Memorandum

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DATE:	August 15, 2012
TO:	Linda Dorn and Terrie Mitchell, Sacramento Regional County Sanitation District
Cc:	Tom Grovhoug, LWA
SUBJECT:	Information for Consideration by the State Water Resources Control Board during the Update/Review of the Bay-Delta Plan

In advance of several informational workshops to be held in September 2012, the State Water Board has requested that written information be submitted by interested participants regarding ecosystem changes, the low salinity zone, pelagic fishes, and salmonids to inform potential changes to the Bay-Delta Plan. In particular, the State Board is interested in information regarding the level of scientific certainty or uncertainty regarding the scientific and technical information that has emerged since, or was not addressed in, the 2009 Staff Report and the 2010 Delta Flow Criteria Report.

Per your request, I have prepared this memorandum summarizing currently available scientific and technical information pertaining to recently and currently hypothesized direct and indirect effects of ammonia in the upper San Francisco Estuary (the legal Delta plus Suisun Bay). The purpose of this memorandum is to differentiate between lines of investigation where there currently seems to be reasonable agreement among scientists regarding effects of ammonia on Bay-Delta pelagic organisms, and lines of investigation where there is currently disagreement or uncertainty regarding the effects of ammonia.

Over the last several years, a series of hypotheses have been advanced regarding potential effects of ammonia on pelagic organisms in the upper San Francisco Estuary (SFE; used herein to refer to the freshwater Delta and the brackish estuary). Agencies and interested parties have energetically funded ammonia research addressing specific hypotheses that broadly fall into two main categories:

1. Direct effects of ammonia on pelagic fishes or their invertebrate prey through acute or chronic toxicity
2. Indirect effects of ammonia on the pelagic food web, via alterations of phytoplankton biomass or species composition.

Information from these efforts has continued to emerge since the State Board issued the 2009 Staff Report and the 2010 Delta Flow Criteria Report. Recent reviews of the available evidence regarding ammonia have been conducted through the San Francisco Bay Nutrient Numeric Endpoint development process (in Spring 2011)[1], by the San Francisco Regional Water Quality Control Board during the NPDES permit renewal process for Central Contra Costa Sanitary District WWTP in (in Spring 2012)[2], and via the "other stressors" report by the NRC Committee on Sustainable Water and Environmental Management in the California Bay-Delta (Spring 2012).[3] None of these reviews have concluded that enough is known about the potential role of ammonia in the SFE ecosystem to warrant a conclusion that it is a key driver of the pelagic organism decline (POD) or that it is responsible for other undesirable changes in the estuarine food web. Also, two studies which have been highly leveraged since 2010 by some stakeholders as evidence of deleterious effects of ammonia in the SFE (a chronic toxicity study involving the copeopod *Pseudodiaptomus forbesi*,[4] and a correlation exercise using pre-existing monitoring data[5]) were recently shown to be flawed by independent reviewers (see below). Such events illustrate the large uncertainties that currently surround many viewpoints regarding ammonia in the SFE and the potential for hypotheses to take on unwarranted 'lives of their own' in a highly politicized scientific arena.

Table 1 lists several key hypotheses that are the subject of recent and/or ongoing research in the SFE regarding potential effects of ammonia, and a snapshot of the information regarding their current status. More detail about the information from experiments, field studies, or analyses that pertain to each hypothesis is provided in the order that they are listed in the table. The memorandum focuses on publicly available information resulting from experiments or monitoring conducted in the Delta, as opposed to studies from other systems.

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[1] McKee, Lester; Sutula, Martha; Gilbreath, Alicia; Beagle, Julie; Gluchowski, David; and Hunt, Jennifer. 2011. Nutrient Numeric Endpoint Development for the San Francisco Bay Estuary; Literature Review and Data Gaps Analysis (June 2011).

[2] Order No. R2-2012-0016, NPDES No. CA 0037648, Central Contra Costa Sanitary District Wastewater Treatment Plant

[3] National Research Council. 2012. Sustainable Water and Environment Management in the California Bay-Delta. Prepared by the Committee on Sustainable Water and Environmental Management in the California Bay-Delta; Water Science and Technology Board; Ocean Studies Board; Division on Earth and Life Studies; National Research Council. 231 pp.

[4] Teh, S., I. Flores, M. Kawaguchi, S. Lesmeister, and C. Teh. 2010. Full life-cycle bioassay approach to assess chronic exposure of Pseudodiaptomus forbesi to ammonia/ammonium. Oral presentation given to POD Contaminant Workteam, July 2010.

[5] Glibert, Patricia. 2010. Long-Term Changes in Nutrient Loading and Stoichiometry and Their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, California; Reviews in Fisheries Science, Vol. 18, Issue 2 (August 2010).

Table 1. Status of Key Hypotheses Regarding Effects of Ammonia in the Bay-Delta Ecosytem

	Hypothesis	Current Status	Key Relevant Information
Toxicity	1. Ammonia concentrations exceed current US EPA criteria for acute or chronic toxicity	False	Ambient data sets for unionized ammonia have been compared to current EPA criteria by multiple stakeholders and regulators. Ambient concentrations throughout the freshwater Delta and Suisun Bay meet current US EPA criteria.
	2. The US EPA acute criterion is not protective of Delta smelt	False	The UC Davis Aquatic Toxicity Laboratory conducted studies which showed (1) Delta smelt are similar to salmonids in their acute ammonia sensitivity, and (2) that levels of ammonia acutely toxic to Delta smelt are not lower than the current US EPA criterion.
	3. Ammonia concentrations in the Delta cause chronic toxicity to Delta smelt	Not tested. Unlikely.	Laboratory methods are not available for conducting chronic toxicity tests using Delta smelt using US EPA approved chronic test endpoints. However, because Delta smelt show similar acute sensitivity to ammonia as salmonids, and ammonia levels in the Delta do not exceed the US EPA chronic criterion for water bodies with salmonids, it is unlikely that ambient levels of ammonia in the Delta and brackish estuary causes chronic toxicity to Delta smelt. Use of US EPA-approved Genus Acute-Chronic Ratios (ACRs) for fish do not indicate that ambient ammonia concentrations in the Delta pose risk of chronic toxicity.
	4. Ammonia concentrations in the Delta are acutely toxic to key calanoid copepods	False	Ammonia concentrations in the Delta are much lower than acute thresholds reported for the two copepod species *Eurytemora affinis* and *Pseudodiaptomus forbesi.*
	5. Ammonia concentrations in the Delta cause chronic toxicity to key calanoid copepods	Unknown. A previous report was flawed.	A non-peer reviewed report from the UC Davis Aquatic Toxicity Laboratory[6] that alleged potential for chronic toxicity to *Pseudodiaptomus forbesi* in the Sacramento River has been described as seriously flawed in a review by an ecotoxicity testing firm.[7]

[6] Teh, S., I. Flores, M. Kawaguchi, S. Lesmeister, and C. Teh. 2010. Full life-cycle bioassay approach to assess chronic exposure of Pseudodiaptomus forbesi to ammonia/ammonium. Oral presentation given to POD Contaminant Workteam, July 2010.

[7] A Critical Review of "Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of Pseudodiaptomus forbesi to Ammonia/Ammonium – Final Report. Teh et al., August 31, 2011." Prepared by Pacific EcoRisk, Inc., December 26, 2011.

	Hypothesis	Current Status	Key Relevant Information
Indirect food web effects	6. Ammonium concentrations above 4 μM delay the uptake of nitrate by phytoplankton (especially diatoms).	Observed in several studies using small container experiments	Experiments using river or bay water in small containers have shown that ammonium above certain concentrations can delay uptake of nitrate by phytoplankton. Cell size data from incubation experiments imply that diatoms are affected.
	7. Ammonium inhibition of nitrate uptake significantly affects primary production in the Bay-Delta.	No consensus Conflicting evidence has been published.	Field surveys in which short term primary production rates are directly measured (e.g. using carbon isotope tracers) do not consistently demonstrate an inverse relationship between ammonia concentrations (above or below the nitrate-uptake inhibition threshold) and primary production rates. In addition, some longer-term incubations (days) using Sacramento River water resulted in higher phytoplankton biomass when starting ammonium concentrations were above (not below) 4 μM and when ammonium concentrations were higher than nitrate concentrations. Ammonia concentrations below 4 μM do not consistently coincide with phytoplankton blooms in the estuary.
	8. Ammonia has contributed to a deleterious change in phytoplankton composition in the SFE.	Unknown A highly publicized report was statistically flawed.	Current studies in the Delta do not indicate that ammonia concentrations are well-linked to outbreaks of hazardous (toxin-producing) phytoplanton or levels of toxin in the water. Published reports from the Delta to support other postulated effects of ammonia on phytoplankton species composition are currently limited to correlation analysis (not direct experimental evidence). One highly publicized correlation analysis was statistically flawed.
	9. The copepods eaten by Delta smelt (and other pelagic fish) are reliant on diatoms.	False	The principal type of copepods that pelagic fishes eat in the SFE (calanoid copepods) do not rely on diatoms – or even on other phytoplankton – as a direct food source. Calanoid copepods are omnivorous. They also prefer *moving* prey (ciliates, dinoflagellates, flagellated phytoplankton) over non-moving prey (such as diatoms). In addition, diatoms can cause genetic defects in copepod offspring when adults directly consume diatoms. Recent investigation implies that the detrital food web (organic matter-> bacteria -> microzooplankton -> copepods -> fish) may contribute more to fish production in the SFE than commonly believed.

Hypotheses 1-2. Ambient ammonia levels cause acute toxicity to Delta smelt.

Current Status: False

Starting in the 2006, the Interagency Ecological Project (IEP) and the Central Valley Regional Water Quality Control Board funded several investigations by the UC Davis Aquatic Toxicity Lab to determine whether ambient levels of ammonia in the Delta could cause acute toxicity to Delta smelt.[8] As a result of these investigations, and screening work conducted by other investigators using monitoring data, there is now ample evidence that ambient ammonia concentrations throughout the upper SFE are not high enough to cause acute toxicity to Delta smelt or to the wide range of aquatic organisms explicitly protected by current U.S. EPA ammonia criteria. This characterization of ambient conditions applies not only to "POD" years (2002 onward), but also to the entire 35-year period for which long-term monitoring data are available. The characterization also applies to the entire reach of the Sacramento River below the SRWTP discharge (e.g., River Mile 44 and points downstream).

The current U.S. EPA acute criterion for ammonia that applies to water bodies with salmonids was specifically derived to protect rainbow trout. Because repeated rounds of testing indicate that delta smelt have similar acute sensitivity to ammonia as rainbow trout (Werner et al. 2008b, 2009b),[9] the current U.S. EPA acute criterion is appropriately considered protective of delta smelt. The acute thresholds for delta smelt are compared to the thresholds for the three most sensitive fish in the 2009 EPA data base in Table 2.

Two recent studies indicate that ambient concentrations of ammonia throughout the estuary, including in the Sacramento River below the SRWTP, meet current U.S. EPA ammonia criteria:

- In 2010, I compared U.S. EPA acute and chronic criteria with ambient ammonia concentrations from almost 12,000 grab samples taken throughout the freshwater and

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[8] Werner I., L. Deanovic, D. Markiewicz, M. Stillway, N. Offer, R. Connon, and S. Brander. 2008. Pelagic Organism Decline (POD): *Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2006-2007*. Final Report. 30 April 2008.
Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2008b. *The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*. Final Report to the Central Valley Regional Water Quality Control Board. September 26, 2008.
Werner I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009a. *Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt [2008]*. Final Report to the Central Valley Regional Water Quality Control Board, Rancho Cordova, CA.
Werner I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009b. *Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt - 2009*. Final Report to the Central Valley Regional Water Quality Control Board, Rancho Cordova, CA.
Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2010a. *Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species*. Draft Final Report Submitted to the Central Valley Regional Water Quality Control Board on August 23, 2010.
Werner I., D. Markiewicz, L.A. Deanovic, R.E. Connon, S. Beggel, S.J. Teh, M. Stillway, and C. Reece. 2010b. *Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010*. Final Report.

