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Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt - 2009

FINAL REPORT

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Acknowledgments

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Table of Contents

1. Executive Summary

This follow-up study was performed as a collaborative effort between the Central Valley Regional Water Quality Control Board (CVRWQCB) and the UC Davis Aquatic Toxicology Laboratory (UCD-ATL), with assistance from the Sacramento Regional Wastewater Treatment Plant (SRWTP), to assess the potential toxicity of ammonia and treated wastewater effluent from the Sacramento Regional Wastewater Treatment Plant to larval delta smelt (*Hypomesus transpacificus)*. In addition, acute ammonia/um effect concentrations were determined and compared for larval and juvenile delta smelt.

Following up on a pilot study performed in 2008 (Werner et al., 2009), an additional exposure experiment (Experiment III) was conducted on June 11-18, 2009 with two series of increasing concentrations of total ammonia and ammonium (ammonia/um). The two sources of ammonia/um were 1) SRWTP effluent, and 2) a stock solution of ammonium chloride. Experiment III consisted of four NH_4Cl concentrations (2.0-8.0 mg/L ammonia/um) and 4 SRWTP effluent concentrations (2.0-8.0 mg/L ammonia/um). The dilution water used for both test series was ambient water collected from the Sacramento River at Garcia Bend upstream of the SRWTP. Garcia Bend water was collected daily, one day prior to being used for testing throughout the 7-d flow-through test. SRWTP effluent in the form of 24-h composite samples was also collected daily. Control treatments for delta smelt consisted of water obtained from the delta smelt culturing facility, upstream Garcia Bend Sacramento River water (field control) and delta smelt culturing facility water adjusted with de-ionized water to the conductivity of Sacramento River water (low-EC control). Experiment III was conducted concurrently with larval delta smelt and larval fathead minnow (*Pimephales promelas)*. Reference toxicant tests were performed for both species to account for differences in organism sensitivity. Test protocol specified that delta smelt survival in hatchery water controls be at least 60 percent for the test results to be considered acceptable.

Control survival of 47-d old delta smelt larvae in Experiment III was above 60%, and thus met test acceptability criteria. Mean 7-d survival in hatchery water, low conductivity (EC) water (EC=177 µS/cm) and Garcia Bend water after 7 d was 82.4±9.9 %, 85.2±7.1%, and 73.5±10.6%, respectively. Significant effects on 7-d survival were detected in SRWTP effluent at ≥3.92 mg/L and in NH_4Cl at ≥7.71 mg/L total ammonia/um. Toxicity of SRWTP effluent was significantly higher than toxicity of ammonia/um alone. No significant reduction in 7-d survival or growth was detected in larval fathead minnow tests performed concurrently with Experiment III. LC50 experiments showed that juveniles were less sensitive to ammonia/um than larval delta smelt, with 96-h LC50s of 52.3 mg/L for juvenile (149 DPH), and 11.63 (51 DPH) and 11.81 (47 DPH) mg/L for larval fish.

2. Background

Contaminants and their potential deleterious effects to fish in the Sacramento-San Joaquin Delta are of particular interest due to negative long-term population trends and a possible step decline in numbers of several pelagic fish species in the years 2000-2001 (Feyrer et al., 2007). This trend, known as the pelagic organism decline (POD), has been the focus of an increasing number of investigations over the past several years, but no single cause has so far been identified. Delta smelt (*Hypomesus transpacificus*) is one of the species of concern in the POD. It is endemic to the Delta and has been federally listed as threatened since 1993.

