
May-04	--	2.5 ± 0.07	--	2.0	2.8	--	1.8 ± 0.07	6.4 ± 0.1	6.0 ± 0.5	1.8	1.6 ± 0.07	6.2 ± 0.2	--	--
Dec-08	--	2.0 ± 0.2	--	2.0	2.8	--	1.4	--	5.6 ± 0.2	--	1.3	5.6	6.9	--
Mar-09	--	2.0 ± 0.2	--	1.7 ± 0.1	--	--	--	--	5.5 ± 0.01	--	1.4 ± 0.1	5.8 ± 0	7.0 ± 0.2	--
Apr-09	--	2.0 ± 0.1	--	1.7 ± 0.1	--	--	--	--	5.5	--	1.4 ± 0.1	5.7	7.0 ± 0.5	--
Oct-09	--	--	--	-	--	--	--	--	--	--	--	--	6.7	--
Apr-10	--	2.3	--	1.6	--	--	--	--	--	--	1.5	--	6.6	--
July-10	--	--	--	-	--	--	--	--	6.2	--	1.3	--	7.4	--
July-10	--	2.1 ± 0.1	--	1.8 ± 0.2	--	--	--	1.4 ± 0.1	5.4 ± 0.4	--	6.2	5.7 ± 0.1	7.2 ± 0.1	--

Table 3. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 4.1, northern San Francisco Bay, California, October 1996–February 2010.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; δ, per mil; %, percent; --, no data; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
10/17/1996	1	10	23.27	0.446	0.045	10.6	--	--	--	--	--
10/17/1996	2	12	21.46	0.481	0.040	11.2	--	--	--	--	--
10/17/1996	3	14	20.44	1.001	0.072	11.2	--	--	--	--	--
10/17/1996	4	19	19.65	0.615	0.032	9.2	--	--	--	--	--
10/17/1996	5	20	18.38	0.513	0.026	10.6	--	--	--	--	--
10/17/1996	6	28	17.63	0.632	0.023	12.6	--	--	--	--	--
10/17/1996	7	19	16.27	0.342	0.018	11.4	--	--	--	--	--
11/5/1997	1	31	17.09	0.909	0.029	12.4	--	--	--	--	--
11/5/1997	2	35	21.03	1.885	0.054	11.2	--	--	--	--	--
11/5/1997	3	28	23.40	2.076	0.074	11.2	--	--	--	--	--
10/12/1998	1	5	22.20	0.210	0.042	5.7	--	--	--	--	--
10/12/1998	2	5	24.20	0.280	0.056	5.4	--	--	--	--	--
11/27/2001	1	71	9.97	0.318	0.004	16.0	47.0	12.1	4.52	-27.19	13.09
11/27/2001	2	58	11.60	0.388	0.007	16.0	45.7	11.5	4.64	-27.11	12.94
12/18/2001	1	85	10.00	0.322	0.004	14.0	43.6	10.4	4.89	-26.60	12.17
12/18/2001	2	73	11.82	0.435	0.006	12.0	43.6	10.5	4.85	-26.39	12.61
3/23/2002	1	56	9.97	0.358	0.006	8.0	49.7	12.8	4.53	-27.16	12.92
3/23/2002	2	6	19.98	0.268	0.045	6.6	39.9	9.6	4.84	-29.45	6.72
5/8/2002	1	74	8.55	0.408	0.006	5.0	32.1	8.2	4.57	-28.35	8.71
5/8/2002	2	45	9.49	0.332	0.007	5.0	61.3	15.1	4.74	-26.39	9.67
6/5/2002	1	50	9.51	0.218	0.004	6.0	44.3	11.1	4.70	-26.65	10.29
6/5/2002	2	38	10.46	0.221	0.006	5.9	42.2	10.7	4.71	-26.74	10.19
6/5/2002	3	34	11.44	0.256	0.008	5.3	42.8	10.6	4.65	-26.64	10.22
7/17/2002	1	35	10.58	0.177	0.005	13.0	54.2	14.1	4.47	-27.08	11.30
7/17/2002	2	28	11.41	0.177	0.006	13.0	44.2	10.9	4.72	-26.71	12.34
7/17/2002	3	21	12.45	0.172	0.008	13.0	36.2	9.3	4.52	-26.67	12.76
8/22/2002	1	32	11.40	0.257	0.008	11.0	40.1	9.6	4.86	-26.94	11.89
8/22/2002	2	19	13.53	0.211	0.011	12.0	27.6	6.9	4.69	-26.01	11.78
8/22/2002	3	17	14.45	0.228	0.013	11.0	46.6	10.9	4.98	-26.92	11.98
9/11/2002	1	21	13.49	0.269	0.013	10.2	41.4	9.8	4.70	-26.20	11.86
9/11/2002	2	14	15.58	0.250	0.018	10.8	40.7	9.8	4.69	-26.22	11.73

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
9/11/2002	3	10	17.35	0.245	0.024	10.4	40.6	9.9	4.85	-26.22	11.88
10/9/2002	1	24	12.45	0.253	0.011	10.1	41.4	9.8	4.98	-26.38	11.99
10/9/2002	2	18	13.57	0.209	0.012	10.1	41.0	9.6	4.81	-26.29	11.99
10/9/2002	3	17	14.44	0.220	0.013	8.9	41.0	9.5	4.95	-26.44	12.14
11/14/2002	1	28	14.99	0.459	0.016	11.0	51.7	12.7	4.74	-26.86	11.43
11/14/2002	2	20	16.97	0.377	0.019	10.0	42.1	10.2	4.84	-26.43	12.15
12/11/2002	1	12	17.55	0.315	0.026	11.0	33.4	7.7	5.06	-26.35	12.75
12/11/2002	2	7	19.18	0.221	0.032	10.0	64.6	14.2	5.29	-26.95	12.44
12/11/2002	3	6	20.47	0.227	0.038	10.0	37.5	9.2	4.75	-26.74	11.55
1/8/2003	1	18	13.80	0.223	0.012	8.9	35.0	8.6	4.74	-23.88	11.19
1/8/2003	2	10	17.60	0.228	0.023	7.9	45.9	11.6	4.63	-26.85	11.54
1/8/2003	3	6	20.29	0.190	0.032	7.8	53.6	14.0	4.46	-26.56	11.97
2/20/2003	1	6	16.50	0.098	0.016	7.8	46.7	12.0	4.57	-27.78	10.13
2/20/2003	2	6	17.44	0.107	0.018	8.0	48.1	11.6	4.85	-27.64	10.42
2/20/2003	3	6	19.27	0.145	0.024	7.9	46.5	11.9	4.55	-27.53	10.30
3/19/2003	1	33	10.98	0.225	0.007	7.6	45.6	11.8	4.51	-29.67	7.10
3/19/2003	2	10	16.40	0.207	0.021	6.0	44.8	11.8	4.44	-28.71	8.16
3/19/2003	3	4	19.39	0.111	0.028	7.0	44.3	11.6	4.45	-28.26	8.96
7/16/2003	1	35	11.49	0.234	0.007	6.2	44.7	11.1	4.68	-28.05	10.20
7/16/2003	2	20	12.53	0.180	0.009	6.7	42.5	10.8	4.61	-27.95	9.91
7/16/2003	3	15	13.55	0.179	0.012	6.7	42.2	10.7	4.60	-27.84	9.91
8/13/2003	1	22	11.52	0.179	0.008	6.2	43.6	10.5	4.85	-27.57	11.03
8/13/2003	2	26	12.56	0.268	0.010	6.4	42.9	10.5	4.75	-27.67	10.94
8/13/2003	3	22	13.52	0.247	0.011	6.2	43.9	10.8	4.73	-27.56	11.16
9/10/2003	1	21	12.55	0.219	0.010	7.5	42.3	10.5	4.66	-27.31	11.03
9/10/2003	2	17	13.56	0.222	0.013	7.7	43.5	10.4	4.89	-27.40	11.11
9/10/2003	3	10	17.51	0.243	0.024	7.3	41.6	10.3	4.72	-27.19	10.91
10/16/2003	1	14	16.52	0.300	0.021	8.0	39.9	9.9	4.69	-26.76	12.33
10/16/2003	2	10	17.37	0.242	0.024	8.4	40.1	9.6	4.88	-26.67	12.26
10/16/2003	3	5	20.38	0.216	0.043	7.1	39.9	9.4	4.96	-26.72	12.42
11/19/2003	1	47	10.50	0.244	0.005	10.7	39.5	9.4	4.98	-27.40	10.95
11/19/2003	2	37	11.38	0.245	0.007	9.7	39.1	9.4	5.07	-27.37	10.99
11/19/2003	3	7	16.59	0.178	0.025	7.7	38.5	9.5	5.35	-27.14	11.56
12/17/2003	1	8	15.45	0.161	0.020	7.9	42.4	9.7	5.08	-27.27	10.91
12/17/2003	2	10	17.68	0.270	0.027	7.9	43.3	9.8	5.17	-27.28	11.08
12/17/2003	3	10	18.51	0.289	0.029	7.8	43.5	9.7	5.22	-27.27	11.23

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
1/13/2004	1	22	11.54	0.151	0.007	7.1	43.9	10.8	4.73	-27.29	10.71
1/13/2004	2	13	14.52	0.205	0.016	6.2	43.7	10.1	5.07	-27.22	11.04
1/13/2004	3	15	16.46	0.318	0.021	7.1	44.7	10.2	5.08	-27.18	10.85
2/11/2004	1	35	11.48	0.234	0.007	6.8	42.9	10.5	4.76	-27.91	9.52
2/11/2004	2	19	13.45	0.223	0.012	6.4	43.3	10.4	4.83	-27.51	10.03
2/11/2004	3	10	16.39	0.203	0.020	6.7	43.9	10.3	4.98	-27.38	10.46
3/10/2004	1	47	10.38	0.299	0.006	5.5	44.6	10.8	4.83	-28.65	8.28
3/10/2004	2	33	11.36	0.262	0.008	5.5	43.4	10.4	4.89	-28.58	7.99
4/21/2004	1	49	10.01	0.377	0.008	4.4	46.1	10.3	5.23	-29.89	5.69
4/21/2004	2	44	11.95	0.539	0.012	3.9	46.5	10.3	5.27	-29.78	5.72
4/21/2004	3	14	16.43	0.404	0.029	4.8	44.9	10.8	4.83	-29.24	7.20
5/19/2004	1	83	9.30	0.423	0.005	4.8	44.2	10.3	5.03	-26.57	8.87
5/19/2004	2	45	10.82	0.345	0.008	4.9	43.6	10.2	4.98	-26.69	8.78
5/19/2004	3	23	13.07	0.321	0.014	5.1	43.8	10.1	5.05	-26.81	8.96
6/23/2004	1	63	10.21	0.337	0.005	9.1	42.1	10.4	4.69	-27.12	10.45
6/23/2004	2	34	11.50	0.251	0.007	8.2	41.7	10.4	4.67	-27.08	10.38
6/23/2004	3	27	12.47	0.248	0.009	8.7	42.7	10.5	4.72	-27.25	10.31
6/23/2004	4	21	13.28	0.230	0.011	7.8	41.6	10.4	4.67	-27.18	10.46
6/23/2004	5	17	14.51	0.252	0.015	7.8	40.9	10.0	4.75	-27.21	10.14
7/27/2004	1	28	11.43	0.207	0.007	8.3	43.3	10.7	4.70	-27.23	10.82
7/27/2004	2	27	12.47	0.243	0.009	9.3	43.7	10.9	4.67	-27.15	10.89
7/27/2004	3	17	14.57	0.222	0.013	7.9	44.4	11.0	4.72	-27.10	10.49
8/25/2004	1	29	11.49	0.211	0.007	8.2	43.9	10.5	4.82	-26.74	10.89
8/25/2004	2	27	12.45	0.237	0.009	9.5	44.1	11.0	4.69	-26.68	10.74
8/25/2004	3	31	13.49	0.339	0.011	8.7	43.6	10.7	4.75	-26.70	10.80
9/15/2004	1	19	13.57	0.248	0.013	7.3	41.7	9.8	4.97	-26.13	11.38
9/15/2004	2	11	15.38	0.207	0.019	7.3	41.8	9.6	5.10	-26.18	11.43
9/15/2004	3	7	16.55	0.162	0.023	6.8	42.1	9.5	5.15	-26.13	11.38
11/4/2004	1	17	14.06	0.249	0.015	6.8	42.1	9.3	5.28	-26.61	11.24
11/4/2004	2	11	16.61	0.248	0.023	6.7	41.4	9.0	5.34	-26.54	11.05
11/4/2004	3	8	18.34	0.239	0.030	5.6	40.4	8.7	5.39	-26.55	10.98
12/14/2004	1	13	14.49	0.195	0.015	8.0	42.6	9.9	4.90	-26.87	10.80
12/14/2004	2	11	15.43	0.179	0.016	8.3	44.3	9.9	5.23	-27.01	10.46
12/14/2004	3	9	16.36	0.175	0.019	7.9	42.0	9.6	5.11	-26.88	10.48
1/12/2005	1	14	15.36	0.221	0.016	6.4	41.9	10.0	4.88	-27.00	9.93
1/12/2005	2	11	16.47	0.208	0.019	6.3	42.0	10.1	4.84	-26.98	9.85