[9] Op. Cit.

brackish estuary from 1974 to the present.[10] The dataset included monitoring results from the IEP, USGS, DWR, USFWS, the District, and the UC Davis Aquatic Toxicology Lab (Figure 1). In this large dataset, ammonia concentrations *never* exceeded the U.S. EPA acute criterion; the chronic criterion was exceeded *only twice* in the available record (one sample each in 1976, 1991). Margins of safety were large: the chronic criterion exceeded ambient concentrations by average factors of 40 and 80, in the brackish and freshwater estuary, respectively.

- Region 5 staff conducted ambient water sampling at 21 sites in the freshwater Delta between March 2009-February 2010 (Foe et al. 2010).[11] None of their measurements of ammonia exceeded the U.S. EPA acute or chronic criterion. In addition, Region 5 staff screened their ambient data using an ultra-conservative, hypothetical chronic criterion for delta smelt, which they created by using the highest of 3 Acute to Chronic Ratios (ACRs) (20.7, 9.7, 6.5) for fathead minnow contained in U.S. EPA (1999)[12]. Although such use of an ACR of 20.7 conflicts with the U.S. EPA interpretation of fathead minnow data,[13] and although U.S. EPA does not use ACRs for single species to derive chronic criteria[14], the hypothetical chronic criterion so derived was not exceeded by any of the ambient concentrations measured in the Regional Board study.

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[10] Engle, D. (2010) Testimony before State Water Resources Control Board Delta Flow Informational Proceeding. Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web. Testimony submitted to the State Water Resources Control Board, February 16, 2010.

[11] Foe, C., A. Ballard, and S. Fong (2010) Nutrient Concentrations and Biological Effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, July 2010.

[12] USEPA. 1999. *1999 Update of Ambient Water Quality Criteria for Ammonia*. EPA 822-R-99-014. United States Environmental Protection Agency, December 1999.

[13] U.S. EPA used the geometric mean of all three available ACRs (20.7, 9.7, 6.5) to characterize the acute:chronic sensitivity of fathead minnow (*Pimephales*), not the highest of the available ACRs (20.7). This was done because U.S. EPA considered the test that yielded the ACRs of 20.7 to be flawed (see U.S. EPA 1999 pp. 53-54). The resulting Genus Mean ACR (GMACR) for fathead minnow is 10.86.

[14] Five GMACRs for fish genera have survived vetting by the U.S. EPA and were published in both the 1999 (see reference above) and 2009 (U.S. EPA, Draft 2009 Update Aquatic Life Ambient Water quality Criteria for Ammonia – Freshwater. EAP-822-D-09-001. December 2009) U.S. EPA ammonia criteria documents (*Pimephales* - 10.86, *Catostomus* - <8.33, *Ictaluris* - 2.712, *Lepomis* - 7.671, *Micropterus* - 7.688). All five GMACRs are used by U.S. EPA in the derivation of the chronic ammonia criterion - not just the GMACR for fathead minnow.

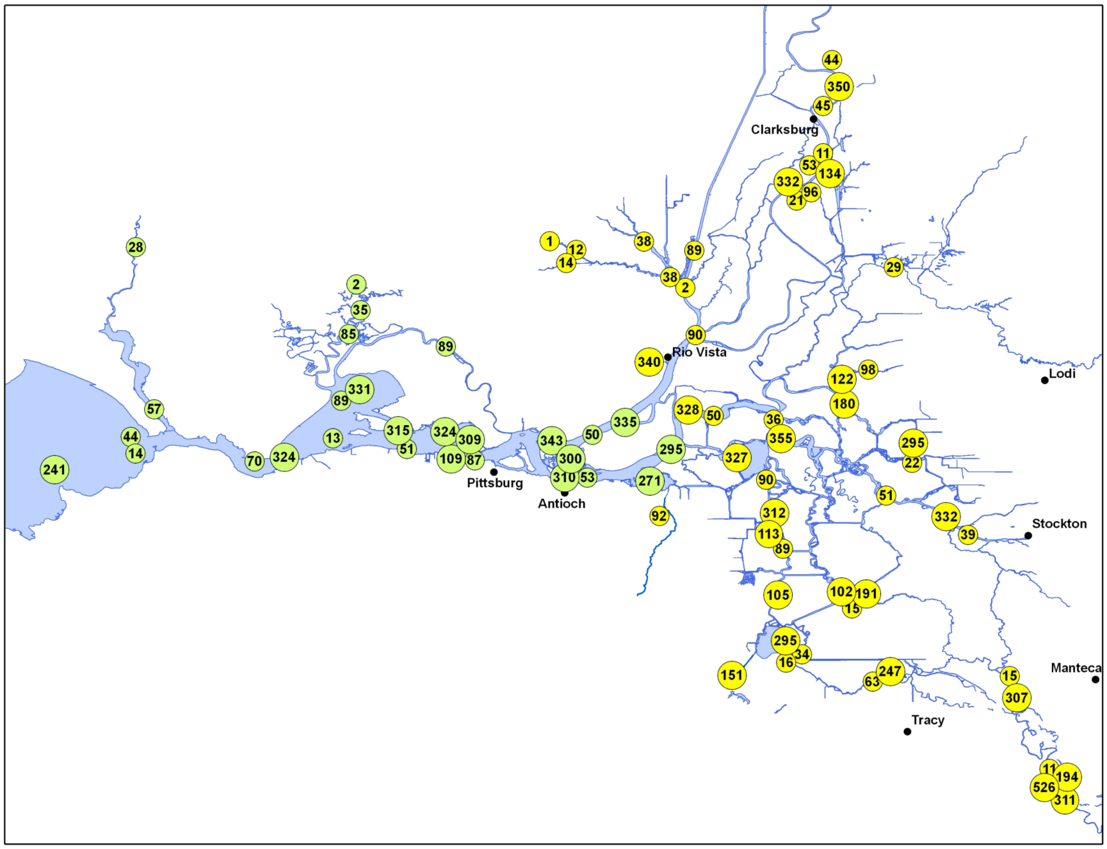

Figure 1. Long-term estuarine (green symbols) and freshwater (yellow symbols) monitoring stations in the Upper San Francisco Estuary providing co-occurring measurements of pH, water temperature, and total ammonia. Values inside symbols are numbers of monthly or bi-weekly grab samples taken during the period 1974-2010. Stations were classified as estuarine or freshwater based on procedures in the California Toxics Rule. In this dataset, ammonia concentrations *never* exceeded the U.S. EPA acute criterion; the chronic criterion was exceeded *only twice* (one sample each in 1976, 1991). Figure is from Engle (2010).[15]

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[15] Op. Cit.

Table 2. Acute Ammonia Toxicity Thresholds for Fish (mg N/L). Delta smelt thresholds are from Werner et al. (2008, 2009).

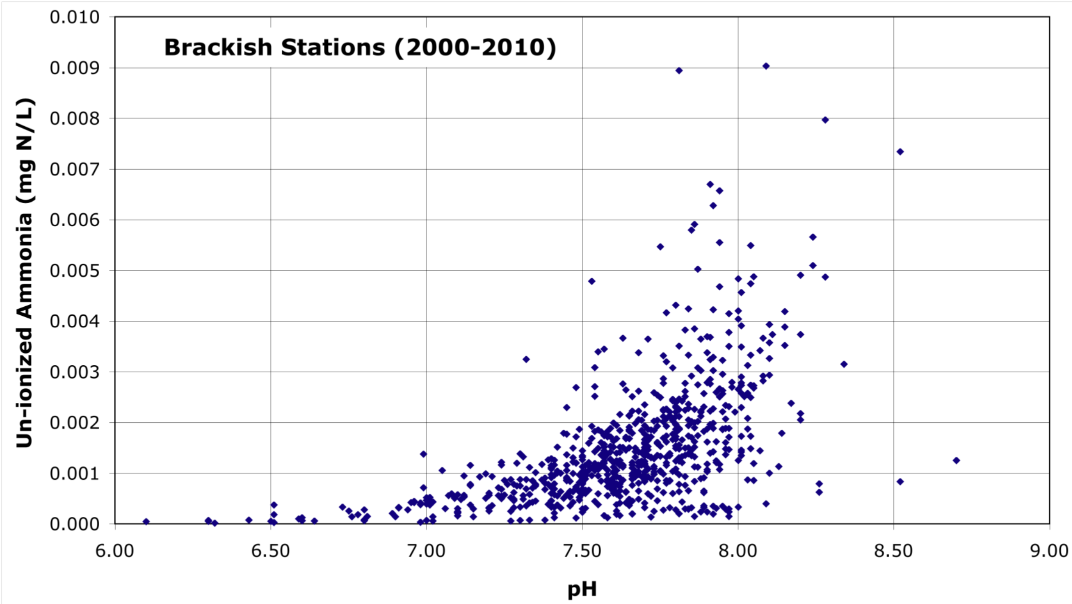
Taxon		Acute Threshold (mg N/L)*
Delta smelt	Juveniles (149 days old)	52.30 (96-hr LC50)
	Larvae (51 days old)	11.63 (96-hr LC50)
	Larvae (47 days old)	11.81 (96-hr LC50)
Species with the lowest mean acute thresholds in USEPA 2009	Mountain whitefish	12.09
	Guadalupe bass	12.70
	Lost River Sucker	13.19
*Ammonia in the Sacramento River at Hood <1.00 mg N/L		

Hypotheses 3. Ambient ammonia levels cause chronic toxicity to Delta smelt.

Current Status: Unknown, Not Tested Using US EPA Chronic Endpoints

Owing to a lack of experimental protocols that have sufficient control survival rates, chronic toxicity tests using US EPA-accepted chronic endpoints have not been conducted. Werner et al. (2008b, 2009b)[16] have expressed an opinion that repeated excursions of pH above 8.0 in the Delta equate to a potential for chronic toxicity for delta smelt. This gross generalization is not evaluated using ambient data in Werner et al. (*ibid.*), and does not constitute a valid basis for inferring chronic toxicity in the estuary. Because total ammonia concentrations and water temperature vary widely within pH strata across the estuary, ambient pH alone is an inappropriate basis for gauging whether un-ionized ammonia concentrations are of concern. Plots of pH versus un-ionized ammonia for both the brackish estuary and the freshwater Delta for the years 2000-2010 (SRCSD 2010) indicate that un-ionized ammonia concentrations span the full range of ambient values (low to high) when pH >8.0.

[16] Op. Cit.



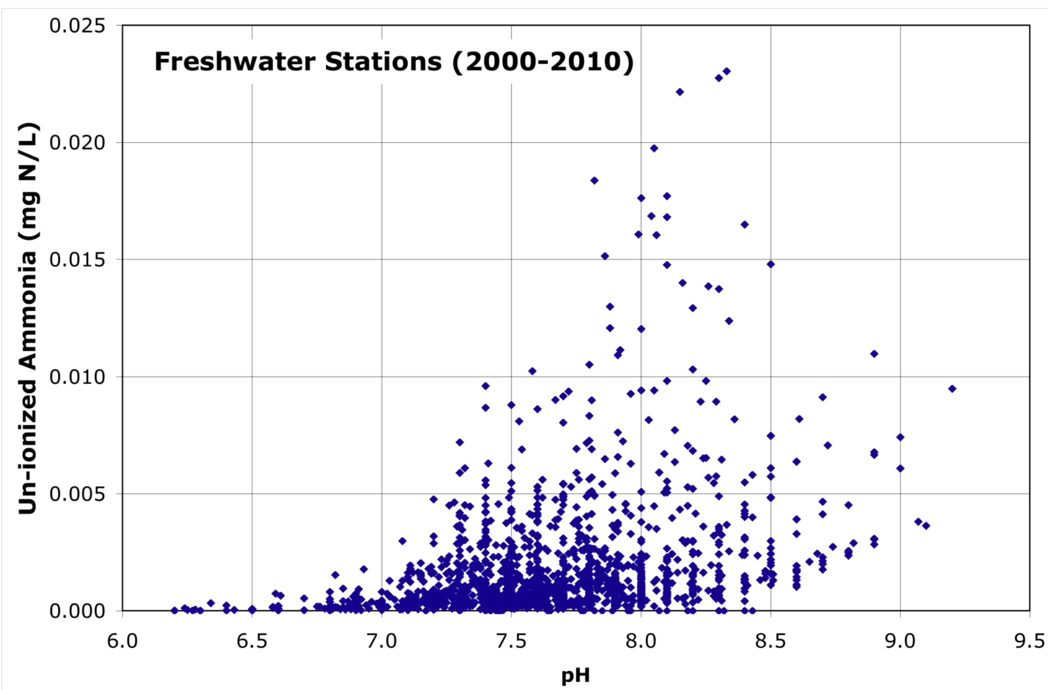


Figure 2. Relationship between field pH and un-ionized ammonia (mg N/L) at brackish stations (upper panel) (Sherman Island to San Pablo Bay) and at freshwater stations (lower panel) in the upper San Francisco Estuary during 2000-2010. Dataset is described in Engle & Lau (2010). Data from eighteen stations used by the IEP, DWR-MWQI, and UC Davis ATL POD project are represented. All of the un-ionized ammonia concentrations in the data set (across all pH) are well below the 96-hr LC10s for 47-d old Delta smelt (0.084, 0.105 mg N/L un-ionized ammonia; Werner et al. 2009[17]). Figure is from SRCSD (2010).[18]

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[17] Op. Cit.