The term ammonia/um refers to two chemical species which are in equilibrium in water (NH_3, un-ionized and NH_4^+, ionized) according to $NH_3 + H^+ \Longleftrightarrow NH_4^+$. Tests for ammonia/um usually measure total ammonia plus ammonium, while the toxicity is primarily attributable to the un-ionized form. In general, more un-ionized ammonia and greater toxicity exist at higher pH, because its relative proportion increases with increasing pH according to the following equations (US EPA, 1985):

$$1 / (1 + 10^{\,pKa-pH}) = \%\ NH_3$$
$$\text{where: } pKa = 0.0902 + [2729.9/(°C+273.2)]$$

Temperature will affect this equilibrium, but to a far lesser extent than pH. Acute fish toxicity of ammonia decreases with increasing temperature, but toxicity of total ammonia/um shows no correlation with temperature (US EPA, 1999). This is probably due to an increase in the permeability of biological membranes such as gills by a factor of 2-3 for each 10°C increase in water temperature (Eddy, 2005). Throughout this report, we refer to the sum of ammonia and ammonium as ammonia/um, and to the un-ionized form as ammonia.

The Sacramento River drains into delta smelt spawning and larval nursery areas, thus toxicants present in river water could potentially affect early life stages of delta smelt found downstream. Werner et al. (2008) found that ambient ammonia concentrations were greatest (<0.012 mg/L) at Grand Island (POD site 711), near the Sacramento River confluence with the Deep Water Shipping Channel. Ammonia concentrations in the Sacramento River at Hood were lower (<0.004 mg/L) than at Grand Island, likely due to the lower pH of the river water at Hood. During the 2006-07 monitoring period, the pH range measured at Hood was 7.0-7.6 (7.55 ± 0.32; mean and standard deviation (sd)), while pH at Grand Island was 6.6-8.3 (7.28 ± 0.18; mean, sd). Water temperature in the river was 6.1-25°C (16.0 ± 5.0; mean, sd; Werner et al. 2008). Treated effluent discharged into the river by Sacramento Regional Wastewater Treatment Plant (SRWTP) contained ammonia/um at an average concentration of 24 ± 3.4 mg/L in 2006-2007, and maximum ambient concentrations in the Sacramento River downstream of the point of discharge are approximately 1 mg/L ammonia/um. For 2007-2008, SRWTP reports weekly ammonia concentrations of 0.004 ± 0.002 mg/L and 0.6 ± 0.3 mg/L ammonia/um (SRWTP, unpublished data). For comparison, the pH-dependent US EPA acute water quality criterion (criterion maximum concentration, CMC) for ammonia/um when salmonids are present ranges from 4.37 mg/L at pH 8.3 to 46.84 mg/L at pH 6.6. The pH- and temperature-dependent chronic water quality criteria (30-day average) for water bodies where early life stages of fish are present range from 0.827 mg/L ammonia/um at pH 8.3 and T=24°C (0.075 mg/L ammonia, at 150 µS/cm), to 6.57 mg/L at pH 6.6 and T=0-14°C (0.0034 mg/L ammonia at 6.1°C and 150 µS/cm) (USEPA 1999). The highest 4-day average within the 30-d period should not exceed 2.5 times the chronic criteria.

A pilot study performed in 2008 detected no acute effects on 7-d survival of larval delta smelt at ammonia concentrations up to 2 mg/l and ~9.5 % SRWTP effluent. The second experiment performed in 2008 to address potential interactions between effluent and ammonia toxicity to delta smelt using ammonia concentrations up to 8 mg/L did not yield conclusive data, however 42.5% of fish survived the 7-d exposure to 7.95 mg/L ammonia/um from SRWTP effluent (~26.9% effluent). This study was intended to repeat that experiment using slightly different treatments (Table 1) to increase statistical power. In addition, conductivity and pH in ammonia-chloride treatments was adjusted daily to reflect conditions in effluent treatments. The study presented here is a screening level study designed with conservative methods to determine if further studies are necessary. It is a collaborative effort between the Central Valley Regional Water Quality Control Board (CVRWQCB) and the UC Davis Aquatic Toxicology Laboratory (UCD-ATL), with the cooperation of SRWTP.

The following hypotheses were addressed:

1. Larval delta smelt survival is negatively impacted by one or more contaminant(s) that are positively correlated with ammonia/um from SRWTP.
2. Toxic effect thresholds for ammonia/um are life-stage dependent.