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
1/12/2005	3	8	17.41	0.187	0.023	5.6	66.3	15.4	5.03	-26.92	9.84
2/24/2005	1	24	13.25	0.221	0.009	6.3	42.5	10.8	4.60	-27.50	8.59
2/24/2005	2	14	15.57	0.200	0.014	7.0	41.8	10.4	4.68	-27.22	9.34
2/24/2005	3	15	16.79	0.266	0.018	6.1	43.0	11.0	4.56	-27.25	9.64
3/23/2005	1	25	12.37	0.220	0.009	5.0	43.8	10.9	4.69	-28.89	7.82
3/23/2005	2	10	15.91	0.156	0.016	5.6	43.2	11.0	4.57	-28.32	8.67
3/23/2005	3	6	17.88	0.133	0.022	5.7	43.0	10.9	4.62	-28.30	8.49
4/13/2005	1	28	11.94	0.272	0.010	5.1	44.1	10.3	4.98	-30.13	6.36
4/13/2005	2	18	15.39	0.307	0.017	5.2	43.7	10.9	4.68	-28.93	7.55
4/13/2005	3	11	16.27	0.281	0.026	4.7	35.4	8.6	4.79	-28.81	7.75
5/11/2005	1	70	8.50	0.388	0.006	3.3	44.9	9.3	5.62	-28.58	7.05
5/11/2005	2	50	9.51	0.379	0.008	3.1	44.6	9.7	5.38	-28.51	7.21
5/11/2005	3	37	10.45	0.359	0.010	3.4	44.3	9.8	5.29	-28.40	7.19
6/22/2005	1	75	9.19	0.382	0.005	3.8	45.1	9.7	5.45	-29.19	6.91
6/22/2005	2	40	10.96	0.348	0.009	4.0	44.9	9.2	5.70	-29.16	7.06
6/22/2005	3	20	13.02	0.268	0.013	4.1	42.7	9.0	5.56	-28.93	7.25
8/10/2005	1	34	12.10	0.326	0.010	7.8	40.9	10.3	4.62	-27.84	9.87
8/10/2005	2	21	14.15	0.301	0.014	7.2	41.2	10.2	4.71	-27.87	9.83
8/10/2005	3	9	17.77	0.246	0.027	6.7	40.1	9.5	4.93	-27.70	9.80
9/8/2005	1	21	14.02	0.355	0.017	7.7	42.5	10.5	4.71	-27.31	9.81
9/8/2005	2	14	15.49	0.287	0.021	8.2	42.3	10.7	4.61	-27.37	10.02
9/8/2005	3	9	17.71	0.266	0.030	7.2	41.3	10.1	4.75	-27.20	9.77
10/13/2005	1	15	14.09	0.258	0.017	7.7	43.3	9.5	5.35	-27.45	9.62
10/13/2005	2	10	16.39	0.226	0.023	7.2	43.4	9.9	5.10	-27.61	9.50
10/13/2005	3	8	17.57	0.210	0.026	6.8	43.1	9.7	5.16	-27.53	9.79
11/9/2005	1	19	14.38	0.351	0.018	7.8	41.0	9.3	5.17	-27.29	9.92
11/9/2005	2	10	17.51	0.266	0.027	7.5	41.3	9.6	5.02	-27.35	9.91
11/9/2005	3	7	18.96	0.244	0.035	7.0	40.9	9.0	5.32	-27.29	9.96
12/8/2005	1	16	16.19	0.360	0.022	8.3	41.6	9.6	5.07	-27.43	9.73
12/8/2005	2	10	17.43	0.264	0.026	7.6	41.3	9.4	5.14	-27.29	9.86
12/8/2005	3	8	18.47	0.242	0.030	7.6	41.1	9.7	4.93	-27.34	10.02
1/11/2006	1	22	11.22	0.146	0.007	6.5	44.3	10.4	4.96	-27.40	8.86
1/11/2006	2	11	16.48	0.235	0.021	6.1	43.8	9.9	5.16	-27.25	9.49
1/11/2006	3	6	18.48	0.159	0.026	6.1	44.2	10.4	4.95	-27.22	9.64
2/15/2006	1	46	10.18	0.258	0.006	4.9	43.9	10.1	5.05	-29.63	8.00
2/15/2006	2	24	13.19	0.291	0.012	5.3	44.3	10.4	4.97	-29.06	8.48

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
2/15/2006	3	8	17.46	0.201	0.025	4.2	44.5	10.7	4.85	-28.59	8.69
3/16/2006	1	32	10.93	0.302	0.009	3.9	44.9	10.5	4.99	-30.63	7.11
3/16/2006	2	8	16.94	0.223	0.028	3.7	42.0	11.0	4.46	-29.15	8.03
3/16/2006	3	5	19.56	0.218	0.044	3.9	41.5	10.7	4.51	-29.57	7.54
5/10/2006	1	45	9.08	0.273	0.006	2.9	47.5	10.2	5.44	-31.70	6.90
5/10/2006	2	21	11.59	0.287	0.014	2.9	47.7	10.8	5.13	-31.55	6.85
5/10/2006	3	8	13.68	0.185	0.023	2.0	46.3	10.3	5.24	-31.27	6.73
8/16/2006	1	14	13.57	0.285	0.020	4.4	45.0	9.6	5.49	-26.93	7.50
8/16/2006	2	9	16.57	0.243	0.027	4.9	44.0	10.1	5.08	-26.61	7.39
8/16/2006	3	7	17.47	0.215	0.031	5.0	43.8	10.1	5.07	-26.66	7.39
9/13/2006	1	43	9.94	0.258	0.006	6.2	42.4	10.5	4.69	-27.15	8.20
9/13/2006	2	14	15.50	0.233	0.017	6.0	41.1	10.5	4.56	-26.76	8.17
9/13/2006	3	10	17.34	0.221	0.022	6.3	42.1	10.7	4.58	-26.58	8.33
10/18/2006	1	10	14.56	0.146	0.015	5.9	42.5	10.2	4.85	-27.27	8.74
10/18/2006	2	7	17.32	0.159	0.023	5.8	41.9	10.4	4.72	-27.15	8.93
10/18/2006	3	6	18.25	0.171	0.029	5.4	42.5	10.0	4.94	-27.15	8.95
11/15/2006	1	21	12.80	0.202	0.010	7.4	41.4	10.3	4.70	-27.39	9.21
11/15/2006	2	10	17.58	0.222	0.022	6.3	40.7	9.9	4.79	-27.17	8.91
11/15/2006	3	8	18.62	0.218	0.027	6.2	40.5	9.7	4.86	-27.17	8.99
12/13/2006	1	44	10.66	0.269	0.006	6.3	40.9	10.0	4.78	-28.09	8.76
12/13/2006	2	13	15.23	0.236	0.018	6.5	39.9	9.6	4.83	-27.59	8.93
12/13/2006	3	10	17.42	0.240	0.024	6.1	39.8	9.6	4.82	-27.53	8.87
1/10/2007	1	58	9.01	0.225	0.004	8.2	42.8	10.3	4.86	-28.54	8.11
1/10/2007	2	17	12.83	0.190	0.011	6.7	42.0	10.1	4.84	-27.84	8.71
1/10/2007	3	9	16.71	0.199	0.022	6.3	42.0	10.1	4.84	-27.73	8.74
2/7/2007	1	46	9.04	0.168	0.004	8.0	43.0	10.6	4.73	-28.51	8.25
2/7/2007	2	22	11.36	0.151	0.007	8.2	43.6	10.7	4.76	-28.02	8.45
2/7/2007	3	6	17.18	0.138	0.023	6.4	41.8	10.2	4.77	-27.68	8.51
4/4/2007	1	80	8.83	0.258	0.003	5.6	44.2	10.7	4.81	-29.32	7.24
4/4/2007	2	30	10.97	0.167	0.006	5.9	43.2	10.8	4.64	-28.93	7.67
4/4/2007	3	12	15.62	0.189	0.016	5.3	43.4	11.4	4.44	-28.17	8.18
7/17/2007	1	79	8.53	0.216	0.003	7.2	43.1	10.2	4.91	-27.57	9.86
7/17/2007	2	60	9.53	0.224	0.004	8.3	43.5	10.5	4.81	-27.57	9.84
7/17/2007	3	40	10.42	0.181	0.005	9.2	43.5	10.6	4.80	-27.27	10.03
8/21/2007	1	81	9.28	0.300	0.004	9.8	43.4	9.9	5.14	-27.16	10.03
8/21/2007	2	40	11.42	0.258	0.006	9.6	43.8	10.0	5.14	-27.02	10.10