Hypothesis 4. Ammonia causes acute toxicity to key calanoid copepods.

Current Status: False

Allegations that ambient ammonia concentrations can cause acute toxicity to Delta copepods (*Eurytemora affinis* or *Pseudodiaptomus forbesi*) rely on test results from Teh et al. (2009)[19] using misrepresentative pH for the Sacramento River. This experimental issue is described by the Central Valley Regional Water Quality Control Board staff in a summary of the 2009 Ammonia Summit (Foe 2009)[20]. In this summary, Foe acknowledged that the test pH associated with toxicity in Dr. Teh's experiments (7.2) was not representative of ambient pH levels in the Sacramento River:

> *Ten percent mortality occurred to both species at ambient ammonia concentrations present in the river below the SRWTP. **However, toxicity was only observed at a lower pH (7.2) than commonly occurs in the River (7.4 to 7.8)**. Toxicity was not observed when toxicity testing was done at higher pH levels.* (Foe 2009, p. 2, emphasis added)

When environmentally representative pH is considered, test results using *E. affinis* and *P. forbesi* do not indicate a potential for acute toxicity in the Sacramento River or the Delta. The LC10s[21] for *E. affinis* and *P. forbesi* obtained at the most environmentally relevant test pH used (pH 7.6) were both about 5 mg N/L total ammonia.[22] This concentration (5 mg N/L) is about five times higher than the maximum concentrations observed in the Sacramento River from RM-44 and points downstream. These LC10s are higher than the 99.91-% percentile of ammonia concentrations occurring 350 feet below the SRWTP diffuser[23]. In other words, ambient concentrations of total ammonia in the Sacramento River essentially never exceed the lowest acute thresholds (LC10) thus far reported for *E. affinis* or *P. forbesi* for representative pH conditions.

The lack of reasonable potential for acute toxicity for *E. affinis* or *P. forbesi* in the rest of the Delta is reflected by long-term monitoring data; in terms of *un-ionized* ammonia, the LC10 for representative pH 7.6 (0.08 mg N/L un-ionized ammonia) is well above the 99th percentile for freshwater concentrations of un-ionized ammonia in the freshwater Delta for 2000-2010

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[18] Sacramento Regional County Sanitation District Comments on Draft Nutrient Concentration and Biological Effects in the Sacramento-San Joaquin Delta, Central Valley Regional Water Quality Control Board, May 2010. Letter submitted to Chris Foe, Central Valley Regional Water Quality Control Board, June 14, 2010 (SRCSD 2010).

[19] Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009a. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

[20] Foe, C. 2009. *August 2009 Ammonia Summit Summary*. Technical Memo to Jerry Bruns and Sue McConnell, Central Valley Regional Water Quality Control Board, 24 September 2009.

[21] LC10 is the concentration at which it is estimated there is 10% mortality.

[22] LC10s in Teh (2009) were 5.02 and 5.16 mg N/L total ammonia for E. affinis and P. forbesi, respectively.

[23] Larry Walker Associates, 2009 Anti-Degradation Analysis for Proposed Discharge Modification to the Sacramento Regional Wastewater Treatment Plant, DRAFT; prepared for Sacramento Regional County Sanitation District, May 2009.

(0.014 mg N/L un-ionized ammonia, Figure 3)[24]. Finally, only one of the ambient ammonia measurements in the entire data set illustrated in Figure 3 exceeds the preliminary 96-h LOEC for 3-day-old nauplii of *P. forbesi* reported in a Nov. 11, 2010 Letter from S. Teh to C. Foe.[25]

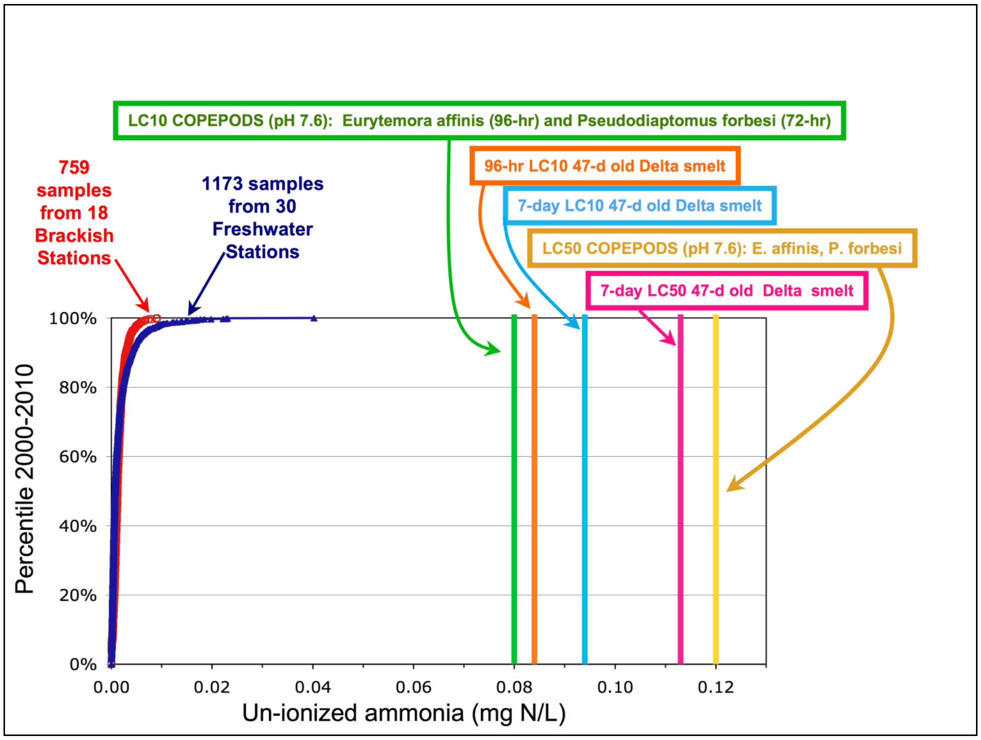

Figure 3. Ranked distribution of ambient concentrations of un-ionized ammonia from estuarine stations (red circles) and freshwater stations (blue triangles) in the upper San Francisco Estuary for 2000-2010. Monitoring stations are illustrated in Figure 1. Included are acute effects thresholds for un-ionized ammonia from exposure tests using Delta smelt and the adult copepods *Eurytemora affinis and Pseudodiaptomus forbesi*. Preliminary 96-h LC10 for juvenile copepods (3-day-old P. forbesi nauplii; 0.030 mg N/L un-ionized ammonia, reported in Nov. 2010. Figure is adapted from Engle (2010a).[26]

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[24] Engle, D. (2010a) Testimony before State Water Resources Control Board Delta Flow Informational Proceeding. Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web. Testimony submitted to the State Water Resources Control Board, February 16, 2010.

[25] Only one of the ambient ammonia measurements in the data set in Figure 3 exceeds the LOEC on the basis of un-ionized ammonia (LOEC is 0.03 mg N/L un-ionized ammonia at reported test conditions of pH 7.8 and temperature 20°C).

[26] Figure 3 in Engle (2010a) was adapted by adding the LC10 and LC50 for *Pseudodiaptomus forbesi* from Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Hypothesis 5. Ambient ammonia levels cause chronic toxicity to copepods.

Current Status: Unknown, Previous Reports Flawed

In August 2011, Teh et al. released a report[27] describing the the results of preliminary chronic tests (30-day full life cycle tests) using *P. forbesi*, conducted during the summer 2010. Even prior to the release of a draft report, the lowest test concentration from his experiments (0.36 mg N/L total ammonia), which the investigators interpreted as a LOEC, was being widely cited as a potential chronic threshold for the Delta, an outcome that was inappropriate for several reasons:

- The test results and report were not peer-reviewed, nor vetted by the US EPA.
- The test result concentration (0.36 mg/L) does not represent an EC20 for the species. EC20s are the thresholds used by the U.S. EPA (2009) for derivation of the chronic ammonia criterion.
- There were irregularities in the test results, for which the investigators offered no explanation. For example, an inverse relationship was observed between toxicity and test pH, which is opposite from the expected responses for organisms included in the U.S. EPA ammonia database. Also, a dose-response was not observed in the chronic test based on the number of nauplii surviving to adulthood.
- The tests were conducted with a novel test organism (a copepod species), for which there are (to this day) no established protocols and no comparable test results from other laboratories.

In late 2011, in providing comments on the proposed NPDES permit for Central Contra Costa Sanitary District (CCCSD), the State Water Contractors and San Luis & Delta Mendota Water Authority (Water Agencies) cited Dr. Teh's research regarding ammonium toxicity of *P. forbesi* as a basis for requiring CCCSD to install new treatment facilities to remove ammonia and nitrate from its effluent.[28] Pacific EcoRisk, a California firm specializing in toxicity testing, prepared a technical review of the August 2011 final report by Teh et al.[29] The review identified the following major issues with the work of Teh and his coauthors:

- Serious flaws in the testing methodology, quality control, data interpretation and reporting.
- Disagreement with the copepod toxicity endpoint derived by Teh et al. (0.36 mg/L), including the inability to reproduce the authors' toxicity endpoints using standard toxicity software and the raw data provided in Teh et al. (2010).
- Erroneous and inaccurate conclusions as stated in the August 2011 final report.

Using the raw data provided by Teh et al. and standard toxicity test software, Pacific EcoRisk calculated that a more appropriate chronic threshold derived from the data set would fall into the range 0.79 (LOEC for juveniles) to as high as 3.23 mg/L TAN (LOEC for adults). These

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[27] Op. Cit.

[28] Comments on Tentative Order No. R2-2011-XXX (NPDES No. CA0037648) for the Central Contra Costa Sanitary District Wastewater Treatment Plant. Oct. 31, 2011.

[29] A Critical Review of "Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of Pseudodiaptomus forbesi to Ammonia/Ammonium – Final Report. Teh et al., August 31, 2011." Prepared by Pacific EcoRisk, Inc., December 26, 2011.

concentrations are higher than ambient ammonia concentrations in the Sacramento River or Suisun Bay. Pacific Ecorisk concludes:

> *"However, the problems associated with Teh et al.'s experimental methodology for Subtasks 3-3 and 3-4-1 and significant questions regarding the analysis of the resulting data do indicate that the quality of the work should preclude the resulting 'critical threshold' data (i.e., NOECs, LOECs, and point estimates [e.g., ECx, LCx, and ICx values]) from being used for regulatory purposes."* (Pacific Ecorisk, p.8)

Hypotheses 6. Ammonium concentrations >4 μM inhibit nitrate uptake

Current Status: Observed in Several Studies Using Short-term Experiments

Experiments conducted by Drs. Richard Dugdale, Alex Parker, and their colleagues at the Romberg Tiburon Center, San Francisco State University, have shown that ammonium concentrations above about 4 μM (0.56 mg N/L) can delay the uptake of nitrate by phytoplankton from the upper SFE.[30],[31],[32],[33] These experiments are conducted by placing water from the SFE in small containers, dosing them with isotope-labeled ammonium and nitrate, incubating them for short periods (hours) at a single light level, and then evaluating the amount of isotope that becomes incorporated into the particulate (and thus presumably phytoplankton) material in the water. This interaction between ammonium and nitrate uptake is referred to as ammonia inhibition, and the frequently observed threshold (~4 μM, equating to ~0.56 mg ammonia-N/L) is now referred to in Delta venues as the "Dugdale' threshold.