3. Materials and Methods

3.1 Test Animals

H. transpacificus were obtained from the UC Davis Fish Culture and Conservation Laboratory (FCCL) in Byron, CA. Larval fish were transported to UCD-ATL in black 2-gal buckets at a maximum density of 150 fish per bucket. Juvenile fish were transported in 20-gal carboys with a maximum of 130 fish per carboy. Containers were placed in coolers packed lightly with ice to maintain a temperature of 16 ± 2ºC during transport. The control water used in both the ammonia/um exposures and the copper reference toxicant tests were made from water obtained from the hatchery. Water from FCCL was also used for control and low conductivity control treatments. This water was pumped directly from the intake channel of the H.O Banks Pumping Facility near Byron, CA, then passed through a series of sedimentation beds containing natural vegetation to allow any suspended solids in the water to precipitate. The less turbid water was then exposed to an ozonation system to kill any potentially harmful microbes. Ozonated FCCL water was transported to UCD-ATL, and appropriate control waters were prepared for the test one day before fish were collected.

Larval fathead minnows (*P. promelas*) were obtained from AquaTox Inc., Arkansas. Upon receipt at the lab, the animals were acclimated to laboratory control water and placed in a temperature controlled water bath maintained at 25 ± 2º C.

3.2 Ammonia/um – SRWTP Effluent Test

3.2.1 Test Design

Exposure experiments were conducted with larval delta smelt and larval fathead minnow (*Pimephales promelas*). The experiment with SRWTP effluent was conducted on June 11-18, 2009 (Experiment III). It consisted of two series of increasing concentrations of ammonia/um. Concentrations selected were based on ammonia/um effect concentrations determined in a related study conducted in 2008. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) determined in 2008 were 5 and 9 mg/L ammonia/um, respectively, corresponding to 0.066 and 0.105 mg/L ammonia (UCD-ATL, unpublished data). The sources of ammonia/um were 1) the SRWTP effluent, and 2) a concentrated stock solution of ammonium chloride (4,000 ppm NH_4Cl). Experiment III consisted of four concentrations of ammonia/um from NH_4Cl (2.0-8.0 mg/L) and four concentrations (2.0-8.0 mg/L) of ammonia/um from SRWTP effluent (Table 1). The dilution water used for both test series was ambient water collected from the Sacramento River at Garcia Bend, approximately 2 miles upstream from the SRWTP. Garcia Bend water was collected daily, one day prior to being used for testing throughout the 7-d test. SRWTP effluent in the form of 24-h composite samples was also collected daily. Delta smelt hatchery water serves as the control treatment and performance control. Additional reference treatments were: 1. Water from Sacramento River at Garcia Bend; 2. Low EC/turbidity control consisting of hatchery water diluted with de-ionized water to match EC and turbidity of Sacramento River at Garcia Bend. A mixture of antibiotics directed at gram-negative and gram-positive bacteria was added to all treatments, and an additional hatchery water control added that contained no antibiotics.

Table 1. Treatment list for larval delta smelt test.

Experiment II
Sac River at Garcia Bend (SRGB)
SRGB w/ 2.00 mg/L NH_3-N from NH_4Cl
SRGB w/ 4.00 mg/L NH_3-N from NH_4Cl
SRGB w/ 6.00 mg/L NH_3-N from NH_4Cl
SRGB w/ 8.00 mg/L NH_3-N from NH_4Cl
SRGB w/ 2.00 mg/L NH_3-N from SRWTP
SRGB w/ 4.00 mg/L NH_3-N from SRWTP
SRGB w/ 6.00 mg/L NH_3-N from SRWTP
SRGB w/ 8.00 mg/L NH_3-N from SRWTP
Low Conductivity Treatment to match SRGB conductivity and turbidity
Hatchery Water Control to match rearing conductivity and 11 NTU
Hatchery Water Control to match rearing conductivity and 11 NTU, without antibiotics