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
8/21/2007	3	28	12.40	0.218	0.008	7.4	43.3	10.4	4.87	-26.84	10.14
9/12/2007	1	27	10.48	0.166	0.006	9.1	43.0	9.7	5.19	-26.61	10.46
9/12/2007	2	28	11.46	0.204	0.007	9.4	42.5	9.5	5.23	-26.47	10.52
9/12/2007	3	21	12.38	0.188	0.009	9.0	41.9	9.6	5.09	-26.40	10.36
10/24/2007	1	42	10.18	0.212	0.005	10.0	43.9	9.8	5.22	-27.01	10.12
10/24/2007	2	27	12.43	0.227	0.008	10.0	43.3	9.8	5.14	-26.86	10.27
10/24/2007	3	13	14.51	0.167	0.013	9.9	43.4	9.7	5.23	-26.69	10.41
11/15/2007	1	35	11.48	0.232	0.007	10.0	41.9	9.5	5.13	-26.71	10.22
11/15/2007	2	30	12.47	0.240	0.008	10.0	41.7	9.7	5.02	-26.87	10.20
11/15/2007	3	25	13.41	0.231	0.009	8.5	42.2	9.8	5.00	-26.83	10.19
12/12/2007	1	48	9.86	0.223	0.005	12.0	43.1	10.1	4.99	-26.94	10.06
12/12/2007	2	25	12.51	0.215	0.009	11.0	41.7	9.8	4.96	-26.84	10.16
12/12/2007	3	22	13.32	0.220	0.010	10.0	42.8	9.7	5.14	-26.81	10.03
2/13/2008	1	35	10.46	0.152	0.004	8.6	43.8	11.6	4.38	-27.14	10.16
2/13/2008	2	30	11.50	0.171	0.006	8.4	43.5	11.4	4.44	-27.03	10.28
2/13/2008	3	21	13.37	0.180	0.009	7.5	42.7	11.0	4.54	-27.21	10.03
5/7/2008	1	58	9.47	0.390	0.007	7.4	40.6	10.0	4.72	-27.32	9.16
5/7/2008	2	40	10.64	0.360	0.009	8.2	41.7	10.6	4.59	-27.43	9.52
5/7/2008	3	10	14.30	0.180	0.018	9.2	40.2	10.5	4.46	-27.41	9.44
6/18/2008	1	52	9.62	0.254	0.005	13.0	39.6	10.3	4.47	-27.08	11.36
6/18/2008	2	31	11.82	0.261	0.008	12.0	38.9	10.2	4.44	-26.98	10.89
6/18/2008	3	8	15.63	0.145	0.018	12.0	39.2	10.3	4.46	-26.79	10.92
7/16/2008	1	42	10.64	0.280	0.007	12.0	39.3	9.9	4.63	-26.77	11.53
7/16/2008	2	30	12.88	0.329	0.011	12.0	38.9	10.1	4.51	-26.60	11.71
7/16/2008	3	18	14.18	0.267	0.015	12.0	38.7	9.7	4.67	-26.66	11.21
9/17/2008	1	75	9.93	0.452	0.006	11.0	39.0	9.0	5.04	-26.97	11.75
9/17/2008	2	22	12.48	0.254	0.012	9.6	39.7	9.2	5.05	-26.94	11.49
9/17/2008	3	13	15.35	0.284	0.022	10.0	37.9	8.8	5.02	-26.60	11.74
10/16/2008	1	28	12.44	0.286	0.010	8.6	36.2	8.1	5.20	-26.93	11.87
10/16/2008	2	17	14.73	0.306	0.018	7.9	36.7	8.4	5.11	-26.68	11.30
10/16/2008	3	9	16.30	0.216	0.024	8.0	36.7	8.0	5.37	-26.82	12.05
11/19/2008	1	30	12.90	0.331	0.011	8.7	37.6	8.8	4.99	-26.93	11.71
11/19/2008	2	17	14.43	0.261	0.015	9.0	36.9	8.2	5.25	-26.77	11.63
11/19/2008	3	11	16.40	0.257	0.023	8.5	36.6	7.8	5.48	-26.89	11.33
12/17/2008	1	60	9.89	0.304	0.005	12.0	37.1	8.9	4.87	-27.20	11.97
12/17/2008	2	23	13.15	0.260	0.011	10.0	35.8	8.7	4.78	-26.85	11.72

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
12/17/2008	3	12	15.40	0.219	0.018	11.0	36.3	8.6	4.94	-26.72	11.82
1/14/2009	1	62	9.43	0.204	0.003	10.0	43.1	10.5	4.81	-27.51	11.40
1/14/2009	2	42	11.10	0.225	0.005	13.0	42.5	10.4	4.75	-27.33	11.44
1/14/2009	3	19	14.50	0.220	0.012	10.0	41.9	10.1	4.84	-27.14	11.70
2/11/2009	1	53	8.47	0.157	0.003	13.0	44.3	10.9	4.74	-28.59	9.69
2/11/2009	2	37	10.38	0.179	0.005	12.0	45.4	11.3	4.70	-28.31	10.05
2/11/2009	3	18	12.50	0.148	0.008	11.0	42.9	10.6	4.72	-28.13	10.11
3/11/2009	1	56	9.13	0.185	0.003	7.2	45.3	11.4	4.64	-28.17	9.67
3/11/2009	2	35	11.28	0.198	0.006	7.6	45.6	11.2	4.75	-28.17	10.10
3/11/2009	3	14	15.08	0.191	0.014	6.5	45.3	11.3	4.69	-27.99	9.71
4/15/2009	1	56	9.00	0.174	0.003	11.0	46.1	11.8	4.56	-28.90	8.99
4/15/2009	2	37	10.46	0.176	0.005	9.3	45.2	11.7	4.52	-28.87	8.99
4/15/2009	3	16	12.33	0.115	0.007	9.6	45.5	11.9	4.44	-27.98	10.47
5/20/2009	1	53	8.59	0.199	0.004	6.4	46.1	11.8	4.56	-28.90	8.99
5/20/2009	2	27	10.39	0.161	0.006	6.8	45.2	11.7	4.52	-28.87	8.99
5/20/2009	3	16	12.82	0.156	0.010	6.9	45.5	11.9	4.44	-27.98	10.47
6/24/2009	1	56	9.15	0.225	0.004	8.9	42.5	10.3	4.79	-28.10	9.85
6/24/2009	2	40	10.53	0.232	0.006	8.7	40.9	10.1	4.73	-28.05	10.13
6/24/2009	3	24	12.05	0.187	0.008	8.9	42.1	10.3	4.77	-27.79	10.03
7/22/2009	1	63	9.09	0.245	0.004	11.0	44.7	10.9	4.77	-27.70	10.42
7/22/2009	2	46	10.56	0.283	0.006	12.0	41.8	10.3	4.73	-27.66	10.57
7/22/2009	3	27	12.22	0.234	0.009	10.0	41.5	10.2	4.73	-27.47	10.55
8/26/2009	1	62	9.71	0.291	0.005	9.1	44.4	10.4	4.99	-26.97	11.16
8/26/2009	2	40	11.93	0.314	0.008	8.9	43.6	10.2	4.98	-26.89	11.25
8/26/2009	3	21	13.49	0.228	0.011	7.9	43.3	10.3	4.92	-26.87	11.28
9/23/2009	1	32	12.03	0.253	0.008	10.0	42.8	9.9	5.04	-26.75	11.05
9/23/2009	2	21	13.44	0.225	0.011	9.7	42.3	9.8	5.02	-26.82	11.22
9/23/2009	3	15	14.91	0.217	0.014	8.6	43.8	10.0	5.10	-26.84	11.46
10/28/2009	1	27	11.54	0.184	0.007	10.0	43.3	10.0	5.03	-26.75	10.89
10/28/2009	2	26	13.21	0.263	0.010	9.9	43.5	10.1	5.01	-26.67	11.18
10/28/2009	3	13	15.05	0.187	0.014	8.8	42.9	10.1	4.95	-26.64	11.51
11/17/2009	1	52	10.77	0.288	0.006	9.9	42.8	9.8	5.09	-27.02	10.95
11/17/2009	2	27	12.62	0.214	0.008	9.7	42.7	9.7	5.13	-27.00	10.95
11/17/2009	3	20	13.89	0.211	0.011	8.9	42.5	9.9	4.99	-26.85	11.15
12/2/2009	1	28	12.53	0.254	0.009	9.8	39.6	9.5	4.86	-27.08	10.90
12/2/2009	2	21	13.50	0.229	0.011	10.0	39.3	9.5	4.83	-26.99	11.10

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
12/2/2009	3	16	14.85	0.209	0.013	9.8	35.2	9.0	4.58	-27.06	12.40
1/6/2010	1	27	12.48	0.226	0.008	11.0	39.4	9.7	4.74	-27.21	10.62
1/6/2010	2	22	13.96	0.254	0.012	10.0	42.9	10.6	4.72	-27.16	10.90
1/6/2010	3	10	15.47	0.147	0.015	11.0	41.4	10.3	4.68	-27.09	11.11
2/24/2010	1	25	12.00	0.159	0.006	7.4	43.1	10.7	4.68	-27.73	10.17
2/24/2010	2	17	13.60	0.152	0.009	6.2	43.5	11.1	4.57	-27.52	10.34
2/24/2010	3	11	15.27	0.131	0.012	5.9	44.7	11.4	4.57	-27.29	11.00

Table 4. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 6.1, northern San Francisco Bay, California, October 1995–August 2000.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; δ, per mil; --, no data; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
10/23/1995	1	9	21.97	0.457	0.051	16.5	--	--	--	--	--
10/23/1995	2	11	19.85	0.449	0.041	13.4	--	--	--	--	--
10/23/1995	3	10	17.98	0.306	0.031	13.6	--	--	--	--	--
10/17/1996	1	16	20.93	0.652	0.041	15.0	--	--	--	--	--
10/17/1996	2	19	20.18	0.727	0.038	15.0	--	--	--	--	--
10/17/1996	3	42	17.10	0.950	0.023	17.0	--	--	--	--	--
10/17/1996	4	53	15.08	0.782	0.015	18.0	--	--	--	--	--
10/17/1996	5	67	13.66	0.755	0.011	18.0	--	--	--	--	--
11/5/1997	1	29	18.76	1.043	0.036	14.6	--	--	--	--	--
11/5/1997	2	34	21.01	1.737	0.051	14.0	--	--	--	--	--
11/5/1997	3	21	22.64	1.214	0.058	13.4	--	--	--	--	--
6/16/1998	1	24	11.96	0.376	0.016	4.7	--	--	--	--	--
6/16/1998	2	12	19.73	0.684	0.057	4.4	--	--	--	--	--
6/16/1998	3	9	22.23	0.739	0.082	6.2	--	--	--	--	--
10/12/1998	1	29	13.61	0.351	0.012	12.0	--	--	--	--	--
10/12/1998	2	32	16.23	0.524	0.016	12.0	--	--	--	--	--
10/12/1998	3	14	17.60	0.298	0.021	13.0	--	--	--	--	--
3/10/1999	1	7	20.00	--	--	7.4	--	--	--	--	--
4/13/1999	1	18	14.66	--	--	7.8	--	--	--	--	--
4/13/1999	2	14	17.24	--	--	7.0	--	--	--	--	--
4/13/1999	3	12	20.54	--	--	7.6	--	--	--	--	--
5/4/1999	1	38	7.91	--	--	3.5	--	--	--	--	--
5/4/1999	2	14	18.98	--	--	6.6	--	--	--	--	--
5/4/1999	3	6	21.18	--	--	7.0	--	--	--	--	--
6/8/1999	1	50	11.51	--	--	6.8	--	--	--	--	--
7/7/1999	1	45	13.18	0.463	0.010	10.8	43.0	9.0	5.50	-24.81	11.50
8/18/1999	1	36	13.76	0.442	0.012	10.5	38.0	9.0	4.81	-24.33	12.29
8/18/1999	2	30	15.97	0.490	0.016	9.5	41.0	10.0	4.75	-24.34	12.54
9/15/1999	1	50	10.88	0.376	0.007	10.6	42.0	10.0	5.02	-24.02	11.74
9/15/1999	2	30	15.04	0.541	0.018	8.2	36.0	8.0	5.04	-24.08	11.94
10/20/1999	1	37	13.05	0.320	0.009	14.0	41.0	9.0	5.05	-25.06	11.44