Hypotheses 7. Ammonium inhibition significantly affects primary production in the SFE

Current Status: No Consensus, Conflicting Evidence has Been Published

There are significant doubts regarding whether ammonium inhibition of nitrate uptake, as observed in short-term small container experiments, is ecologically significant. In other words, the scientific community is not convinced that ammonium inhibition of nitrate uptake explains patterns in primary production in the SFE. Information which informs this viewpoint falls into the numbered categories below, several of which are elaborated on in subsequent numbered paragraphs. This information includes three academic articles that appeared in the academic literature in 2012 that directly address ammonia inhibition or patterns of primary production using datasets from the Delta (Parker et al. 2012a, Parker et al. 2012b, Kimmerer et al. 2012).

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[30] Dugdale, R; Wilkerson, F; Hogue, V; and Marchi, A. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. Estuarine, Coastal and Shelf Science, 73: 17-29.

[31] Wilkerson, F.P. R.C. Dugdale, V. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. Estuaries and Coasts 29(3):401-416.

[32] Parker, A.E., V. E. Hogue, F.P. Wilkerson, and R.C. Dugdale. 2012a. The effect of inorganic nitrogen speciation on primary production in the San Francisco Estuary. *Estuarine, Coastal and Shelf Science* doi:10.1016/j.ecss.2012.04.001.

[33] Parker, A.E., R.C. Dugdale, and F.P. Wilkerson. 2012b. Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary. *Mar. Pollut. Bull*. 64: 574–586.

1. Algal blooms are not always observed in the SFE when ammonium falls below 4 µM.
2. Although an unusually large algal bloom in Suisun Bay in 2010 has been ascribed by Dugdale and colleagues to lower than usual ammonia concentrations, the bloom was initially dominated by a previously rare benthic diatom (*Entomoneis* sp.),[34] leading Kimmerer et al. (2012) to speculate that the surge in abundance of this taxon might represent another "change of state" in the estuary.
3. Ammonia concentrations above the proposed inhibition threshold of 4 µM were shown in one study shown to stimulate growth of N-limited phytoplankton as they enter the Delta in the Sacramento River.
4. No one has explained frequently observed longitudinal decreases in phytoplankton biomass that can occur in the Sacramento River starting *far above* the SRWTP discharge. Consequently, it is possible that declines in phytoplankton biomass sometimes observed *below* the SRWTP discharge are caused by factor(s) *unrelated to ammonium inputs* that may operate in the Sacramento River starting upstream from the legal Delta and extending downstream toward the confluence zone.
5. Studies in which carbon uptake has been directly measured have not revealed a consistent relationship between carbon uptake (primary production) and ammonia concentrations in the SFE. Some of the longitudinal data from the Sacramento River contradicts hypotheses that ammonia causes a decrease in phytoplankton biomass or primary production rates, or that it changes the cell size or taxonomic composition of phytoplankton.
6. Due to the overwhelming impact of benthic grazing by the invasive clam *Corbula amurensis* on phytoplankton biomass during the summer and fall in Suisun Bay it seems unlikely that there will be a consistent return of historic summer-fall phytoplankton biomass in the brackish Delta as long as the estuary remains colonized by *Corbula*—regardless of other physical or chemical changes that may occur in the estuary.

Algal blooms are not always observed in the SFE when ammonium falls below 4 µM. In the time series of Wilkerson et al. (2006) and Dugdale et al. (2007), algal blooms occurred in Suisun Bay only twice out of five periods when ammonium concentrations fell below 4 µM (Figure 4), and one of the blooms (Spring 2003) failed to yield chlorophyll-a levels above 10 µg/L - a level which is commonly quoted as a threshold for nutritional adequacy for Delta zooplankton. This pattern illustrates that other factors frequently prevent blooms in Suisun Bay even when ammonium concentrations are below the "Dugdale" threshold. Because drawdown of ammonium has been documented by Wilkerson et al. (2006)[35] during the onset of blooms, time series limited to measurements of ammonium and chlorophyll-a at customary intervals (weekly or less frequently) cannot rule out the possibility that low ammonium concentrations *in situ* are the *result* of algal uptake of ammonium during the beginning of a bloom triggered by non-nutrient factors, rather than the *cause* of the bloom.

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[34] Kimmerer W.J., A.E. Parker, U.E. Lidström, and E.J. Carpenter. 2012. Short-Term and Interannual Variability in Primary Production in the Low-Salinity Zone of the San Francisco Estuary. *Estuaries and Coasts* ,35:913–929.

[35] Op. Cit.

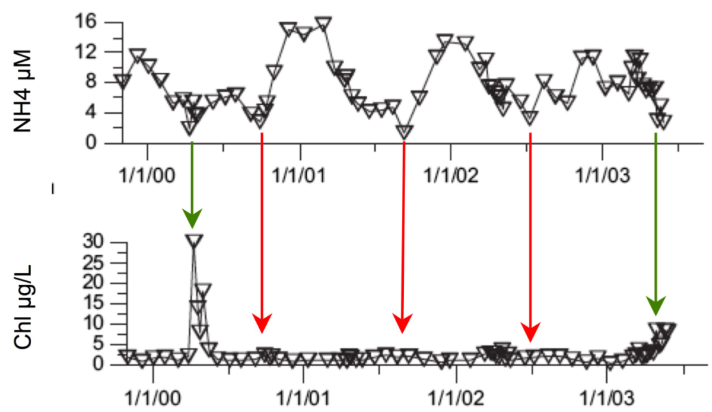

Figure 4. Time series of ammonium and chlorophyll-a from Suisun Bay. Green arrows indicate where ammonium concentrations below a 4 µM threshold were accompanied by increases in chlorophyll-a. Red arrrows show periods when similarly low ammonium concentrations were not accompanied by increases in chlorophyll-a. Panels are from Figure 1 in Dugdale et al. (2007); identical time series presented in Wilkerson et al. (2006). Figure is from SRCSD (2010)[36].

Ammonia concentrations above the proposed inhibition threshold of 4 µM have been shown to stimulate growth of N-limited phytoplankton as they enter the Delta in the Sacramento River. Five-day "grow-out" experiments were conducted by Parker et al. (2010)[37] using water collected above and below the SRWTP discharge in November 2008, and March and May 2009. The grow-out experiments were intended to eliminate light limitation, but by design also eliminate other environmental factors (such as settling, in-situ grazing) that potentially affect riverine phytoplankton biomass in transport through the Delta. During three out of four of the grow-out experiments, phytoplankton grew *better* in water collected at RM-44 below the SRWTP discharge than they did in water collected above the discharge, despite the fact that ammonium concentrations at RM-44 were well above the “Dugdale threshold” of 4 µM[38] (Figure 5).

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[36] Op. Cit.

[37] Parker, A.E., A. M. Marchi, J. Davidson-Drexel, R.C. Dugdale, and F.P. Wilkerson. 2010. Effect of ammonium and wastewater effluent on riverine phytoplankton in the Sacramento River, CA. Final Report. Technical Report for the California State Water Resources Board, May 29, 2010.

[38] Ammonium concentrations in RM-44 water used in the grow-out experiments were: July 2008 - 9.06 µM; November 2008 - 71.87 µM; March 2009 - 12.47 µM; May 2009 - 9.54 µM (Table 19-22 in Parker et al. (2010).

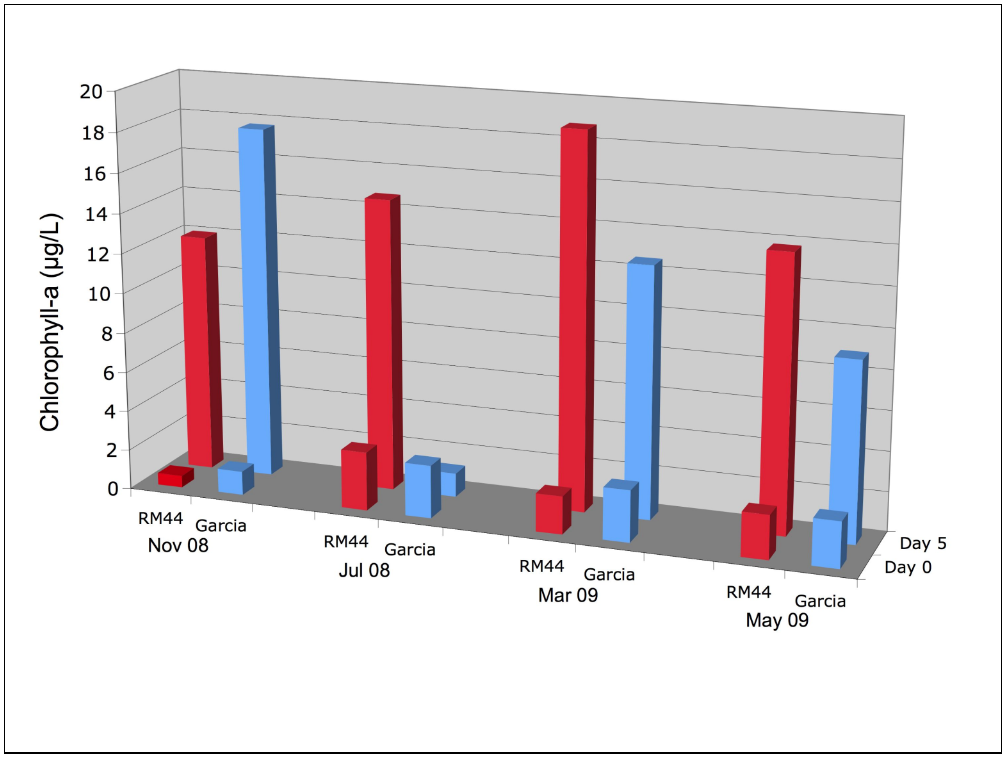

Figure 5. Results of 5-day grow-out experiments using water collected below the SRWTP discharge (RM44, red bars) and above the SRWTP discharge (Garcia Bend, blue bars). In three out of four experiments (July 2008, March 2009, May 2009) phytoplankton biomass (chlorophyll-a) was higher after 5 days in water collected below the SRWTP discharge (RM44) than in water collected above the discharge (Garcia). Initial ammonium concentrations in RM-44 water used in the grow-out experiments were well above the "Dugdale" threshold of 4 μM and were as follows: July 2008 - 9.06 μM; November 2008 - 71.87 μM; March 2009 - 12.47 μM; May 2009 - 9.54 μM. Figure was created using data from Tables 19-21 in Parker et al. (2010).[39]

These results of the grow-out experiments led Parker et al. (2010) to paint a picture of *nitrogen-limited phytoplankton* upstream from the SRWTP, which potentially benefit from the ammonia introduced at the discharge.

> *Results from experimental grow-outs suggest that after removing light limitation phytoplankton bloom magnitude in the Sacramento River at RM-44* [downstream of SRWTP discharge] *and GRC* [upstream of SRWTP discharge] *is likely determined by dissolved inorganic nitrogen (DIN) availability. Grow-out experiments conducted at RM-44 produced more chlorophyll-a than experimental grow-outs conducted at GRC. Phytoplankton appeared to take advantage of additional DIN, whether supplied as NO3 or NH4 in experiments conducted with water from GRC, or in the form of NH4 supplied in the wastewater effluent (at RM-44) to produce greater biomass.* (Parker et al. 2010, p. 26.)

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[39] Op. Cit.

Parker and colleagues did not describe the Sacramento River grow-out experiments in the scientific article they recently published regarding their work in the Sacramento River in 2009 (Parker et al. 2012b).[40] This omission introduces a bias in the newer article in favor of the thesis that ammonia concentrations above 4 µM depress primary production. Another recent publication (using 2005 and 2006 data from the brackish estuary) by Parker et al. (2012a)[41] *did* include time courses of chlorophyll-a concentrations over a 4-day (96-hr) period from other "grow out" experiments, so the omission of the results from analogous Sacramento River grow out experiments in Parker et al. (2012b) is apparently not based on methodological preferences.