3.2.2 Sample Preparation

On seven consecutive days, CVRWQCB staff collected 55-60 gal (approx. 220 L) of water from mid-channel in the Sacramento River at Garcia Bend (SRGB) in 5-gal clear plastic cubitainers. Samples were collected using a battery-operated bilge pump with a 20 ft hose mounted on a buoy. The pump and hose were flushed with river water for a minimum of three minutes each day prior to collecting the samples. Cubitainers were rinsed with river water three times prior to filling. On the same day, 5-6 gal of SRWTP effluent (24-h composite sample) were provided by SRWTP in 1-gal amber plastic cubitainers. Samples were transported on ice to UCD-ATL. Within one hour of sample delivery to UCD-ATL, the SRWTP effluent from different cubitainers was composited in a large LDPE (Low Density Poly Ethylene) or HDPE (High Density Poly Ethylene) container. Ambient SRGB water was composited in a 55 gal HDPE container. Subsamples of 22 L were used to prepare ammonia/um exposure concentrations (Table 1) for the larval delta smelt and one parallel larval fathead minnow test. Each day of the experiment, a stock solution of ammonium chloride (15.352g/L NH_4Cl) was used to prepare exposure solutions. Dilutions of SRWTP effluent were also prepared daily. After each solution was thoroughly stirred, total ammonia/um was measured. In instances where measurements were more than ± 8% of the target concentration, the sample was either spiked with additional ammonium chloride or SRWTP effluent, or diluted with SRGB to adjust concentrations.

3.2.3 Measurement of Water Quality Parameters

Sample Receipt: The following water quality parameters were measured upon sample receipt: turbidity, pH, temperature, total hardness (mg/L as $CaCO_3$), alkalinity (mg/L as $CaCO_3$), specific conductance (SC), dissolved oxygen (DO), and ammonia/um. Ammonia/um was measured within 30 min of sample receipt. Data are shown in Table 2.

Ammonia/um – WWTP Effluent Test: Each day, total ammonia/um, hardness, alkalinity, pH, DO, electrical conductivity (EC), SC, turbidity and temperature were measured in fresh test solutions prior to animal exposure. During the test, ammonia/um, turbidity, pH, DO, EC and temperature were measured in exposure aquaria twice daily at 9:00 AM and 4:00 PM. For measurements during exposure, a subsample was obtained by pooling approximately 50 ml from each of the replicate tanks per treatment. Detailed water quality data for both experiments are presented in the Appendix (Tables A3-A26).

Ammonia/um was measured using a HACH DR/890 Colorimeter Meter and a HACH AmVer™ Low Range Ammonia Test 'N Tube™ Reagent Set 0-2.5 mg/L N (HACH Inc., Catalog # 26045-45). This low-range reagent kit was used for the majority of ammonia/um measurements because it was found to be more accurate than the high range kit (HACH AmVer™ High Range Ammonia Test 'N Tube™ Reagent Set 0-50 mg/L N, Catalog # 26069-450). When concentrations exceeded the low range maximum, samples were diluted with de-ionized water.

3.3 Tests with Delta Smelt

3.3.1 Ammonia/um – Effluent Exposures

No standard test protocols exist for delta smelt, and procedures were based on protocols developed at the UCD-ATL. Delta smelt hatchery water served as the control treatment and performance control, and the test acceptability criterion was ≥60% mean survival. Survival in ammonia and effluent treatments was statistically compared to survival in Sacramento River water from Garcia Bend.