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
10/20/1999	2	18	17.66	0.478	0.026	11.0	42.0	10.0	5.07	-24.93	11.91
10/20/1999	3	9	19.49	0.248	0.028	13.0	42.0	10.0	4.97	-24.83	12.08
11/10/1999	1	30	15.08	0.449	0.015	12.5	40.0	9.0	5.10	-24.98	11.58
11/10/1999	2	25	18.11	0.553	0.022	13.0	40.0	9.0	5.17	-24.99	11.59
11/10/1999	3	10	21.00	0.420	0.042	12.0	39.0	9.0	5.27	-25.16	11.67
8/9/2000	1	63	10.51	0.236	0.004	13.1	44.0	10.0	4.88	-25.14	10.55
8/9/2000	2	43	13.27	0.339	0.008	10.8	44.0	10.0	4.95	-25.00	10.51

Table 5. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 8.1, northern San Francisco Bay, California, May 1995–February 2010.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; δ, per mil; %, percent; --, no data; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
5/3/1995	1	15	13.02	0.325	0.022	7.6	--	--	--	--	--
5/3/1995	2	13	11.46	0.207	0.016	7.0	--	--	--	--	--
5/3/1995	3	26	9.86	0.264	0.010	6.8	--	--	--	--	--
10/23/1995	1	9	17.46	0.264	0.029	14.5	--	--	--	--	--
10/23/1995	2	19	15.55	0.393	0.021	14.9	--	--	--	--	--
10/23/1995	3	19	12.56	0.211	0.011	16.8	--	--	--	--	--
12/15/1995	1	21	13.63	0.274	0.013	16.9	--	--	--	--	--
1/12/1996	1	14	17.00	0.315	0.023	13.2	--	--	--	--	--
1/12/1996	2	18	15.36	0.297	0.016	16.3	--	--	--	--	--
1/12/1996	3	35	13.33	0.410	0.012	16.7	--	--	--	--	--
2/8/1996	1	13	17.29	0.300	0.023	18.6	--	--	--	--	--
2/8/1996	2	15	15.67	0.270	0.018	18.6	--	--	--	--	--
2/8/1996	3	19	14.36	0.268	0.014	19.5	--	--	--	--	--
3/7/1996	1	24	15.28	0.363	0.015	11.4	--	--	--	--	--
3/7/1996	2	23	12.92	0.223	0.010	11.1	--	--	--	--	--
4/4/1996	1	15	16.80	0.546	0.036	13.3	--	--	--	--	--
4/4/1996	2	19	15.42	0.522	0.027	12.3	--	--	--	--	--
4/4/1996	3	20	14.50	0.542	0.027	12.9	--	--	--	--	--
5/2/1996	1	12	17.83	0.444	0.037	10.6	--	--	--	--	--
5/2/1996	2	16	16.39	0.444	0.028	11.6	--	--	--	--	--
5/2/1996	3	20	15.35	0.490	0.024	11.1	--	--	--	--	--
6/13/1996	1	12	18.63	0.500	0.042	11.3	--	--	--	--	--
6/13/1996	2	25	15.32	0.530	0.021	10.4	--	--	--	--	--
6/13/1996	3	35	11.21	0.250	0.007	13.1	--	--	--	--	--
7/18/1996	1	25	18.12	0.735	0.029	10.3	--	--	--	--	--
7/18/1996	2	23	16.13	0.481	0.021	11.4	--	--	--	--	--
7/18/1996	3	41	11.28	0.274	0.007	13.0	--	--	--	--	--
8/14/1996	1	29	17.76	0.881	0.030	10.8	--	--	--	--	--
8/14/1996	2	27	14.46	0.414	0.015	12.4	--	--	--	--	--
8/14/1996	3	43	10.69	0.250	0.006	14.8	--	--	--	--	--
9/19/1996	1	15	17.80	0.404	0.027	12.4	--	--	--	--	--
9/19/1996	2	31	11.73	0.277	0.009	15.6	--	--	--	--	--
10/17/1996	1	39	15.71	0.717	0.018	17.0	--	--	--	--	--

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
10/17/1996	2	42	13.40	0.479	0.011	20.0	--	--	--	--	--
10/17/1996	3	69	12.49	0.613	0.009	20.0	--	--	--	--	--
10/17/1996	4	51	10.76	0.323	0.006	22.0	--	--	--	--	--
11/1/1996	1	40	18.50	1.202	0.030	16.0	--	--	--	--	--
11/1/1996	2	43	15.44	0.693	0.016	18.0	--	--	--	--	--
11/1/1996	3	40	11.68	0.309	0.008	19.0	--	--	--	--	--
12/18/1996	1	20	18.21	0.522	0.026	13.7	--	--	--	--	--
12/18/1996	2	22	15.65	0.346	0.016	12.8	--	--	--	--	--
12/18/1996	3	53	11.81	0.371	0.007	17.8	--	--	--	--	--
1/29/1997	1	14	17.16	0.261	0.019	11.2	--	--	--	--	--
1/29/1997	2	25	13.85	0.246	0.010	11.6	--	--	--	--	--
1/29/1997	3	53	11.16	0.286	0.005	12.2	--	--	--	--	--
4/23/1997	1	10	19.63	0.764	0.076	11.0	--	--	--	--	--
4/23/1997	2	16	16.86	0.812	0.051	10.6	--	--	--	--	--
4/23/1997	3	19	14.86	0.641	0.034	10.4	--	--	--	--	--
5/15/1997	1	14	21.73	1.916	0.137	6.4	--	--	--	--	--
5/15/1997	2	27	19.82	2.754	0.102	5.9	--	--	--	--	--
5/15/1997	3	36	16.77	2.523	0.070	6.4	--	--	--	--	--
6/11/1997	1	18	19.73	1.476	0.082	6.9	--	--	--	--	--
6/11/1997	2	42	15.83	1.350	0.032	8.0	--	--	--	--	--
6/11/1997	3	58	13.42	0.920	0.016	10.1	--	--	--	--	--
7/16/1997	1	14	21.74	1.293	0.092	7.4	--	--	--	--	--
7/16/1997	2	25	17.60	1.041	0.042	8.4	--	--	--	--	--
7/16/1997	3	46	12.39	0.393	0.009	13.0	--	--	--	--	--
8/6/1997	1	17	18.86	0.869	0.051	8.0	--	--	--	--	--
8/6/1997	2	46	13.14	0.452	0.010	13.0	--	--	--	--	--
8/6/1997	3	81	10.83	0.400	0.005	15.0	--	--	--	--	--
10/1/1997	1	--	--	0.601	--	21.0	--	--	--	--	--
10/1/1997	2	--	--	0.547	--	16.0	--	--	--	--	--
10/1/1997	3	--	--	0.559	--	9.5	--	--	--	--	--
11/5/1997	1	47	13.71	0.580	0.012	18.8	--	--	--	--	--
11/5/1997	2	23	17.01	0.597	0.026	15.2	--	--	--	--	--
11/5/1997	3	21	20.78	1.114	0.053	12.0	--	--	--	--	--
9/2/1998	1	45	10.14	0.370	0.008	16.0	--	--	--	--	--
9/2/1998	2	37	11.76	0.448	0.012	15.0	--	--	--	--	--
10/12/1998	1	29	10.24	0.150	0.005	11.0	--	--	--	--	--
10/12/1998	2	36	11.97	0.292	0.008	14.0	--	--	--	--	--
10/12/1998	3	20	14.03	0.275	0.014	14.0	--	--	--	--	--

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
11/11/1998	1	45	10.31	0.376	0.008	14.0	--	--	--	--	--
11/11/1998	2	18	14.83	0.469	0.026	14.0	--	--	--	--	--
6/8/1999	1	50	9.05	--	--	11.0	--	--	--	--	--
6/8/1999	2	46	10.01	--	--	10.0	--	--	--	--	--
6/8/1999	3	45	11.04	--	--	6.5	--	--	--	--	--
7/7/1999	1	60	10.52	0.358	0.006	14.8	46.0	10.0	5.27	-23.23	11.06
7/7/1999	2	39	12.39	0.351	0.009	10.2	41.0	9.0	5.51	-23.13	11.17
7/7/1999	3	39	13.62	0.497	0.013	11.0	44.0	9.0	5.55	-23.18	10.90
8/18/1999	1	40	12.43	0.326	0.008	9.7	42.0	10.0	4.90	-23.28	11.55
8/18/1999	2	34	13.47	0.342	0.010	11.7	42.0	10.0	4.82	-23.16	11.80
8/18/1999	3	30	14.75	0.416	0.014	9.9	51.0	12.0	4.84	-23.04	11.76
9/15/1999	1	30	13.44	0.327	0.011	7.5	44.0	11.0	4.76	-23.17	11.77
9/15/1999	2	24	14.37	0.298	0.012	8.9	36.0	8.0	4.92	-22.94	11.68
9/15/1999	3	19	15.41	0.297	0.016	8.6	34.0	8.0	4.87	-22.92	11.72
10/20/1999	1	35	13.37	0.342	0.010	16.0	42.0	10.0	4.82	-23.32	11.42
10/20/1999	2	25	15.45	0.365	0.015	16.0	44.0	11.0	4.85	-23.20	11.56
10/20/1999	3	19	16.74	0.421	0.022	14.0	42.0	10.0	4.85	-23.13	11.58
11/9/1999	1	61	10.48	0.325	0.005	17.0	38.0	9.0	5.00	-23.51	10.73
11/9/1999	2	32	13.25	0.326	0.010	14.0	41.0	10.0	4.97	-23.31	11.10
11/9/1999	3	26	15.20	0.396	0.015	13.0	38.0	9.0	4.91	-23.25	11.31
1/11/2000	1	56	10.04	0.320	0.006	14.0	41.0	10.0	5.00	-24.15	9.63
1/11/2000	2	44	12.55	0.435	0.010	12.0	38.0	9.0	4.83	-23.90	10.02
1/11/2000	3	32	15.11	0.541	0.017	12.0	42.0	10.0	4.80	-23.70	10.09
2/9/2000	1	80	8.91	0.284	0.004	17.0	44.0	10.0	4.94	-24.38	9.64
3/8/2000	1	54	9.97	0.272	0.005	8.1	42.0	10.0	4.78	-24.76	9.32
3/8/2000	2	37	11.75	0.305	0.008	7.7	42.0	10.0	4.83	-24.63	9.44
3/8/2000	3	18	14.44	0.262	0.015	7.9	37.0	9.0	4.66	-24.40	9.61
5/17/2000	1	55	9.07	0.381	0.007	5.4	--	--	--	--	--
6/14/2000	1	66	10.16	0.364	0.005	9.0	41.0	9.0	5.25	-23.22	11.28
6/14/2000	2	49	11.79	0.440	0.009	8.6	33.0	8.0	5.12	-22.99	11.24
6/14/2000	3	21	13.85	0.356	0.017	7.8	34.0	7.0	5.32	-23.18	11.21
7/19/2000	1	60	10.48	0.306	0.005	11.7	41.0	10.0	5.01	-23.12	10.21
7/19/2000	2	50	11.95	0.369	0.007	9.6	43.0	10.0	5.14	-23.00	10.24
7/19/2000	3	34	14.27	0.518	0.015	9.8	40.0	9.0	5.08	-22.96	10.84
8/9/2000	1	66	9.81	0.248	0.004	14.4	45.0	11.0	4.94	-23.20	10.01
8/9/2000	2	54	11.49	0.335	0.006	11.8	42.0	10.0	4.86	-22.98	11.65
8/9/2000	3	42	13.11	0.411	0.010	11.8	42.0	10.0	5.06	-23.02	10.85
9/6/2000	1	79	9.90	0.321	0.004	18.8	45.0	11.0	4.94	-23.12	10.05