Several lines of evidence contradict hypotheses that ammonia decreases phytoplankton biomass or primary production rates, or that it changes the cell size or taxonomic composition of phytoplankton. Multiple longitudinal transects measuring nutrients and algal biomass in the Sacramento River from above Sacramento (I-80 bridge) to Suisun Bay were conducted by Regional Board staff (Foe et al. 2010)[42] and Parker et al. (2009, 2010)[43,44] in 2008-2009. Both studies revealed that although chlorophyll-a often declines in the downstream direction from the I-80 above Sacramento to Rio Vista, no step decline is associated with the SRWTP discharge. For example, in the data shown in Figure 6 more phytoplankton biomass (green line) was lost from river water *above* the SWRTP discharge than below it; and most of the decline in diatoms (blue bars) occurred *upstream* of the SRWTP - a field result which directly contradicts the ammonium-inhibition hypotheses for the freshwater Delta. The Central Valley Regional Water Quality Control Board acknowledged that factors unrelated to the SRWTP discharge are needed to explain declines in chlorophyll-a (and other indices of phytoplankton biomass) which were observed between the Yolo/Sacramento County line and the Rio Vista locale during the 2008-2009 field studies.

> *"The decrease in chlorophyll a appears to commence above the SRWTP. The average annual decline in pigment between Tower Bridge in the City of Sacramento and Isleton is about 60 percent. The cause of the decline is not known, but has been variously attributed to algal settling, toxicity from an unknown chemical in the SRWTP effluent, or from ammonia. The SRWTP discharge cannot be [the] cause of pigment decline upstream of the discharge point, and may not be contributing to the decline downstream of the discharge point."*
> (NDPES Permit for SRWTP, Order No. R5-2010-0114 , p. J-7)

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[40] Parker, A.E., R.C. Dugdale, and F.P. Wilkerson. 2012b. Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary. *Mar. Pollut. Bull*. 64: 574–586.

[41] Parker, A.E., V. E. Hogue, F.P. Wilkerson, and R.C. Dugdale. 2012a. The effect of inorganic nitrogen speciation on primary production in the San Francisco Estuary. *Estuarine, Coastal and Shelf Science* doi:10.1016/j.ecss.2012.04.001.

[42] Foe, C., A. Ballard, and S. Fong. 2010. Nutrient concentrations and biological effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, Final Report, July 2010.

[43] Parker A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J.Davidson-Drexel, J. Fuller, & S. Blaser. 2009. *Transport and Fate of Ammmonium Supply from a Major Urban Wastewater Treatment Facility in the Sacramento River, CA*. 9th Biennial State of the San Francisco Estuary Conference, Oakland, CA, September 29-October 1, 2009.

[44] Op. Cit.

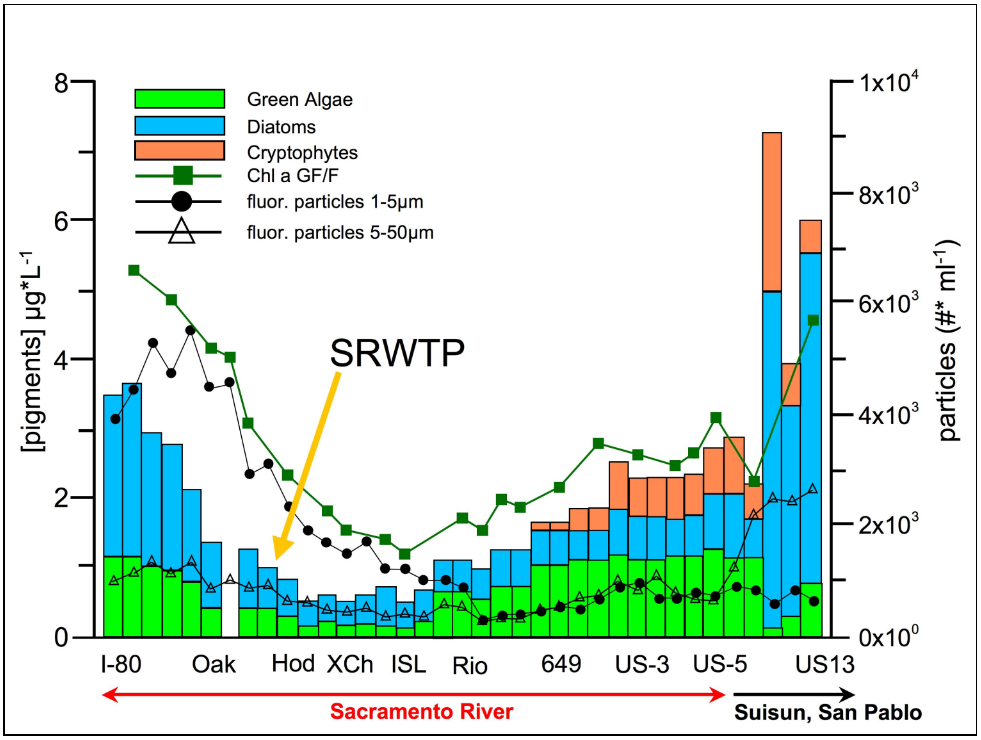

Figure 6. Longitudinal patterns in chlorophyll-a (green squares), biomass of major phytoplankton taxa (colored bars), concentration of small phytoplankton (black circles), and concentration of large phytoplankton (open triangles). Figure is from Parker et al. 2009.[45]

Additional transect data from Parker et al. (2010)[46] (the report to the Regional Board) also contradict elements of the ammonium inhibition hypothesis and indicate that the location of the SRWTP discharge cannot explain patterns in phytoplankton biomass, cell size, or taxonomic composition in the Sacramento River. Figure 7, which is from the report, reveals that a downstream decrease in large phytoplankton (assumed by the investigators to be diatoms) - when it occurs - did not begin (nor did it accelerate) below the SRWTP discharge (the SRWTP discharge is located between stations GRC and R44). Small phytoplankton did not increase in relative abundance below the SRWTP discharge. In other words, ammonium inputs at the SRWTP discharge did not apparently influence the relative abundance of large phytoplankton (green bars in the figure, presumed to be diatoms) and small phytoplankton (red bars in the figure) in the Sacramento River in this study. These field data contradict the hypothesis that ammonia will cause small phytoplankton to out-compete large (diatom) phytoplankton. In the recent scientific article that addresses the same data research project, Parker et al. (2012b) did not present the data from the May 2009 transects that were described in the 2010 report to the Regional Board and which appear in Figure 7 below.

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[45] Op. Cit.

[46] Op. Cit.

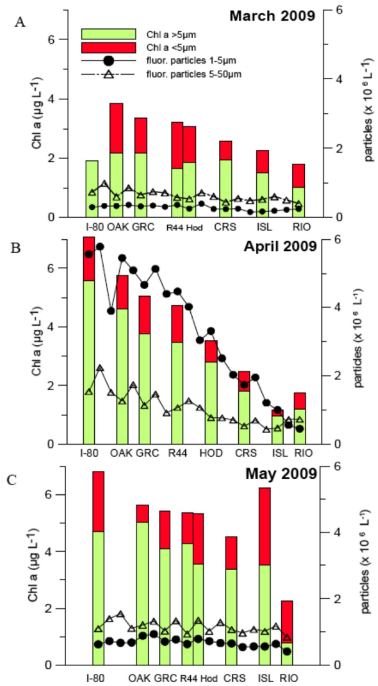

Figure 7. Longitudinal patterns in biomass of large phytoplankton (green bars and open triangles) and small phytoplankton (red bars and closed circles) in the Sacramento River between the I-80 bridge and Rio Vista during Spring 2009; large phytoplankton are presumed by the investigators to include most of the diatoms. Data show that the location of the SRWTP discharge (located between station GRC and R44) does not explain the overall patterns in algal biomass or cell size in the river. Figure is from Parker et al. (2010).[47]

Short-term rate measurements reported from the same study (Parker et al. 2010) also contradict elements of the ammonium inhibition hypothesis. *Rate measurements in* Figure 8 *show that primary production rates (black triangles) do not consistently decline in the Sacramento River, and when they do, the decline is not initiated when water flows past the SRWTP discharge*. The field data clearly show that ammonium uptake rates (orange diamonds) are *not* inversely related to primary production rates (brown triangles). *These field data directly contradict the hypothesis that ammonium uptake causes a decrease in primary production in the river*. These field data demonstrate that predictions about phytoplankton growth responses and ammonium uptake based on multiple-day, small container experiments in Wilkerson et al. (2006) and Dugdale et al. (2007) should not be presumed valid outside the laboratory.

[47] Op. Cit.

The recent article Parker et al. (2012b)[48] (addressing the Sacramento River research discussed above) omits some available data that contradicts the ammonium inhibition theory. In Parker et al. (2012b), carbon and nitrogen uptake rate measurements are tabulated that presumably include the results in Figure 8 for March and April 2009, and extend the longitudinal data set downstream from Rio Vista (RIO in the figure) out into Suisun Bay. However, data from May 2009 were omitted from the 2012 article. This is potentially a source of bias in the 2012 article because longitudinal patterns in May 2009 were inconsistent with the conclusions of the 2012 article. For example, carbon and NH4 uptake rates were ***not*** lower in May below the SRWTP compared to upstream stations, and patterns in carbon and NH4 uptake rates going from upstream to downstream mirror each other (the ammonium inhibition hypothesis predicts that carbon and NH4 uptake rates will be inversely related).

In addition, the results for stations upstream from the SRWTP discharge (and for other reaches) were averaged together in the 2012 article. This obscured the fact that there were declines in N and C uptake in April 2009 *within the reach of the river above the discharge* which cannot be ascribed to ammonia inhibition of N or C uptake. By focusing on subreach *averages* in the 2012 article, potentially important trends that occur in the river upstream from the SRWTP (the "Upper River" in the article) are not addressed – and questions regarding non-ammonium (and perhaps non-light-related) influences on phytoplankton that may operate above *and* below the SRWTP are not raised.

Finally, there are some puzzling discrepancies between some of the data in the 2012 article and the previous 2010 report which potentially bias the 2012 article.[49] These are important discrepancies because differences in several parameters between the "Upper River" and the "SRWTP" subreach are vital support for the authors' conclusions about the effects of SRWTP effluent on primary production, and the values in the previous 2010 Report indicate smaller differences between the subreaches and were therefore less supportive of the authors' hypotheses.

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[48] Parker, A.E., R.C. Dugdale, and F.P. Wilkerson. 2012b. Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary. *Mar. Pollut. Bull*. 64: 574–586.

[49] Example data discrepancies which lead to larger differences between the "Upper River" and the "SRWTP" subreach in the later publication: The mean carbon uptake rate for the "Upper River" for March 2009 in the 2012 article is much higher (14.13 µmol/L/d, see Table 2) than the values for stations in the same subreach from the 2010 Report would suggest (8.8, 9.6. 10.08 µmol/L/d for I-80, OAK, GRC, in Table 12). Along the same lines, the mean NO3 uptake rate for March for the "Upper River" in the 2012 article (1.57 µmol/L/d, see Table 3) is not consistent with the March values for the corresponding stations in the 2010 Report (0.34, 0.41, 0.38 µmol/L/d, see Table 12). The specific uptake rate for NH4 in the 2012 article for the "SRWTP reach" in April (0.25/d) is much lower than the corresponding values in the 2010 Report would suggest (1.19, 0.60/d for RM-44 and HOD, see Table 15).