After arrival of larval delta smelt at UCD-ATL, fish used in ammonia/um and low conductivity control treatments were acclimated for two days to the specific conductance of Sacramento River water. The transport buckets containing the fish were placed into a temperature-regulated water bath maintained at 16ºC. One-liter beakers were used to carefully collect fish from the buckets, and fish were gently poured into a glass pan containing water at a depth of approximately 2 cm. Fish were then gently scooped up using 100 mL beakers and released into 2.5-gal exposure tanks at random, by submerging the beaker and allowing fish to swim freely into the tanks. Ten to twelve fish were placed into each of the test tanks (4 replicates per treatment) containing 7 L of hatchery water for a 48-h EC acclimation period (Werner et al., 2008). Fish in all tanks except laboratory controls were acclimated with hatchery water diluted with distilled water to match the conductivity of SRGB, while the fish in the laboratory control treatment were acclimated to the exposure chambers at a conductivity matching the fish's rearing conditions. Nanno 3600™, a concentrated *Nannochloropsis* algae solution (68 billion cells per ml; Reed Mariculture, Inc. Campbell, CA) was added to increase the turbidity of the acclimation water to minimize stress. Antibiotics (Maracyn and Maracyn-2, Virbac AH Inc., Fort Worth TX) were added at the manufacturer's recommended dose throughout the acclimating and testing period. Final concentrations were 5.3 mg/L Maracyn (erythromycin) and 0.26 mg/L Maracyn-2 (minocycline). A more detailed description of the acclimation procedure is provided by Werner et al. (2007). At test initiation, the acclimation water was drawn down from 7 L to approximately 2 L to allow for an accurate count of living fish. If more than 10 fish were alive in a replicate, the extra fish were counted, but were not removed from the tank in order to minimize handling stress. During the exposure period, water was renewed daily by means of a drip system at a rate of 1 mL/min. Turbidity of hatchery control water was adjusted daily to 12 NTU using Nanno 3600™ to match rearing conditions. Turbidity and EC of Low EC Control water was adjusted to match Garcia Bend conditions. Dead fish were counted and removed daily, as well as any excess food and detritus. The feeding behavior of fish was monitored throughout the duration of the test. On day 4 and at test termination, the number of surviving fish was recorded.

Table 2. Water quality parameters measured upon sample receipt of 100% effluent from the Sacramento Regional Water Treatment Plant and of ambient river water from the Sacramento River at Garcia Bend for use in an *H. transpacificus* exposure initiated on 6/11/09 (Experiment III).

Water	Test Day	Date	Temp (°C)	EC (µS/cm)	SC (µS/cm)	DO (mg/L)	Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)	pH	Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Sac. River at Garcia Bend	0	6/11/2009	13.2	117	150	9.1	0.03	0.000	7.67	8.44	36	66
Sac. River at Garcia Bend	1	6/12/2009	16.1	117	141	9.5	0.05	0.001	7.73	7.93	56	62
Sac. River at Garcia Bend	2	6/13/2009	14.0	107	136	10.0	0.01	0.000	7.83	7.8	52	62
Sac. River at Garcia Bend	3	6/14/2009	14.8	114	142	10.8	0.00	0.000	7.78	12.2	52	60
Sac. River at Garcia Bend	4	6/15/2009	14.4	107	134	9.7	0.00	0.000	7.88	13.3	52	56
Sac. River at Garcia Bend	5	6/16/2009	12.5	113	147	9.7	0.01	0.000	7.95	10.5	56	58
Sac. River at Garcia Bend	6	6/17/2009	13.6	118	149	9.8	0.07	0.001	7.59	27.2	56	62
SRWTP	0	6/10/2009	7.2	516	784	10.5	30	0.023	6.76	6.27	164	178
SRWTP	1	6/11/2009	6.0	525	810	10.4	26	0.015	6.68	6.49	152	172
SRWTP	2	6/12/2009	7.6	530	792	10.1	29	0.029	6.86	4.72	172	174
SRWTP	3	6/13/2009	7.7	510	756	11.1	24	0.023	6.84	4.61	132	154
SRWTP	4	6/14/2009	8.0	483	716	10.4	24	0.021	6.79	4.73	120	154
SRWTP	5	6/15/2009	7.5	475	714	10.3	30	0.032	6.89	4.61	128	140
SRWTP	6	6/16/2009	13.1	594	766	9.4	25	0.039	6.87	5.24	128	172

3.3.2 LC50 Tests

In addition to effect concentrations of ammonia/um to delta smelt larvae determined in 2008 for IEP-POD, this study compared the sensitivity of larval and juvenile delta smelt to ammonia/um. Four-day tests consisting of a control treatment and five concentrations of ammonia/um (5, 10, 20, 40, 80 mg/L total ammonia/um) were performed. One test with juvenile delta smelt was carried out for 7 d. UV-treated and filtered Delta water obtained from the UC Davis Fish Conservation and Culture Laboratory (FCCL), Byron, CA, adjusted to SC 900 µS/cm and pH 7.9 was used for all LC50 experiments.