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
9/6/2000	2	44	11.84	0.281	0.006	16.8	43.0	10.0	4.95	-23.06	11.37
9/6/2000	3	22	14.57	0.293	0.013	12.4	40.0	9.0	5.04	-22.88	11.53
10/11/2000	1	57	11.23	0.383	0.007	13.0	41.0	10.0	4.92	-23.06	10.99
10/11/2000	2	35	12.56	0.323	0.009	12.2	44.0	10.0	4.94	-22.97	11.07
10/11/2000	3	16	14.98	0.245	0.015	12.3	40.0	10.0	4.95	-22.89	10.94
11/8/2000	1	56	9.74	0.247	0.004	13.9	40.0	10.0	4.81	-23.44	10.32
11/8/2000	2	43	11.55	0.318	0.007	12.1	36.0	9.0	4.79	-23.30	10.61
11/8/2000	3	41	13.17	0.451	0.011	11.4	35.0	8.0	4.82	-23.13	11.68
12/13/2000	1	43	10.07	0.211	0.005	14.4	41.0	10.0	4.67	-23.22	11.09
2/6/2001	1	54	9.19	0.232	0.004	18.0	--	--	--	--	--
2/26/2001	1	77	8.90	0.296	0.004	20.0	47.0	11.9	4.61	-24.12	9.86
3/22/2001	1	94	8.70	0.365	0.004	17.0	35.2	8.8	4.66	-24.12	10.50
3/22/2001	2	51	9.79	0.250	0.005	16.0	46.8	12.1	4.50	-23.88	10.76
4/24/2001	1	77	9.17	0.409	0.005	15.0	46.0	11.3	4.75	-23.14	11.39
4/24/2001	2	64	10.47	0.471	0.007	15.0	44.1	10.7	4.81	-23.32	11.35
4/24/2001	3	27	11.89	0.263	0.010	15.0	48.1	12.4	4.54	-22.92	11.61
5/22/2001	1	31	12.42	0.465	0.015	9.2	45.3	10.3	5.12	-20.59	11.73
6/19/2001	1	63	10.73	0.468	0.007	14.0	46.0	11.5	4.69	-22.08	11.78
6/19/2001	2	50	12.91	0.620	0.012	9.3	36.1	8.9	4.72	-22.07	11.67
6/19/2001	3	17	14.72	0.323	0.019	9.6	38.0	9.4	4.74	-21.90	11.91
7/18/2001	1	56	12.64	0.678	0.012	12.0	40.3	10.0	4.73	-22.73	11.53
7/18/2001	2	46	14.78	0.755	0.016	13.0	40.7	10.0	4.75	-22.48	11.97
7/18/2001	3	11	16.77	0.273	0.025	11.0	40.0	9.7	4.80	-22.32	11.99
9/11/2001	1	65	12.29	0.630	0.010	16.5	40.0	10.3	4.54	-22.86	12.27
9/11/2001	2	47	14.77	0.753	0.016	13.0	39.8	10.2	4.56	-22.66	12.34
9/11/2001	3	19	16.09	0.356	0.019	17.0	33.5	8.5	4.60	-22.58	12.29
10/16/2001	1	47	10.74	0.678	0.014	16.0	38.8	10.2	4.45	-23.16	12.09
10/16/2001	2	37	12.60	0.349	0.009	14.0	37.4	9.7	4.49	-22.85	12.15
10/16/2001	3	41	13.69	0.510	0.012	10.0	39.9	10.0	4.64	-22.80	12.31
11/27/2001	1	24	11.68	0.157	0.007	18.0	37.7	9.8	4.47	-22.93	12.14
11/27/2001	2	14	15.56	0.190	0.014	12.0	31.0	8.1	4.47	-22.84	12.11
12/18/2001	1	33	12.10	0.284	0.009	17.0	44.7	11.7	4.44	-23.01	12.41
5/8/2002	1	52	8.52	0.213	0.004	12.0	47.6	11.7	4.72	-26.01	9.80
5/8/2002	2	40	9.63	0.236	0.006	12.0	30.7	7.7	4.67	-23.55	11.34
6/5/2002	1	72	8.52	0.273	0.004	14.0	46.1	11.1	4.67	-23.88	11.33
6/5/2002	2	49	9.44	0.234	0.005	13.7	45.2	11.2	4.62	-23.88	11.12
6/5/2002	3	37	10.44	0.249	0.007	11.7	44.7	11.1	4.69	-23.73	11.18
7/17/2002	1	52	9.51	0.247	0.005	15.0	44.7	10.8	4.84	-26.51	12.15

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
7/17/2002	2	35	10.53	0.224	0.006	16.0	29.3	7.1	4.81	-26.40	12.15
7/17/2002	3	31	11.47	0.271	0.009	15.0	32.1	8.3	4.51	-26.48	12.50
8/22/2002	1	47	9.50	0.210	0.004	16.0	59.9	15.6	4.47	-26.11	12.40
8/22/2002	2	46	10.49	0.275	0.006	16.0	29.1	7.8	4.35	-23.67	12.35
8/22/2002	3	35	11.45	0.267	0.008	15.0	40.5	10.6	4.46	-23.74	12.25
9/11/2002	1	54	9.54	0.304	0.006	12.6	42.6	9.9	4.91	-22.89	11.78
9/11/2002	2	37	10.48	0.268	0.007	10.0	41.4	9.4	4.82	-22.91	11.59
9/11/2002	3	28	11.33	0.261	0.009	9.9	41.7	9.7	4.79	-22.97	11.69
10/9/2002	1	43	10.56	0.268	0.006	12.5	43.4	9.8	4.91	-23.30	11.57
10/9/2002	2	34	11.52	0.265	0.008	11.7	43.1	9.3	4.99	-23.37	11.69
10/9/2002	3	30	12.50	0.313	0.010	8.9	43.8	9.6	5.01	-23.37	11.79
11/14/2002	1	60	10.52	0.423	0.007	13.0	35.1	8.4	4.84	-26.51	12.19
11/14/2002	2	40	11.43	0.417	0.010	12.0	53.8	13.1	4.79	-26.66	12.25
11/14/2002	3	29	12.47	0.337	0.012	11.0	30.6	7.5	4.76	-23.39	11.46
12/11/2002	1	50	9.55	0.280	0.006	13.0	51.3	11.8	5.06	-26.89	12.01
12/11/2002	2	38	10.45	--	--	15.0	33.2	8.0	4.81	-24.04	10.70
12/11/2002	3	30	11.43	0.294	0.010	14.0	45.9	11.7	4.58	-24.02	11.27
1/8/2003	1	44	10.56	0.309	0.007	15.0	45.3	11.5	4.60	-26.90	10.40
1/8/2003	2	30	11.45	0.276	0.009	16.0	36.8	9.1	4.70	-24.22	9.60
1/8/2003	3	21	12.45	0.246	0.012	14.0	45.0	11.3	4.66	-24.07	11.19
2/20/2003	1	43	9.60	0.200	0.005	13.8	46.5	11.9	4.57	-25.09	9.54
2/20/2003	2	37	10.50	0.190	0.005	15.8	41.9	9.9	4.96	-25.61	11.08
2/20/2003	3	21	12.58	0.170	0.008	19.4	40.6	9.4	5.01	-25.53	11.00
3/19/2003	1	50	9.45	0.264	0.005	13.6	46.0	11.6	4.58	-25.41	9.81
3/19/2003	2	40	10.57	0.302	0.008	14.6	45.3	11.6	4.54	-25.20	9.86
3/19/2003	3	22	11.53	0.195	0.009	14.9	46.4	11.9	4.54	-25.17	10.03
6/18/2003	1	40	9.49	0.161	0.004	12.1	37.9	9.0	4.88	-25.60	11.71
6/18/2003	2	40	10.47	0.194	0.005	12.2	40.2	10.0	4.70	-25.39	11.75
6/18/2003	3	29	11.41	0.184	0.006	11.8	39.5	9.4	4.89	-25.52	12.04
7/16/2003	1	38	9.54	0.154	0.004	11.0	42.5	10.4	4.74	-24.81	11.07
7/16/2003	2	46	10.55	0.253	0.006	10.9	42.4	10.3	4.78	-24.83	10.99
7/16/2003	3	35	11.39	0.245	0.007	10.6	41.8	10.1	4.82	-24.69	11.07
8/13/2003	1	40	9.54	0.151	0.004	12.8	43.9	10.5	4.86	-25.06	11.22
8/13/2003	2	30	10.42	0.140	0.005	11.8	44.6	10.6	4.89	-25.00	11.49
8/13/2003	3	30	11.47	0.186	0.006	11.2	43.8	10.5	4.62	-25.01	11.53
9/10/2003	1	34	11.54	0.223	0.007	12.8	41.9	10.2	4.79	-24.44	11.36
9/10/2003	2	30	12.45	0.266	0.009	12.3	41.7	10.2	4.77	-24.34	11.45
9/10/2003	3	25	13.45	0.289	0.012	10.3	41.1	10.0	4.80	-24.31	11.62