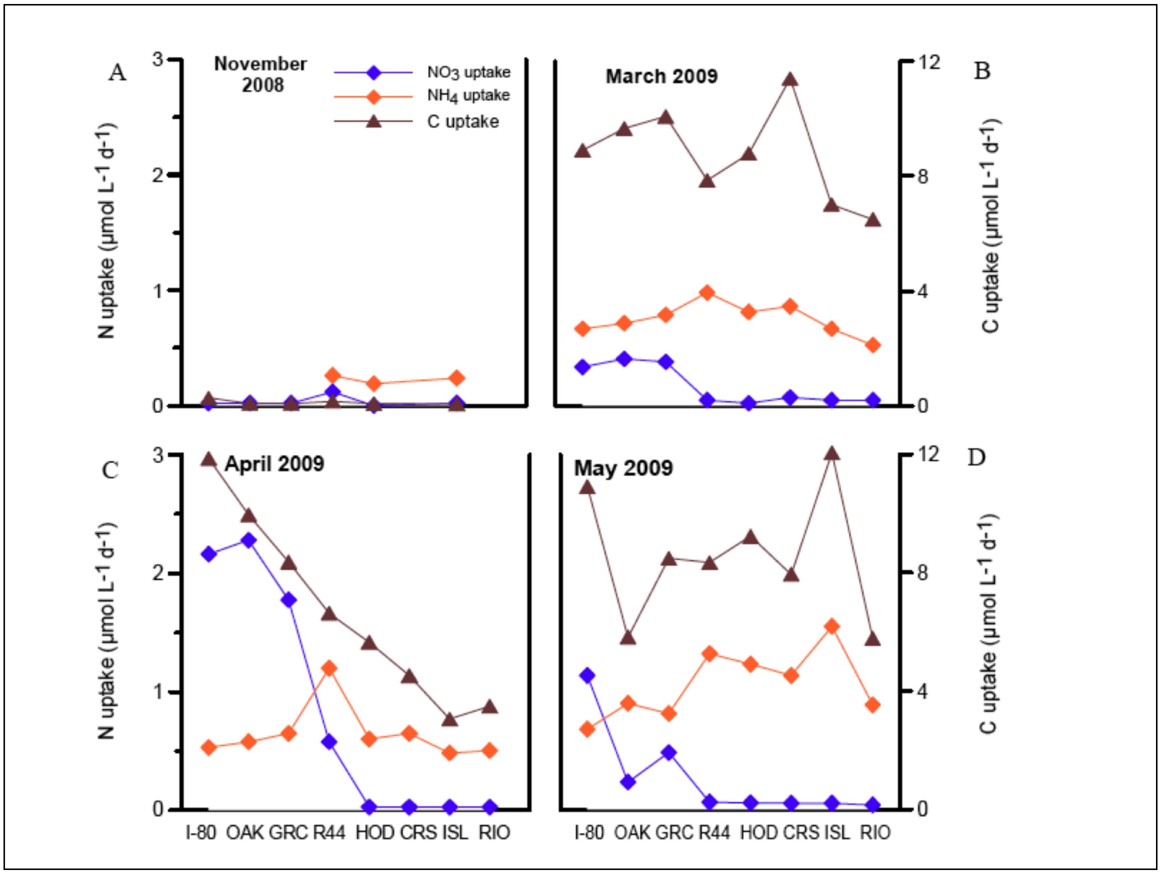

Figure 8. Primary production (C uptake; triangles) and phytoplankton uptake rates of ammonium (orange symbols) and nitrate (blue symbols) made during 24-hr incubations of Sacramento River water collected during four transects between I-80 bridge and Rio Vista. Data do not reveal an inverse relationship between primary production and ammonium uptake. Data further show that longitudinal patterns in primary production are not explained by the SRWTP discharge (located between GRC and R44). Figure is from Parker et al. (2010).[50]

A new article presenting direct measurements of carbon uptake taken at multiple depths at stations in the low salinity zone in 2006 and 2007 (Kimmerer et al. 2012)[51] reports that light availability explained the majority of the variation in primary productivity in the LSZ, and that "ammonium contributed nothing to the relationships" (see Table 3 in the article). However, the authors also report that ammonium concentrations were above 4 µM during their investigation, which may have precluded the ability to detect a relationship between ammonium and primary production that might occur at lower concentrations.

[50] Op. Cit.

[51] Op. Cit.

It appears unlikely that historic summer-fall phytoplankton biomass will consistently reoccur in the brackish Delta as long as the estuary remains colonized by *Corbula*—regardless of other physical or chemical changes that may occur in the estuary. Due to the overwhelming impact of benthic grazing by the invasive clam *Corbula amurensis* on phytoplankton biomass during the summer and fall in Suisun Bay (Alpine & Cloern 1992, Jassby et al. 2002, Kimmerer 2005, Thompson 2000)[52], it appears unlikely that there will be a consistent return of historic summer-fall phytoplankton biomass in the brackish Delta as long as the estuary remains colonized by *Corbula*—regardless of other physical or chemical changes that may occur in the estuary. Postulated dividends of increased diatom biomass related to ammonia reduction may be currently constrained primarily to the April-May window, when lower benthic grazing rates, increased water temperature, increased thermal stratification, and other factors occasionally provide windows for bloom development. However, what seems lost from many discussions about Suisun Bay is that—historically—*the spring period (Apr-May) was not when the bulk of annual phytoplankton biomass occurred in Suisun Bay*. Instead, prior to the arrival of the clam in 1987, June-September were the months of highest mean phytoplankton biomass in Suisun Bay and the confluence zone (**Figure 9**). Consequently—even if ammonium reductions led to more frequent spring blooms in Suisun Bay—it seems reasonable to conclude that grazing by *Corbula* during summer and fall months will still prevent a consistent recovery of annual algal biomass to levels that occurred in Suisun Bay in the 1970s and early 1980s.

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[52] Alpine, A. E., and J. E. Cloern. 1992. Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol. Oceanogr. 37:946-955.

Jassby A.D., Cloern J.E., Cole B.E. 2002. Annual primary production: patterns and mechanisms of change in a nutrientrich tidal estuary. Limnol Oceanogr 47:698–712

Kimmerer W.J. 2005. Long-term changes in apparent uptake of silica in the San Francisco estuary. Limnol Oceanogr 50:793–798.

Thompson J.K. 2000. Two stories of phytoplankton control by bivalves in San Francisco Bay: the importance of spatial and temporal distribution of bivalves. J Shellfish Res 19:612.

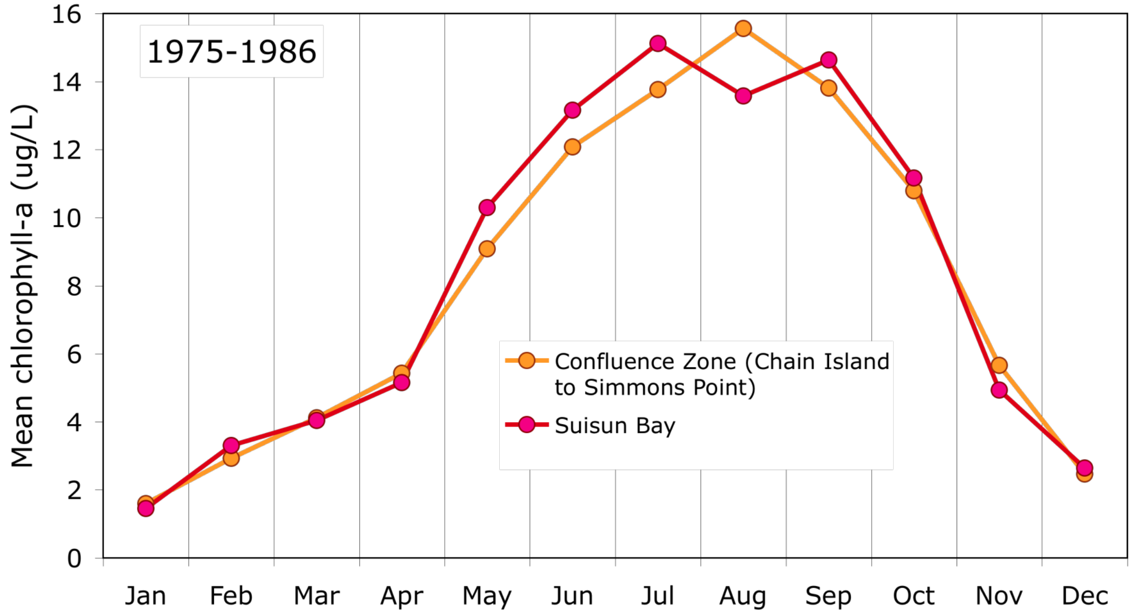

Figure 9. Mean monthly chlorophyll-a concentrations from surface (0-2 m) water samples collected between 1975-1986 at stations used by the IEP, DWR-MWQI, and the USGS. The bulk of annual phytoplankton biomass historically occurred during the same months (June-October) during which *Corbula amurensis* currently controls phytoplankton biomass in the brackish estuary. Figure is from SRCSD (2010)[53].

A lack of consensus regarding the ecological significance of ammonium inhibition is consistent with conclusions reached in a recent report produced through the San Francisco Numeric Nutrient Endpoint (NNE) project underway. The report, prepared in June 2011 for the San Francisco Regional Water Board by the San Francisco Estuary Institute (SFEI) and the Southern California Coastal Water Research Project (SCCWRP), states that the impacts of ammonium on diatom blooms is not well understood and that additional investigation and data synthesis are required to better understand the role of ammonium in the Bay ecosystem. The report, *Nutrient Numeric Endpoint Development for the San Francisco Bay Estuary: Literature Review and Data Gaps Analysis*[54], includes the following statements regarding the current lack of understanding of the role of ammonium inhibition on phytoplankton primary productivity:

> "*…the ecological importance of ammonium inhibition of spring diatom blooms is not well understood relative to factors known to control primary productivity…*"[55]
>
> "*In SF Bay, the biomass associated with phytoplankton, measured as surface water chlorophyll a concentration, varies in space and time in response to nutrient availability*

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[53] Op. Cit.

[54] McKee, L., M. Sutula, A. Gilbreath, J. Beagle, D. Gluchowski, and J. Hunt. 2011. Nutrient Numeric Endpoint Development for the San Francisco Bay Estuary; Literature Review and Data Gaps (June 2011).

[55] Ibid at 147.

from external loads and internal regeneration, grazing, stratification, water temperature, tidal energy, transparency, wind/wave energy, the availability of seed cysts, UV radiation effects on nitrate versus ammonium assimilation perhaps due to disruptions of enzyme pathways, differential uptake of nitrate and ammonium by larger versus smaller cells, inhibition of nitrate uptake by ammonium, predation by benthic invertebrates, and variations in the phase of the Pacific Decadal Oscillation and related changes to top down predation of benthic invertebrates."[56]

"*…the effect of ammonium inhibition on phytoplankton productivity throughout the Bay has not been modeled vis-à-vis other contributing factors…the next logical step is to develop models that synthesize understanding of the relative importance of ammonium and urea versus other factors controlling phytoplankton assemblages*."[57]

"*Elevated ammonium concentrations have been suggested as a major mechanism by which spring diatom blooms appear to be suppressed in the North Bay and Lower Sacramento River…Despite this evidence, the ecological importance of ammonium inhibition of spring diatom blooms is not well understood relative to factors known to control primary productivity, particularly in other regions of the Bay where water column chlorophyll a appears to be increasing. Thus, the linkage between ammonium concentrations and Bay beneficial uses is not at this time universally accepted. San Francisco Bay Technical Advisory Team (TAT) members agree that additional data synthesis is required to better understand the role of ammonium in SF Bay*."[58]

Members of the Technical Advisory Team (TAT) referred to in the excerpt above (which were responsible for scientific review of and input on the NNE report) included Dr. James Cloern, a highly recognized expert in San Francisco Bay ecology, and Dr. Richard Dugdale of the Romberg Tiburon Center, a chief proponent of ammonium inhibition theories for the SFE. The statements from the NNE report cited above are reasonably considered the consensus view regarding ammonium inhibition in the SFE.

Scientific uncertainty regarding the ecological significance of ammonium inhibition is reflected by recent actions by the San Francisco Regional Water Board. In February 2012, the San Francisco Regional Water Board adopted an NPDES permit for Central Contra Costa Sanitary District (CCCSD) without requiring either nitrification or denitrification.[59] Instead, it required CCCSD to participate in several technical studies over the course of three years, including an evaluation of the toxicity of ammonium to copepods, using a methodology acceptable to the Executive Officer of the San Francisco Regional Water Board.

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[56] Ibid at 46 (internal citations omitted).

[57] Ibid at 154.

[58] Ibid at 155.

[59] Order No. R2-2012-0016, NPDES No. CA 0037648, Central Contra Costa Sanitary District Wastewater Treatment Plant

Hypothesis 8. Ammonia has contributed to a deleterious change in phytoplankton composition in the SFE.

Current Status: Unknown; A Previous Report used Flawed Statistics

It is currently unknown whether ammonia has contributed to a changes in phytoplankton composition in the SFE. Postulated effects of ammonium on phytoplankton species composition in the SFE can be placed in two categories:

1. Concerns that ammonia promotes blooms of toxin-producing phytoplankton (principally *Microcystis*).
2. Concerns that ammonia promotes a shift from large diatoms to relatively smaller types of phytoplankton that may be less valuable as food for zooplankton.