H. transpacificus larvae, 51 days post-hatch (DPH) and juveniles (140 DPH) were obtained from the UC Davis FCCL in Byron, CA. The organisms were acclimated a minimum of 24 hours. Antibiotics (Maracyn and Maracyn-2, Virbac AH Inc., Fort Worth TX) were added at the manufacturer's recommended dose throughout the acclimating and testing period. Final concentrations were 5.3 mg/L Maracyn (erythromycin) and 0.26 mg/L Maracyn-2 (minocycline). After the acclimation period, ten larvae or 5 juveniles were randomly loaded into each of four replicate 1-gallon black buckets (larvae) or eight 2-gallon aquaria (juveniles). Tests were performed under low-light conditions in hatchery water filtered through a 1 micron filter (SC: 900 µS/cm, pH 7.9). Water temperature was maintained at 16°C. *H. transpacificus* were fed *Artemia nauplii* three times daily throughout the acclimation and exposure period. Mortality was recorded daily using a small flashlight. At test termination, the number of surviving fish was recorded.

Lethal (LC10, LC20, LC50) effective concentrations were calculated using CETIS v. 1.1.2 (Tidepool Scientific Software, McKinleyville, CA, USA, 2006). NOEC and LOEC were calculated using USEPA standard statistical protocols (USEPA 2002). Lethal Effect Concentrations (LC) were calculated using linear regression, non-linear regression, or linear interpolation methods. For each endpoint, toxicity is defined as a statistically significant difference ($p < 0.05$) to the laboratory control.

Water Quality Parameters: Prior to animal exposure (test days 0 and 2), total ammonia/um, hardness, alkalinity, pH, DO, electrical conductivity (EC), SC, turbidity and temperature were measured in test solutions. For juvenile flow-through test, this was done daily. During the test, and before water was renewed or test take-down, DO, pH, temperature and ammonia/um were measured (days 2, 4, 6 and 7).

3.3.3 Copper Reference Toxicant Tests

Fish from each batch of delta smelt larvae used for the ammonia/um experiments described above underwent a 96-h reference toxicant test with copper to determine the relative sensitivity of the fish. Fish were acclimated to test conditions in the buckets used for transportation from the FCCL to minimize handling stress. Acclimation was for 24 hr in hatchery water adjusted to an SC of 900 µS/cm with Instant Ocean and a pH of 7.9. These conditions as well as the acclimation period were chosen based on the conditions of a previous copper LC50 study, and designed to mimic average conditions in the Delta.

Tests were performed with hatchery water filtered through a 1 micron filter and adjusted to an SC of 900 µS/cm and a pH of 7.9. Copper was dissolved in water and spiked into treatment solutions prior to test initiation and again on day 2, when 80% water was renewed. Tests were conducted in a water bath maintained at 16 °C, surrounded by dark-colored curtains to minimize light-induced stress. One-gal black buckets with lids were used as exposure vessels, each containing 3.5 L of sample water. During testing, lids were allowed to rest on top of the buckets, but were not snapped shut to provide ambient light at less than one ft-candle. Exposure water was not aerated. Fish were fed *Artemia* nauplii three times daily during the acclimation period and experimental exposures.

The reference toxicant tests consisted of four copper concentrations (27, 53, 106 and 213 µg/L Cu^{2+}, nominal) and a control. Concentrations were selected based on the previously determined 96-h LC_{50} for larval delta smelt (85.2 µg/L Cu^{2+}) and set at 0.31, 0.63, 1.25 and 2.5 toxic units. After the acclimation period, ten fish were randomly placed into each of three replicate test containers. Mortality was recorded daily using a small flashlight. On day 2, 80% of test solutions were renewed, and dead fish, excess *Artemia* nauplii and detritus were removed. At the end of the 96-h exposure period, the number of surviving fish was recorded. Water samples were submitted to the Department of Fish and Game, Wildlife Pollution Control Laboratory for analytical determination of copper concentrations.