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
10/16/2003	1	30	11.53	0.246	0.008	10.5	38.5	9.5	4.71	-23.09	11.99
10/16/2003	2	27	12.53	0.294	0.011	9.3	37.9	9.3	4.73	-23.23	12.18
10/16/2003	3	21	13.54	0.270	0.013	8.9	37.6	9.2	4.77	-23.04	12.21
11/19/2003	1	65	9.50	0.281	0.004	11.2	41.4	8.5	4.91	-23.49	11.28
11/19/2003	2	43	10.44	0.258	0.006	11.5	42.0	8.9	4.85	-23.40	11.34
11/19/2003	3	20	12.47	0.203	0.010	9.0	39.2	7.5	4.74	-23.36	11.59
12/17/2003	1	31	10.48	0.183	0.006	10.7	38.3	9.4	4.74	-23.30	11.46
12/17/2003	2	26	11.46	0.179	0.007	11.4	36.1	8.9	4.71	-23.33	11.80
12/17/2003	3	15	14.83	0.249	0.017	9.4	35.9	9.1	4.62	-23.36	11.87
1/13/2004	1	61	9.53	0.284	0.005	13.6	41.4	10.3	4.67	-23.60	11.12
1/13/2004	2	45	10.43	0.268	0.006	13.7	39.5	9.8	4.71	-23.55	11.10
1/13/2004	3	28	11.44	0.208	0.007	13.4	40.6	10.1	4.69	-23.58	11.18
2/11/2004	1	39	9.59	0.206	0.005	14.2	43.3	10.8	4.67	-24.32	9.90
2/11/2004	2	26	10.42	0.194	0.007	14.7	42.9	10.6	4.73	-24.43	9.95
2/11/2004	3	26	11.36	0.231	0.009	12.5	43.4	11.1	4.58	-24.16	10.06
4/21/2004	1	62	10.11	0.538	0.009	6.7	45.4	10.1	5.27	-25.06	9.56
4/21/2004	2	40	11.46	0.464	0.012	7.7	43.7	10.2	4.98	-24.73	9.74
4/21/2004	3	30	12.56	0.456	0.015	7.4	44.0	10.1	5.07	-24.85	9.40
5/19/2004	1	46	10.49	0.329	0.007	9.3	44.5	10.1	5.09	-24.07	10.48
5/19/2004	2	35	11.41	0.306	0.009	9.0	44.3	10.6	4.88	-23.92	10.64
5/19/2004	3	27	12.46	0.321	0.012	8.7	43.8	10.0	5.12	-24.04	10.49
5/19/2004	4	21	13.51	0.317	0.015	8.7	43.2	9.6	5.24	-24.07	10.30
6/23/2004	1	33	11.42	0.263	0.008	10.8	41.0	10.0	4.77	-23.76	11.08
6/23/2004	2	27	12.53	0.272	0.010	10.4	40.0	9.4	4.94	-23.64	11.14
6/23/2004	3	21	13.47	0.257	0.012	8.9	41.0	9.8	4.86	-23.79	11.18
7/27/2004	1	27	11.56	0.232	0.009	12.7	40.5	9.8	4.83	-23.77	11.19
7/27/2004	2	26	12.46	0.277	0.011	12.2	40.2	9.6	4.86	-23.68	11.15
7/27/2004	3	21	13.44	0.273	0.013	11.2	38.6	9.3	4.86	-23.73	11.22
8/25/2004	1	42	11.52	0.387	0.009	10.6	72.5	17.3	4.90	-23.01	11.39
8/25/2004	2	30	13.51	0.418	0.014	10.4	40.3	9.4	4.98	-22.95	11.55
8/25/2004	3	18	15.34	0.345	0.019	10.9	40.1	9.4	4.96	-23.04	11.63
9/15/2004	1	19	12.49	0.277	0.015	9.2	41.3	9.3	5.15	-22.05	11.81
9/15/2004	2	16	14.57	0.344	0.021	9.3	41.6	9.2	5.25	-22.03	11.66
9/15/2004	3	11	15.56	0.274	0.025	9.1	41.3	9.5	5.10	-22.11	11.70
11/4/2004	1	17	14.49	0.308	0.018	8.4	38.3	8.7	5.11	-22.52	11.13
11/4/2004	2	14	15.46	0.325	0.023	7.3	38.6	8.8	5.12	-22.35	11.38
11/4/2004	3	11	16.44	0.290	0.026	8.7	38.5	8.7	5.17	-22.60	11.37
12/14/2004	1	17	13.61	0.269	0.016	10.1	39.2	9.2	4.96	-23.06	10.99

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
12/14/2004	2	17	14.54	0.313	0.018	10.1	39.2	9.6	4.76	-22.98	11.08
12/14/2004	3	11	16.43	0.278	0.025	9.6	39.0	8.8	5.15	-22.98	11.05
1/12/2005	1	16	11.89	0.140	0.009	12.0	42.4	10.3	4.79	-23.08	10.55
1/12/2005	2	10	14.36	0.152	0.015	10.5	42.1	10.3	4.79	-23.11	10.83
1/12/2005	3	8	15.47	0.153	0.019	9.9	41.4	10.2	4.74	-23.00	10.79
3/23/2005	1	23	12.24	0.225	0.010	12.3	41.1	10.4	4.61	-24.87	10.27
3/23/2005	2	13	15.07	0.224	0.017	12.2	40.9	10.7	4.48	-24.42	10.51
3/23/2005	3	8	16.51	0.171	0.021	11.8	39.6	9.9	4.64	-24.53	10.61
4/13/2005	1	63	8.64	0.386	0.006	6.3	44.8	10.3	5.10	-26.15	9.97
4/13/2005	2	21	13.05	0.386	0.018	7.3	44.1	10.4	4.93	-25.39	10.19
4/13/2005	3	6	16.74	0.179	0.030	7.8	44.2	10.9	4.73	-24.86	10.42
5/11/2005	1	55	9.13	0.319	0.006	6.5	44.0	10.1	5.09	-25.22	10.17
5/11/2005	2	46	10.49	0.384	0.008	6.7	43.5	9.7	5.21	-25.13	10.00
5/11/2005	3	35	11.83	0.399	0.011	6.6	44.4	10.3	5.04	-24.91	10.40
6/22/2005	1	68	10.06	0.406	0.006	6.7	43.9	10.1	5.09	-26.09	10.02
6/22/2005	2	50	11.48	0.412	0.008	7.0	44.1	10.5	4.90	-25.81	10.13
6/22/2005	3	50	12.81	0.569	0.011	7.4	44.7	10.7	4.86	-25.82	10.29
8/10/2005	1	44	11.56	0.407	0.009	9.4	39.9	9.5	4.88	-24.94	10.83
8/10/2005	2	22	13.47	0.314	0.014	8.7	39.1	9.3	4.90	-24.69	11.03
8/10/2005	3	16	15.06	0.306	0.019	8.2	38.4	9.0	4.97	-24.66	11.07
9/8/2005	1	64	8.96	0.266	0.004	11.0	40.0	9.5	4.92	-24.54	10.92
9/8/2005	2	30	12.33	0.323	0.011	9.7	38.8	9.3	4.86	-24.55	11.02
9/8/2005	3	18	14.44	0.304	0.017	9.8	37.7	9.1	4.82	-24.49	11.08
10/13/2005	1	63	9.40	0.306	0.005	11.1	41.1	9.6	5.01	-24.26	10.73
10/13/2005	2	34	11.48	0.302	0.009	10.9	39.2	9.2	5.00	-24.33	10.82
10/13/2005	3	21	13.92	0.324	0.015	9.6	38.6	9.0	4.99	-24.33	11.03
11/9/2005	1	53	9.55	0.276	0.005	11.6	37.8	9.0	4.88	-24.24	10.49
11/9/2005	2	32	12.08	0.311	0.010	9.1	36.9	9.0	4.80	-24.24	10.75
11/9/2005	3	17	14.45	0.287	0.017	8.8	36.0	8.6	4.87	-24.44	10.87
12/8/2005	1	49	9.83	0.260	0.005	11.7	38.0	9.1	4.84	-24.13	10.56
12/8/2005	2	29	11.97	0.266	0.009	10.2	36.2	9.0	4.68	-24.10	10.69
12/8/2005	3	23	13.71	0.287	0.012	9.9	35.3	8.6	4.79	-24.17	10.74
1/11/2006	1	45	9.00	0.165	0.004	9.3	45.2	10.5	5.01	-24.32	10.25
1/11/2006	2	24	10.80	0.139	0.006	9.8	45.0	10.3	5.11	-24.32	10.49
1/11/2006	3	15	12.95	0.142	0.009	9.6	44.8	10.5	4.97	-24.25	10.61
2/15/2006	1	39	9.02	0.228	0.006	7.5	46.7	10.9	5.02	-27.82	8.81
2/15/2006	2	28	10.27	0.236	0.008	6.6	46.6	10.9	4.99	-27.56	8.81
2/15/2006	3	13	12.66	0.186	0.014	5.5	46.9	10.8	5.07	-27.66	9.00

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
3/16/2006	1	48	9.19	0.315	0.007	5.1	45.0	10.7	4.89	-27.02	8.30
3/16/2006	2	25	11.48	0.296	0.012	5.2	44.7	10.9	4.78	-27.03	8.36
3/16/2006	3	11	12.83	0.192	0.017	5.4	44.0	10.9	4.71	-26.76	8.52
11/15/2006	1	67	8.93	0.283	0.004	10.0	38.6	9.3	4.84	-24.50	9.77
11/15/2006	2	35	11.23	0.300	0.009	9.6	36.4	8.9	4.77	-24.24	9.94
11/15/2006	3	11	16.70	0.326	0.030	6.4	36.9	8.9	4.86	-24.32	10.33
4/4/2007	1	85	8.51	0.365	0.004	8.5	44.1	10.1	5.09	-23.44	10.44
4/4/2007	2	52	9.36	0.317	0.006	8.5	43.5	9.8	5.19	-23.46	10.51
4/4/2007	3	27	10.44	0.247	0.009	8.0	43.5	9.6	5.27	-23.55	9.84
7/17/2007	1	70	8.61	0.204	0.003	15.0	41.6	9.8	4.97	-23.68	10.90
7/17/2007	2	50	9.49	0.189	0.004	13.0	41.3	9.7	4.98	-23.72	10.83
7/17/2007	3	30	10.46	0.151	0.005	9.3	42.0	9.7	5.05	-23.46	11.00
8/21/2007	1	65	9.56	0.280	0.004	14.0	41.0	9.6	5.01	-23.53	10.68
8/21/2007	2	50	10.51	0.278	0.006	9.2	40.9	9.6	4.95	-23.41	10.72
8/21/2007	3	24	11.47	0.175	0.007	13.0	40.8	9.5	4.99	-23.38	10.89
9/12/2007	1	76	9.16	0.314	0.004	16.5	41.8	9.7	5.03	-23.33	10.74
9/12/2007	2	48	10.56	0.288	0.006	15.0	41.1	9.4	5.09	-23.21	10.73
9/12/2007	3	31	11.89	0.257	0.008	14.0	41.7	9.5	5.14	-23.10	10.93
10/24/2007	1	54	9.57	0.262	0.005	15.0	40.7	9.4	5.05	-23.37	10.50
10/24/2007	2	46	10.50	0.292	0.006	15.0	40.6	9.5	5.01	-23.34	10.57
10/24/2007	3	42	11.47	0.326	0.008	14.0	40.0	9.5	4.93	-23.35	10.57
11/15/2007	1	49	10.50	0.313	0.006	8.8	40.1	9.6	4.89	-23.55	10.40
11/15/2007	2	35	11.41	0.290	0.008	15.0	40.5	9.6	4.90	-23.54	10.42
11/15/2007	3	30	12.45	0.288	0.010	14.0	39.4	9.4	4.89	-23.45	10.51
12/12/2007	1	70	9.29	0.345	0.005	16.5	40.9	9.8	4.89	-23.73	10.12
12/12/2007	2	46	10.48	0.310	0.007	16.0	39.4	9.5	4.83	-23.73	10.25
12/12/2007	3	29	11.93	0.276	0.010	16.0	40.7	9.8	4.86	-23.65	10.11
2/13/2008	1	49	8.54	0.219	0.004	19.0	41.2	10.5	4.56	-24.59	9.79
2/13/2008	2	39	10.55	0.307	0.008	19.0	41.6	10.7	4.54	-24.41	9.82
2/13/2008	3	15	12.36	0.185	0.012	16.0	40.9	10.5	4.56	-24.40	10.02
5/7/2008	1	60	10.00	0.445	0.007	17.0	40.0	10.2	4.57	-23.59	11.12
5/7/2008	2	30	11.44	0.311	0.010	16.0	39.2	10.2	4.47	-23.50	11.07
5/7/2008	3	18	12.80	0.241	0.013	16.0	38.4	9.9	4.53	-23.32	10.94
6/18/2008	1	45	10.62	0.329	0.007	15.0	37.6	9.5	4.60	-24.38	11.21
6/18/2008	2	35	11.42	0.330	0.009	12.0	38.6	9.7	4.63	-24.33	11.32
6/18/2008	3	31	13.09	0.424	0.014	15.0	38.0	9.7	4.58	-24.07	11.71
7/16/2008	1	57	9.94	0.348	0.006	11.0	37.1	9.0	4.81	-24.65	11.05
7/16/2008	2	30	12.51	0.360	0.012	13.0	37.1	8.9	4.84	-24.50	11.24