The available information from the SFE has not confirmed a relationship between ammonium concentrations and (1) the occurrence of toxin-producing phytoplankton, or (2) the occurrence of algal toxins in the water column. In addition, currently available research from the Delta argues against a simplistic association between *Microcystis* and nutrient form or concentration. Studies conducted by Lehman et al. (2008, 2010)[60] and Mioni (2010)[61] in the Delta found no apparent association between ammonium concentrations or NH_4^+:P ratios and either *Microcystis* abundance or toxicity. Instead, it appeared from these studies that water temperature is strongly positively correlated with *Microcystis* abundance and toxicity and that water transparency, flows, and specific conductivity are also potential drivers of *Microcystis* blooms in the Delta. An association between water temperature and *Microcystis* blooms in the Delta is supported by the upward trend in spring-summer mean water temperature in the freshwater Delta between 1996-2005 (Jassby 2008)[62] and would be consistent with observations from other estuaries, where increased residence time (e.g., during drought) and warmer temperatures are acknowledged as factors stimulating cyanobacterial blooms (Pearl et al. 2009; Pearl & Huisman 2008; Fernald et al. 2007).[63]

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[60] Lehman, P.W., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of Microcystis cell density and microcystins concentration in the San Francisco Estuary. Hydrobiologia 600:187-204.

Lehman, P.W., S.J. Teh, G.L. Boyer, M.L. Nobriga, E. Bass, and C. Hogle. 2010. Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia 637:229-248.

[61] Mioni, C.E., and A. Paytan. 2010. *What controls Microcystis bloom & toxicity in the San Francisco Estuary? (Summer/Fall 2008 & 2009)*. Delta Science Program Brownbag Series, Sacramento, CA. May 12, 2010.

[62] Jassby, A. 2008. Phytoplankton in the Upper San Francisco Estuary: recent biomass trends, their causes and their trophic significance. San Francisco Estuary & Watershed Science, Feb. 2008.

[63] Pearl, H.W., K.L. Rossignol, S. Nathan Hall, B.L. Peierls, and M.S. Wetz. 2009. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuaries and Coasts. DOI 10.1007/s12237-009-9137-0.

Paerl, H.W., and J. Huisman. 2008. Blooms like it hot. Science 320:57–58. doi:10.1126/science.1155398.

Fernald, S.H., N.F. Caraco, and J. J. Cole. 2007. Changes in cyanobacterial dominance following the invasion of the zebra mussel *Dreissena polymorpha*: long-term results from the Hudson River Estuary. Estuaries and Coasts 30:163-170.

Cecile Mioni and colleagues recently issued a report to the Regional Board describing recent research in the Delta and Clear Lake (Mioni et. al. 2012).[64] Mioni et al. 2012 used two different types of statistics to evaluate linkages between environmental parameters in the Delta (such as temperature, electric conductivity (EC), nutrients). The authors reported that none of the environmental variables measured in their study (which included ammonium) were significantly correlated with *Microcystis* abundance but suggest surface water temperature was key environmental drivers of algal abundance (in terms of chlorphyll-a). Ammonia and EC were the best predicting environmental variables explaining *A. flos-aquae* abundance and distribution (*A. flos-aqua*e is another species of phytoplankton that can produce toxins). However, the authors state that (1) variables not considered in this study (e.g. DON, stratification, residence time, grazing) may also control cyanobacterial dominance and toxicity and should be investigated, and (2) further work is needed toinvestigate the role of nitrogen sources on cyanobacterial success in the Sacramento-San JoaquinDelta (p. 31).

Two publications by Glibert and colleagues (Glibert 2010, Glibert et al. 2011)[65] employ correlation analysis to allege that temporal patterns in ammonium (and nutrient ratios) in the SFE explain a wide variety of temporal patterns for a wide variety of aquatic biota. The authors' conclusions in both publications are not based on direct experimental evidence of differential phytoplankton growth responses to nutrient ratios in the San Francisco Estuary (SFE). In the first article, Glibert (2010) used an improperly applied statistical transformation (cumulative sums of variability, or CUSUM) to produce artificial and highly misleading correlations between nutrient parameters and biological parameters (phytoplankton, zooplankton, fish abundance). As described in Engle & Suverkropp (2010)[66] Glibert's approach was analytically and conceptually flawed, in the following respects:

> Inadequate Geographic Coverage. Sweeping generalizations are made in Glibert's paper regarding the estuarine food web and the Pelagic Organism Decline (POD) using data from only 1 station in the Freshwater Delta (Hood, IEP station C3) and 2 stations in Suisun Bay (IEP stations D8 and D7).
>
> Violation of Statistical Assumptions. Glibert used a calculation termed *CUSUM* to transform long-term datasets for nutrient concentrations and abundances of selected aquatic organisms, and then performed linear regression using the unordered transformed data for selected pairs of variables. Time series of CUSUM values exhibit features and patterns which diverge in several important ways from those of the underlying measured data and make them

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[64] Mioni, C, R. Kudela, and D. Baxa. 2012. Harmful cyanobacteria blooms and their toxins in Clear Lake and the Sacramento-San Joaquin Delta (California), report prepared for the Central Valley Regional Water Quality Control Board, 110 pp.

[65] Gilbert, Patricia; Long-Term Changes in Nutrient Loading and Stoichiometry and Their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, California; Reviews in Fisheries Science, Vol. 18, Issue 2 (August 2010).

Glibert, P; Fullerton, D; Burkholder, J; Cornwell, J; and Kana, T. 2011. Ecological stoichiometry, biogeochemical cycling, invasive species, and aquatic food webs: San Francisco Estuary and comparative systems. Reviews in Fisheries Science, 19(4): 358-417.

[66] Engle, D. and C. Suverkropp. 2010. Memorandum: Comments for Consideration by the State Water Resources Control Board Regarding the Scientific Article *Long-term Changes in Nutrient Loading and Stoichiometry and their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, California* by Patricia Glibert. 17 pp. July 29, 2010.

inappropriate for standard linear regression. CUSUM series mute seasonal or other short-term variation in a time series (which is meaningful for short-lived organisms like phytoplankton and zooplankton), but exaggerate shifts that occur on long time scales (such as decades). In the statistical literature, CUSUM is primarily used to create charts (or ordered values) for single variables that allow the user to detect change points or determine whether deviations from control points are random or signal a trend. However, the characteristics of CUSUM that lend it to change-point analysis and quality control make it completely inappropriate to perform standard linear regression using paired CUSUM values removed from their respective temporal sequences.

The simple CUSUM correlations that represent the basis for Glibert's conclusions violate virtually every assumption of a standard correlation analysis. CUSUM series are inherently serially correlated, heteroscedastic and non-normally distributed, and the residuals of CUSUM correlations are non-independent (see Engle & Suverkropp 2010 for more detail). In addition to issues surrounding Glibert's use of CUSUM values for correlation analysis, not all of the datasets used by Glibert are even appropriate for customary uses of CUSUM. Autoregressive time series such as flow data are not appropriate for CUSUM change-point analysis. CUSUM change point analysis also assumes that underlying data are homoscedastic and often assumes that data are normally distributed. Glibert did not test raw data for autocorrelation, normality or equal variance prior to the CUSUM transformation. Another requirement of CUSUM analysis is that time series being compared must start and stop at the same point in time. Glibert's correlations were apparently performed by pairing CUSUM series for which underlying data spanned different ranges of years.

<u>Artificial Relationships and Inflated R^2 Values</u>. The CUSUM transformation results in a very limited range of serially correlated data structures, which (if linear regression is performed for pairs of CUSUM series) leads to "correlations" with impressively inflated R^2 values that are largely artificial and can't be interpreted in the same way as standard parametric correlation or regression analysis. Equally important, statistically significant relationships that *are* present in underlying data can be disguised when CUSUM time series are compared instead of real world measurements.

<u>Biased selection of variables</u>. Several obvious pairings of environmental variables were omitted from Glibert's portfolio of CUSUM correlations, including those that were needed for her to claim that nutrient ratios and phytoplankton taxa were statistically related. For example, CUSUM regressions between nutrient *ratios* (TN:TP, NO_3:NH_4, or DIN:DIP) and phytoplankton indices (chl.a or abundances of individual taxonomic groups) were omitted from her analysis. Also, CUSUM trends in nutrient ratios were not directly compared to those for copepod abundance. NO_3:NH_4 trends were not compared to *any* of the biological trends (phytoplankton, copepods, clams, or fish); they were only compared to trends in Delta outflow. As a consequence, even if one were to accept Glibert's CUSUM correlation approach, her publication still does not provide evidence that nutrient ratios and phytoplankton composition are statistically related.

Many well-known alternative hypotheses for the observed changes in plankton composition and fish abundance in the SFE (and in estuaries, generally) - which would have been testable using the CUSUM methodology - were omitted from Glibert's analysis and from discussion in her article. Owing to the peculiarity of the CUSUM transformation, it is likely that a wide variety of non-nutrient environmental factors (essentially any factors which have trended over time in the SFE in concert with changes in fish abundance such as clam abundance, turbidity, or water exports) could be shown as highly correlated with pelagic fish abundance using CUSUM correlations. As an example Figure 10 shows that when subjected to the same analysis used in Glibert's paper, annual water exports perform as well as ammonia concentrations in explaining trends in the summertime abundance of Delta smelt. Although Glibert's CUSUM correlations between fish abundance and ammonia were convenient for focusing attention on ammonia (as opposed to other potential drivers of the food web or the POD), they ultimately signified little with respect to the relative importance of multiple environmental factors which have changed over recent decades in the San Francisco Estuary.

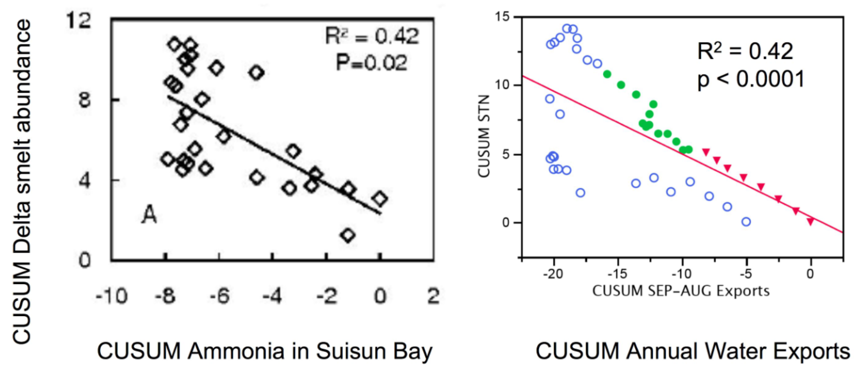

Figure 10. Comparison of correlations using CUSUM ammonia (Suisun Bay) or CUSUM annual Delta water exports (SWP, CVP and Contra Costa Canal combined) as the independent variables (x-axis) and CUSUM values for the Delta smelt Summer Townet Index as the dependent variable (y-axis). Correlation using ammonia is from Glibert (2010) and used data for 1975-2005. Correlation using annual water exports is from Engle & Suverkropp (2010); color coding for subsets of the CUSUM series is as follows: open blue circles for pre-Corbula years (1956-1986), solid green circles for post-*Corbula* years 1987-1999, red triangles for POD years 2000-2007. Details regarding underlying analyses are in Engle & Suverkropp (2010). The correlation coefficient (R^2 value) is the same for both regressions (0.42); both regression lines are significant.

Dr. James Cloern and several other respected Delta scientists published a critique[67] of Glibert (2010) which agreed with several statistical issues identified by Engle & Suverkropp (2010) and included the following observations:

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[67] Cloern, J.E.; A.D. Jassby; J. Carstensen; W.A. Bennett; W. Kimmerer; R. Mac Nally; D.H. Schoellhamer; and M. Winder. 2012. Perils of correlating CUSUM-transformed variables to infer ecological relationships (Breton et al., 2006, Glibert 2010). Limnology and Oceanography 57(2): 665-668

"Glibert (2010) concluded that recent large population declines of diatoms, copepods, and several species of fish were responses to a single factor – increased ammonium inputs from a municipal wastewater treatment plant."

"Glibert's study…contradicts the overwhelming weight of evidence that population collapses of native fish…and their supporting food webs in the San Francisco Estuary are responses to multiple stressors including landscape change, water diversions, introduction of exotic species and changing turbidity."

"…CUSUM transformation, as used by…Glibert (2010), violate the assumptions underlying regression techniques."