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
7/16/2008	3	33	13.44	0.478	0.014	14.0	37.4	9.3	4.68	-24.28	11.30
9/17/2008	1	45	11.06	0.359	0.008	12.0	36.1	8.7	4.84	-24.31	11.23
9/17/2008	2	21	13.48	0.301	0.014	11.0	35.6	8.8	4.71	-24.03	11.78
9/17/2008	3	17	14.41	0.287	0.017	11.0	34.9	8.1	5.02	-24.10	11.08
10/16/2008	1	59	9.70	0.339	0.006	13.0	34.5	8.5	4.75	-24.32	11.32
10/16/2008	2	31	11.81	0.313	0.010	11.0	34.0	8.2	4.83	-24.20	10.93
10/16/2008	3	16	14.11	0.262	0.016	11.0	33.6	8.2	4.77	-24.04	11.53
11/19/2008	1	41	10.83	0.306	0.007	13.0	34.2	8.4	4.75	-24.51	11.09
11/19/2008	2	22	12.76	0.244	0.011	13.0	33.6	8.4	4.66	-24.24	11.39
11/19/2008	3	18	14.82	0.301	0.017	12.0	26.5	6.9	4.48	-23.96	11.54
12/17/2008	1	27	10.51	0.166	0.006	14.0	35.0	8.9	4.57	-24.37	11.17
12/17/2008	2	16	12.49	0.157	0.010	13.0	34.3	9.1	4.37	-24.11	11.74
12/17/2008	3	8	15.04	0.135	0.017	11.0	32.4	8.3	4.53	-23.98	11.51
1/14/2009	1	54	8.50	0.194	0.004	13.0	41.5	10.1	4.79	-24.92	10.50
1/14/2009	2	24	10.57	0.144	0.006	11.0	41.8	10.2	4.80	-24.71	10.80
1/14/2009	3	11	12.85	0.109	0.010	10.0	38.1	9.7	4.60	-24.39	11.02
2/11/2009	1	71	8.54	0.336	0.005	14.0	45.8	11.2	4.75	-25.73	9.26
2/11/2009	2	49	9.56	0.311	0.006	14.0	46.4	11.2	4.82	-25.71	9.39
2/11/2009	3	12	12.14	0.121	0.010	16.0	43.6	11.0	4.60	-25.04	9.88
5/20/2009	1	53	8.60	0.198	0.004	15.0	43.3	10.6	4.77	-24.76	11.34
5/20/2009	2	54	9.50	0.268	0.005	15.0	43.1	10.3	4.88	-24.72	11.27
5/20/2009	3	37	10.37	0.242	0.007	14.0	42.6	10.1	4.94	-24.75	11.30
6/24/2009	1	53	8.93	0.230	0.004	14.0	43.2	10.5	4.78	-24.55	11.41
6/24/2009	2	46	10.57	0.315	0.007	15.0	42.4	10.1	4.91	-24.51	11.28
6/24/2009	3	35	11.49	0.306	0.009	14.0	41.7	10.2	4.78	-24.39	11.43
7/22/2009	1	43	10.80	0.367	0.009	9.5	44.6	10.2	5.08	-23.99	11.24
7/22/2009	2	33	12.10	0.386	0.012	10.0	44.3	10.4	4.95	-23.94	11.18
7/22/2009	3	17	13.80	0.264	0.016	9.8	42.4	9.8	5.03	-23.91	11.39
8/26/2009	1	36	11.48	0.355	0.010	11.0	41.1	9.6	5.00	-23.75	11.43
8/26/2009	2	30	12.52	0.353	0.012	12.0	41.5	9.6	5.03	-23.77	11.60
8/26/2009	3	21	13.88	0.334	0.016	9.9	39.8	9.1	5.10	-23.77	11.56
9/23/2009	1	26	12.57	0.301	0.012	10.0	40.5	9.4	5.03	-23.76	11.33
9/23/2009	2	21	13.50	0.299	0.014	8.2	40.0	8.8	5.28	-23.75	11.06
9/23/2009	3	17	14.28	0.269	0.016	9.3	39.8	9.2	5.04	-23.78	11.30
10/28/2009	1	19	12.57	0.215	0.011	9.6	39.1	9.1	5.00	-23.76	11.61
10/28/2009	2	17	13.50	0.251	0.015	10.0	40.4	9.4	5.03	-23.62	11.66
10/28/2009	3	14	14.32	0.236	0.017	11.0	40.1	9.2	5.11	-23.65	11.75
11/17/2009	1	27	12.59	0.292	0.011	12.0	39.6	9.3	4.98	-23.79	11.55

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
11/17/2009	2	21	13.35	0.270	0.013	10.0	39.5	9.2	5.04	-23.75	11.50
11/17/2009	3	17	14.41	0.280	0.016	11.0	38.5	8.9	5.02	-23.78	11.69
12/2/2009	1	62	9.43	0.307	0.005	8.2	39.6	9.6	4.81	-24.16	10.92
12/2/2009	2	28	13.30	0.375	0.013	7.6	39.9	9.5	4.91	-23.93	11.42
12/2/2009	3	15	14.36	0.264	0.018	7.5	40.2	9.6	4.87	-23.86	11.45
1/6/2010	1	64	8.58	0.197	0.003	15.0	41.5	10.6	4.55	-24.23	10.76
1/6/2010	2	25	13.13	0.306	0.012	13.0	38.9	9.7	4.67	-23.85	11.38
1/6/2010	3	16	14.40	0.248	0.016	12.0	38.3	9.3	4.79	-23.83	11.54
2/24/2010	1	71	8.49	0.266	0.004	14.0	44.6	11.0	4.73	-25.69	10.02
2/24/2010	2	35	11.00	0.273	0.008	14.0	44.1	11.1	4.63	-25.47	10.18
2/24/2010	3	19	13.44	0.258	0.014	12.0	44.4	11.3	4.57	-24.90	10.91

Table 6. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 12.5, northern San Francisco Bay, California, October 1995–November 1999.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; δ, per mil; %, percent; --, no data; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
10/23/1995	1	16	15.87	0.348	0.022	10.2	--	--	--	--	--
10/23/1995	2	23	13.92	0.294	0.013	10.5	--	--	--	--	--
10/23/1995	3	26	12.42	0.270	0.010	12.3	--	--	--	--	--
10/23/1995	4	28	11.42	0.263	0.009	12.1	--	--	--	--	--
10/23/1995	5	36	10.40	0.247	0.007	13.0	--	--	--	--	--
4/4/1996	1	14	15.39	0.279	0.020	8.6	--	--	4.83	-22.47	10.74
4/4/1996	2	32	14.43	0.538	0.017	9.5	--	--	4.86	-22.56	10.60
4/4/1996	3	39	13.47	0.566	0.015	9.7	--	--	4.83	-22.33	10.58
4/4/1996	4	31	12.56	0.387	0.012	9.6	--	--	5.04	-22.56	10.58
4/4/1996	5	22	11.56	0.224	0.010	9.3	--	--	5.00	-22.56	10.38
4/4/1996	6	23	10.49	0.179	0.008	11.0	--	--	5.03	-22.78	10.31
6/13/1996	1	24	15.54	0.643	0.027	9.8	--	--	--	--	--
6/13/1996	2	20	14.52	0.473	0.024	9.1	--	--	--	--	--
6/13/1996	3	18	13.54	0.357	0.020	10.2	--	--	--	--	--
6/13/1996	4	28	12.33	0.395	0.014	11.0	--	--	--	--	--
7/18/1996	1	53	14.86	1.236	0.023	8.8	--	--	--	--	--
7/18/1996	2	80	12.80	1.151	0.014	10.0	--	--	--	--	--
8/14/1996	1	39	15.28	0.972	0.025	7.9	--	--	--	--	--
8/14/1996	2	50	12.41	0.671	0.013	8.1	--	--	--	--	--
9/17/1996	1	25	15.07	0.583	0.023	8.5	--	--	--	--	--
9/17/1996	2	47	13.61	0.771	0.016	6.4	--	--	--	--	--
10/17/1996	1	27	16.79	0.947	0.035	9.3	--	--	--	--	--
10/17/1996	2	63	15.43	1.854	0.029	5.2	--	--	--	--	--
10/17/1996	3	59	14.50	1.293	0.022	9.8	--	--	--	--	--
10/17/1996	4	55	13.44	0.956	0.017	10.5	--	--	--	--	--
12/18/1996	1	67	14.65	1.167	0.017	12.0	--	--	--	--	--
12/18/1996	2	43	13.57	0.589	0.014	8.9	--	--	--	--	--
12/18/1996	3	42	12.38	0.472	0.011	10.4	--	--	--	--	--
11/5/1997	1	63	11.14	0.654	0.010	17.2	--	--	--	--	--
11/5/1997	2	43	13.01	0.705	0.016	14.0	--	--	--	--	--

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
11/5/1997	3	17	16.56	0.564	0.033	13.6	--	--	--	--	--
6/16/1998	1	62	11.10	0.669	0.011	5.8	--	--	--	--	--
6/16/1998	2	16	12.80	0.449	0.028	5.8	--	--	--	--	--
9/2/1998	1	37	10.96	0.414	0.011	11.0	--	--	--	--	--
9/2/1998	2	36	13.03	0.622	0.017	10.0	--	--	--	--	--
10/12/1998	1	35	11.55	0.308	0.009	10.0	--	--	--	--	--
10/12/1998	2	28	12.46	0.307	0.011	9.5	--	--	--	--	--
10/12/1998	3	25	13.45	0.350	0.014	9.2	--	--	--	--	--
9/15/1999	1	79	9.34	0.467	0.006	7.2	41.0	9.0	5.05	-22.77	10.80
10/20/1999	1	46	10.58	0.390	0.009	10.0	40.0	9.0	5.10	-22.52	10.42
10/20/1999	2	40	11.48	0.524	0.013	10.5	40.0	9.0	5.11	-22.64	10.50
10/20/1999	3	35	13.06	0.573	0.016	10.0	44.0	10.0	5.02	-22.74	10.54
11/10/1999	1	60	10.70	0.401	0.007	11.0	37.0	8.0	5.14	-22.69	9.99
11/10/1999	2	40	12.01	0.416	0.010	11.0	40.0	9.0	5.25	-22.67	10.09
11/10/1999	3	30	13.45	0.509	0.017	11.0	39.0	9.0	5.22	-22.58	10.27

Table 7. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 405.1, northern San Francisco Bay, California, July 1999–February 2000.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; %, percent; δ, per mil; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
7/6/1999	1	59	9.36	0.242	0.004	9.9	43.0	9.0	5.61	-23.59	11.46
7/6/1999	2	46	10.48	0.269	0.006	9.5	42.0	9.0	5.46	-23.32	11.22
7/6/1999	3	39	11.46	0.288	0.007	9.2	41.0	9.0	5.41	-23.20	11.57
8/17/1999	1	55	10.04	0.239	0.004	12.4	43.0	10.0	4.86	-23.27	12.10
8/17/1999	2	45	11.43	0.276	0.006	11.6	39.0	9.0	4.89	-23.13	12.14
8/17/1999	3	30	13.06	0.283	0.009	10.5	45.0	10.0	5.13	-23.12	11.99
9/14/1999	1	52	10.38	0.305	0.006	11.3	38.0	9.0	5.04	-22.89	11.68
9/14/1999	2	29	11.95	0.318	0.011	9.4	19.0	4.0	5.30	-22.83	11.25
10/19/1999	1	44	11.36	0.235	0.005	18.0	41.0	10.0	4.81	-23.23	11.71
10/19/1999	2	40	12.53	0.295	0.007	16.0	41.0	10.0	4.83	-23.22	11.74
10/19/1999	3	36	13.53	0.359	0.010	16.0	45.0	11.0	4.77	-23.24	11.71
11/9/1999	1	51	11.40	0.301	0.006	16.0	41.0	10.0	4.74	-23.33	11.52
11/9/1999	2	43	12.47	0.335	0.008	15.0	41.0	10.0	4.77	-23.33	11.44
11/9/1999	3	35	14.03	0.344	0.010	15.0	42.0	10.0	4.84	-23.35	11.44
2/8/2000	1	32	13.08	0.379	0.119	14.0	42.0	10.0	4.83	-24.26	9.98

Table 8. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 411.1, northern San Francisco Bay, California, July 1999–January 2000.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; %, percent; δ, per mil; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
7/6/1999	1	58	9.98	0.316	0.005	9.6	41.0	8.0	5.86	-24.66	11.12
7/6/1999	2	50	11.12	0.354	0.007	9.5	45.0	9.0	5.54	-24.73	11.03
7/6/1999	3	38	11.73	0.297	0.008	9.8	39.0	8.0	5.59	-24.91	10.91
8/17/1999	1	39	12.39	0.299	0.008	8.9	43.0	11.0	4.71	-24.46	12.50
8/17/1999	2	39	13.45	0.388	0.010	10.6	46.0	11.0	4.72	-24.40	12.67
8/17/1999	3	36	14.56	0.413	0.012	10.4	41.0	10.0	4.76	-24.43	12.54
9/14/1999	1	35	13.47	0.458	0.013	9.2	39.0	9.0	5.16	-23.63	12.05
9/14/1999	2	30	14.84	0.488	0.016	8.0	41.0	10.0	4.97	-23.90	12.14
9/14/1999	3	24	15.71	0.458	0.019	8.1	39.0	9.0	5.09	-23.72	12.10
10/19/1999	1	25	15.05	0.457	0.018	12.0	39.0	9.0	5.24	-24.54	12.17
10/19/1999	2	18	17.53	0.445	0.025	11.0	38.0	9.0	5.24	-24.46	12.19
10/19/1999	3	12	19.16	0.371	0.031	11.0	37.0	8.0	5.06	-24.49	12.13
11/9/1999	1	65	10.12	0.288	0.004	14.0	41.0	10.0	4.96	-24.86	11.37
11/9/1999	2	25	15.80	0.382	0.015	10.0	47.0	11.0	5.10	-24.64	11.96
11/9/1999	3	12	18.06	0.257	0.021	11.0	41.0	9.0	5.04	-24.65	11.95
12/14/1999	1	54	9.93	0.171	0.003	15.0	43.0	10.0	4.91	-25.67	10.62
12/14/1999	2	29	15.12	0.313	0.011	13.0	44.0	10.0	4.96	-25.10	11.36
12/14/1999	3	15	18.03	0.270	0.018	12.0	43.0	10.0	5.06	-25.07	11.31
1/11/2000	1	92	9.50	0.412	0.004	15.0	43.0	10.0	5.13	-26.41	9.64
1/11/2000	2	12	17.03	0.280	0.023	11.0	39.0	9.0	4.83	-25.67	10.79

Table 9. Concentrations of selenium and stable isotopes of carbon and nitrogen in the clam *Corbula amurensis* at USGS benthic station 415.1, northern San Francisco Bay, California, July 1999–November 2003.

[Abbreviations: mm, millimeter; g, gram; µg/g, microgram per gram; %, percent; δ, per mil; dw, dry weight]

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
7/6/1999	1	51	12.58	0.610	0.012	7.4	46.0	10.0	5.47	-26.07	11.95
8/17/1999	1	31	13.54	0.403	0.013	10.4	47.0	11.0	4.84	-25.32	12.67
8/17/1999	2	29	15.46	0.487	0.017	10.6	48.0	12.0	4.76	-25.38	12.72
8/17/1999	3	19	17.08	0.440	0.023	9.7	46.0	11.0	4.75	-25.58	12.76
9/14/1999	1	59	9.57	0.355	0.006	8.8	41.0	10.0	4.92	-24.57	10.76
9/14/1999	2	29	14.32	0.581	0.020	8.5	38.0	9.0	5.04	-24.93	11.21
9/14/1999	3	17	17.62	0.537	0.032	7.7	41.0	9.0	5.11	-24.91	11.11
10/19/1999	1	53	9.79	0.366	0.007	9.6	47.0	11.0	5.04	-26.52	11.42
10/19/1999	2	39	11.76	0.440	0.011	9.9	31.0	7.0	5.17	-26.32	11.61
10/19/1999	3	13	17.81	0.535	0.041	9.1	44.0	9.0	5.68	-26.12	12.02
11/9/1999	1	56	10.26	0.264	0.005	8.9	45.0	10.0	5.04	-26.52	10.86
11/9/1999	2	36	11.99	0.272	0.008	8.4	37.0	9.0	4.93	-26.45	10.78
11/9/1999	3	9	18.56	0.454	0.051	6.3	42.0	8.0	6.33	-26.32	11.98
12/12/2000	1	50	11.17	0.383	0.008	8.8	41.0	9.0	5.21	-26.53	11.60
12/12/2000	2	47	12.86	0.552	0.012	8.1	49.0	10.0	5.45	-26.47	10.86
2/26/2001	1	58	10.92	0.479	0.008	8.6	49.6	12.3	4.69	-27.96	10.52
2/26/2001	2	17	14.39	0.248	0.015	8.0	40.4	10.2	4.62	-27.19	11.25
3/22/2001	1	28	9.51	0.108	0.004	8.6	48.1	12.5	4.48	-27.19	11.24
4/24/2001	1	42	10.79	0.183	0.004	10.0	49.1	12.7	4.51	-27.45	11.22
5/22/2001	1	72	8.84	0.220	0.003	9.9	46.4	11.3	4.77	-27.66	11.68
5/22/2001	2	37	11.34	0.232	0.006	11.0	43.9	10.9	4.68	-27.00	12.16
6/19/2001	1	69	9.03	0.234	0.003	12.0	45.6	11.2	4.76	-27.53	12.51
7/18/2001	1	99	9.74	0.552	0.006	9.6	46.4	10.9	4.97	-25.52	12.04
9/11/2001	1	67	8.98	0.251	0.004	11.0	42.8	10.2	4.87	-24.91	13.94
9/11/2001	2	48	10.90	0.295	0.006	11.0	41.6	10.1	4.80	-24.98	14.04
10/16/2001	1	91	9.93	0.526	0.006	11.0	36.8	8.8	4.86	-25.35	13.80
11/27/2001	1	42	8.94	0.151	0.004	14.0	46.6	11.1	4.89	-26.58	12.39
12/18/2001	1	68	10.17	0.219	0.003	12.0	41.7	10.8	4.50	-26.93	13.41
12/18/2001	2	14	13.09	0.134	0.010	12.0	44.2	11.8	4.37	-26.72	13.59
5/7/2002	1	17	9.40	0.079	0.005	8.3	81.1	20.9	4.53	-23.61	11.56
6/4/2002	1	64	8.52	0.175	0.003	8.6	45.9	11.4	4.84	-27.50	11.45

Date	Sample replicate	Individuals per sample	Average shell length (mm)	Total dry weight (g)	Average dry weight (g)	Se (µg/g dw)	% C	% N	Molar C:N	δ ^{13}C	δ ^{15}N
6/4/2002	2	49	9.50	0.204	0.004	8.5	46.2	11.4	4.72	-27.52	11.59
6/4/2002	3	44	10.75	0.250	0.006	9.3	45.5	11.4	4.71	-27.50	11.46
7/16/2002	1	58	9.52	0.270	0.005	12.0	49.8	12.7	4.58	-24.45	11.85
7/16/2002	2	37	10.43	0.223	0.006	9.9	27.4	6.9	4.67	-24.20	11.85
7/16/2002	3	28	11.42	0.207	0.007	12.0	68.1	16.0	4.96	-27.02	12.23
8/21/2002	1	56	8.95	0.217	0.004	9.8	60.5	15.3	4.62	-23.96	12.23
8/21/2002	2	27	11.48	0.212	0.008	9.4	42.5	10.2	4.84	-25.05	12.11
9/10/2002	1	37	10.49	0.240	0.006	9.7	39.3	9.7	5.01	-25.55	12.20
9/10/2002	2	28	11.64	0.251	0.009	7.5	39.6	9.8	5.13	-25.62	12.10
9/10/2002	3	21	12.51	0.223	0.011	7.8	38.7	9.3	5.00	-25.51	12.22
10/8/2002	1	26	10.47	0.154	0.006	9.6	42.9	10.1	5.16	-26.02	12.36
10/8/2002	2	37	11.47	0.337	0.009	8.5	40.8	9.9	5.38	-26.19	12.34
10/8/2002	3	21	12.46	0.208	0.010	8.6	41.8	9.8	5.34	-26.03	12.56
11/13/2002	1	24	11.45	0.192	0.008	8.8	36.4	8.6	4.96	-25.45	12.11
11/13/2002	2	27	12.61	0.315	0.012	8.2	27.3	6.0	5.32	-26.60	12.23
11/13/2002	3	21	13.52	0.290	0.014	8.7	36.5	7.9	5.39	-26.55	12.23
12/10/2002	1	21	12.50	0.217	0.010	8.4	46.6	10.6	5.11	-26.48	12.56
12/10/2002	2	14	14.33	0.231	0.016	7.6	38.9	9.5	4.77	-23.46	11.86
12/10/2002	3	11	15.53	0.226	0.021	7.4	31.1	7.1	5.15	-26.46	12.04
11/18/2003	1	29	10.48	0.184	0.006	5.9	43.1	10.1	5.70	-26.79	11.49
11/18/2003	2	32	11.45	0.269	0.008	5.9	42.5	9.8	5.48	-26.70	11.82
11/18/2003	3	9	15.69	0.192	0.021	4.9	42.5	9.3	6.07	-26.22	11.74

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Publishing support provided by the U.S. Geological Survey
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