"...the p-values for correlations of CUSUM-transformed variables reported by Breton et al. (2006) and Glibert (2010) are incorrect."

"We showed that two CUSUM-transformed variables often have an apparent statistically significant correlation even if none exists between the original untransformed series. Moreover, even if a statistically significant relationship could be established between CUSUM-transformed variables, there is no proven basis for inferring relationships between the original variables. Given these difficulties, we wonder what purpose is served by CUSUM transformation for exploring relationships between two variables. As a real example, Glibert (2010) inferred a strong negative association between delta smelt abundance and wastewater ammonium from regression of CUSUM-transformed time series. However, the Pearson correlation ($r = -0.096$) between the time series (Fig. 1) is not significant, even under the naive IID assumptions ($p = 0.68$)."

Recently a "rejoinder" to the Cloern et al. paper has become available (Lancelot et al. 2011[68]), which is co-authored by Dr. Glibert. Importantly, in the response, Glibert and her coauthors agree with Cloern et al. 2012 that the correlation coefficients in the 2010 paper should be disregarded, which means that the correlations between ammonia and other parameters reported in the article are not statistically significant.

In a more recent article, Glibert et al. (2011)[69], the authors avoided the use of the CUSUM-transformed data and utilized more conventional correlation analysis to support conclusions regarding linkages between ammonia, nutrient ratios, and a variety of SFE biota. In my professional opinion, statistical inference obtained by running dozens of correlation analyses with monitoring data does not demonstrate that the proposed underlying ecological mechanism is operating in the estuary – especially when the independent variable (ammonia) can covary with other factors in the estuary which are not included in the regression model or independently examined. However, some of the ecological hypotheses described in Glibert et al 2011, which involve interactions between nutrient ratios in the water column and the elemental stoichiometry

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[68] Lancelot, C; Grosjean, P; Rousseau, V; Breton, E; and Glibert, P. 2012. Rejoinder to "Perils of correlating CUSUM-transformed variables to infer ecological relationships (Breton et al. 2006, Glibert 2010). Limnology and Oceanography 57(2): 669-670.

[69] Glibert, P; Fullerton, D; Burkholder, J; Cornwell, J; and Kana, T. 2011. Ecological stoichiometry, biogeochemical cycling, invasive species, and aquatic food webs: San Francisco Estuary and comparative systems. Reviews in Fisheries Science, 19(4): 358-417.

of organisms in the food web, could be directly experimentally tested using large mesocosms (not small cubitainers) in which organisms at multiple levels in the food web (hopefully also including benthic organisms which affect nutrient cycling and standing stocks) are held for multiple generations at environmentally relevant densities in chemostat-like circulating systems allowing for trophic interactions such as grazing and predation by secondary and tertiary consumers, and internal nutrient recycling. Until research results such as these are available to review, it will remain unknown whether the stoichiometry-based hypotheses for food-web shifts in the SFE are scientifically defensible. This opinion seems at least superficially consistent with the authors' closing recommendation in Glibert et al. (2011), that "while compelling, the ecological stoichiometric model raises many questions that need further analysis in the San Francisco Estuary…" and "…regulation of the food web by nutrient controls is directly testable, and there is much that needs to be explored to test these relationships directly."

Hypothesis 9. The copepods eaten by delta smelt, and other pelagic fish, are reliant on diatoms.

Status: False

An overly simplistic food-web-related paradigm "diatoms beget copepods beget pelagic fish" has been used to justify much of the attention regarding ammonia and the SFE food web. At least six lines of evidence challenge this simplistic paradigm:

1. Published experiments from the Delta show that key Delta copepods - including the ones that Delta smelt eat - actually prefer *non*-diatom types of phytoplankton and that much of the time they don't consume phytoplankton at all (instead consuming small heterotrophic organisms in the water column)[70]. These feeding experiments indicate that the principal calanoid copepods in the estuary (*Acartia* spp., *E. affinis*, *P. forbesi*) prefer motile prey over non-motile prey, and prefer heterotrophic protistan prey (e.g., ciliates, heterotrophic dinoflagellates) over phytoplankton (Bollens & Penry 2003; Bouley & Kimmmer 2006; Gifford et al. 2007)[71]. Diatoms are not motile (they lack flagella or other means of locomotion). In summary, Delta copepods do not rely on diatoms—or even on phytoplankton—as a direct food source, and frequently discriminate against phytoplankton altogether (even during diatom blooms) depending on season and location in the estuary. Some of the types of phytoplankton preferred by the copepods (e.g., flagellates) are now *more abundant* in the estuary than in previous decades. Greene et al. (2011)[72] published results of feeding experiments that indicated filtration by *Corbula amurensis* can remove 50-

[70] Heterotrophic organisms obtain energy by consuming pre-existing organic matter, as opposed to synthesizing organic matter through photosynthesis.

[71] Bollens, Gretchn C. Rollwagen, Penry, Deborah L. 2003. Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA).

Bouley, P. & Kimmerer, W. J. 2006. Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. *Marine Ecology-Progress Series,* **324,** 219-228.

Gifford, S. M., Rollwagen-Bollens, G. & Bollens, S. M. 2007. Mesozooplankton omnivory in the upper San Francisco estuary. *Marine Ecology-Progress Series,* **348,** 33-46.

[72] Greene, V.E., L. J. Sullivan, J.K. Thompson, and W.J. Kimmerer. 2011. Grazing impact of the invasive clam Corbula amurensis on the microplankton assemblage of the northern San Francisco Estuary. Mar. Ecol. Prog. Ser. 431: 183-193.

90% per day of the population growth capacity of protistan microzooplankton (such as the ciliates and dinoflagellates preferred by calanoid copepods). This study reveals that *Corbula amurensis* must be considered a direct competitor with calanoid copepods for a common food supply, and an important factor regulating calanoid copepod abundance where they co-occur.

2. A large body of literature indicates that direct feeding on diatoms can cause reproductive failure in copepods (see Ianora & Miralto (2010), and references therein)[73]. This potential harmful effect of diatoms on copepods, first described in the early 1990s, has been the subject of considerable research and special workshops and symposia. The harmful effect is caused by organic compounds (oxylipins) that are released from diatom cells when they are broken during feeding - compounds which then induce genetic defects in copepod eggs. The genetic defects are manifested by failure of eggs to hatch or failure of hatched offspring to develop normally. The effect is unrecognized in lab or field studies that rely on egg counts to determine the nutritional status of copepods because the harmful compounds involved do not necessarily effect the numbers of eggs produced, but the viability of the eggs that are produced. There are at least 24 recent experiments indicating harmful effects of diatom grazing for copepod species pertinent to the SFE (i.e., SFE species and their cofamilials; Figure 11).

3. The reproductive implications of food *choices* is virtually unstudied for the copepods of the SFE. For example, a recent review of almost 400 research articles revealed that only three published studies measured egg production or hatching success for SFE-pertinent copepod species fed mixtures of diatoms and non-diatoms (Engle 2011)[74]. In other words, there is essentially no direct evidence that observed changes in phytoplankton composition in the estuary would have had population-level consequences for copepods.

4. Non-diatom classes of phytoplankton (including some groups which are now more abundant in the estuary) include species which are considered highly nutritious for zooplankton. Examples are the cryptophytes (which include *Cryptomonas* and *Rhodomonas* spp.) and *Scenedesmus* spp. (a green alage), which are both used to rear zooplankton in laboratories.

5. Chlorophyll-a levels below 10 µg/L are frequently cited as evidence that zooplankton in the Delta are food limited (Muller-Solger et al. 2002)[75]. However, this threshold is based on growth experiments conducted with a single cladoceran zooplankton species (*Daphnia magna*) and it is unclear whether this threshold is appropriately applied to any of the copepods in this system.

6. The heavy reliance of SFE copepods on non-algal foods indicates that detritus-based pathways for energy transfer may contribute more to the pelagic food web in the Delta than

[73] Ianora, A. & Miralto (2010) A. Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology,* **19,** 493-511.

[74] Engle, D. (2011) How well do we understand the reproductive consequences of copepod diet in the San Francisco Estuary? A survey of the direct evidence. 10th Biennial State of the San Francisco Estuary Conference, Sept. 2011.

[75] Müller-Solger, A.B., A.D. Jassby, and D. C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47:1468-1476.

has been acknowledged. Such information led the IEP to make the following acknowledgement in its 2007 Synthesis of Results:

> *". . . it is possible that the hypothesis that the San Francisco Estuary is driven by phytoplankton production rather than through detrital pathways may have been accepted too strictly."* (Baxter et al. 2008)[76]

This viewpoint appears supported by recent experiments by Rollwagen Bollens et al. (2011)[77] in the brackish estuary (Suisun and Grizzly Bays). Their experiments indicated that heterotrophic microzooplankton (ciliates and flagellates such as are consumed by key SFE calanoid copepods) consumed a substantial amount of both phytoplankton (up to 73% of standing stock) and suspended bacteria. The authors conclude:

> *"However, experimental and field evidence demonstrates that many heterotrophic planktonic protists, particularly small oligotrich ciliates such as we found in Suisun Bay (e.g. Strombidium, Strombilidium), effectively and consistently consume bacterioplankton (Artolozaga et al. 2002; Sherr et al. 1989). Moreover, in the northern SFE, organic carbon available for bacterial growth is primarily of allocthonous origin (Jassby et al. 1993; Murrell and Hollibaugh 2000). Therefore, microzooplankton could be re-packaging and contributing carbon otherwise unavailable to the classic metazoan food web, which could counterbalance losses from reduced trophic efficiency. In conclusion, our results, in combination with those of Gifford et al. (2007), show that microzooplankton may consume a substantial amount of phytoplankton production and are an important prey resource to copepods and cladocerans in Suisun Bay, especially when algal biomass is low."*

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[76] Baxter, R., R. Breuer, L. Brown, M. Chotkowski, F. Feyrer, M. Gingras, B. Herbold, A. Müller-Solger, M. Nobriga, T. Sommer, and K. Souza. 2008. Pelagic organism decline progress report: 2007 Synthesis of results. Interagency Ecological Program for the San Francisco Estuary.

[77] Rollwagen-Bollens, S. Gifford, and S.M.Bollens. 2011. The role of protistan microzooplankton in the Upper San Francisco Estuary planktonic food web: Source or sink? Estuaries and Coasts 34:1026-1038.

Copepod	Diatom	Egg Prod.	Hatching Success	Normal Nauplii	Complete Develop.
Acartia tonsa	*Thalassiosira weissflogii*	−			−
	Thalassiosira pseudo nana	−			−
	Thalassiosira weissflogii	+	+		
	Chaetoceros affinis	−			
	Phaeodacylum tricornutum	−	−		
Acartia hudsonica	*Skeletonema costatum*	+			
Acartia clausi	*Thalassiosira rotula*	+	−		
Centropages typicus	*Thalassiosira rotula*	−	−		
Temora stylifera	*Thalassiosira rotula*	−		−	−
	Skeletonema costatum			−	−
	Phaeodactylum tricornutum			−	−
	Thalassiosira rotula	+	−		
	Thalassiosira weissflogii	+	−		
	Phaeodactylum tricornutum	−	−		
	Skeletonema costatum	−	−		
	Thalassiosira rotula	+	−		
Temora longicornis	*Thalassiosira rotula*				+
	Thalassiosira weissflogii				+
	Leptocylindricus danicus				+
	Skeletonema costatum				+
	Chaetoceros affinis				−
	Chaetoceros decipiens				−
	Chaetoceros socialis				−
	Thalassiosira rotula				−
	Thalassiosira pseudo nana				−
	Thalassiosira rotula	+	−		
	Thalassiosira weissflogii	+	−		
	Chaetoceros affinis	+	−		
	Leptocylindricus danicus	−	−		
	Skeletonema costatum	−	−		

Figure 11. Reproductive consequences of direct feeding on diatoms for Delta copepod taxa. Experiments listed used copepod species from the Delta or their cofamilials. Positive (green) and negative (red) outcomes are indicated for four measures of reproductive success in feeding experiments: egg production (clutch size), hatching success, normal nauplii, and complete development of nauplii. Data are from the review of Ianora & Miralto (2010)[78] and other published literature reviewed in Engle (2011).[79] Figure is from Engle (2011)[80].

[78] Op. Cit.

[79] Op. Cit.

[80] Op. Cit.