Water Quality Parameters: Prior to animal exposure (test days 0 and 2), total ammonia/um, hardness, alkalinity, pH, DO, electrical conductivity (EC), SC, turbidity and temperature were measured in test solutions. During the test, and before water was renewed or test take-down, DO, pH, temperature and ammonia/um were measured (days 2, 4).

3.4 Tests with Larval Fathead Minnow

Tests with larval fathead minnow (*Pimephales promelas)* were conducted concurrently with Experiment I to compare delta smelt test results to a species commonly used in National Pollutant Discharge Elimination System (NPDES) testing. Toxicity testing for larval *P. promelas* followed procedures described in "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (US EPA, 2002). De-ionized water amended with dry salts to EPA moderately hard standards (DIEPAMH) was the laboratory control water used in these tests. For a 7-day test, the test acceptability criterion is 80% control survival.

3.4.1 Ammonia/um – Effluent Exposures

P. promelas were tested concurrently with *H. transpacificus* during the experiment conducted in June 2008 (Experiment I). Treatments consisted of subsamples of the test solutions prepared for the delta smelt exposure, excluding the control treatments. Larval *P. promelas* 7-day chronic tests consist of four replicate 600 mL glass beakers per treatment, each containing 250 mL of sample and ten organisms. Larvae were less than 48 h old at test initiation. Fish were fed three times daily with newly hatched *Artemia* nauplii. Eighty percent of the test solution was renewed daily, at which time debris and dead fish were also removed. Test chambers were incubated in a temperature-controlled water bath maintained at 25 ± 2º C under white fluorescent light with a 16-hour light: 8-hour dark photoperiod. Mortality was recorded daily and at test termination. Water quality measurements (DO, pH, total ammonia and temperature) were measured daily using pooled subsamples from replicate beakers.

3.4.2 Reference Toxicant Tests

Reference toxicant tests with fathead minnow consisted of six concentrations of sodium chloride (NaCl) and a control. The concentrations, ranging from 0.63 to 10 g/L have been used for UCD-ATL's long-term data set for several years. The same protocols used in the ammonia/um exposures were followed in the reference toxicant tests. In addition, biomass was measured for each replicate.

4. Results

4.1 Tests with Delta Smelt

4.1.1 Ammonia/um and SRWTP Effluent Exposures

<u>Experiment III - June 11, 2009:</u> Survival of delta smelt larvae after 7 d was above 60% in the hatchery control treatment, and thus this test met acceptability criteria. Mean control survival in hatchery water, low conductivity (EC) water (EC=177 µS/cm) and Garcia Bend water after 7 d was 82.4±9.9 %, 85.2±7.1%, and 73.5±10.6%, respectively (Tables 3-1 to 3-3). Survival after 7 d in ammonium-chloride and SRWTP effluent treatments was compared to Sacramento River (SRGB) water, and showed significant differences in the 8 mg/l ammonium-chloride treatment, and ≥4 mg/l effluent treatments. In addition, there were significant differences between ammonium-chloride and SRWTP effluent treatments after 4 d (8 mg/l) and 7 d (≥6 mg/l).

Effect concentrations for 4- and 7-d exposures to ammonium-chloride and effluent are shown in Table 3-2 and Figure 1. Results for ammonium-chloride are consistent with LC50 values derived from LC50 tests presented below. While the 96-h LC50 was >7.71 mg/L (>0.116 mg/L un-ionized ammonia), the 7-d LC50 was 7.45 mg/L (0.113 mg/L un-ionized ammonia). For SRWTP effluent, these effect thresholds were lower with 96-h and 7-d LC50s of 7.19 and 5.4 mg/L total ammonia/um, and 0.125 and 0.09 mg/L un-ionized ammonia, respectively.

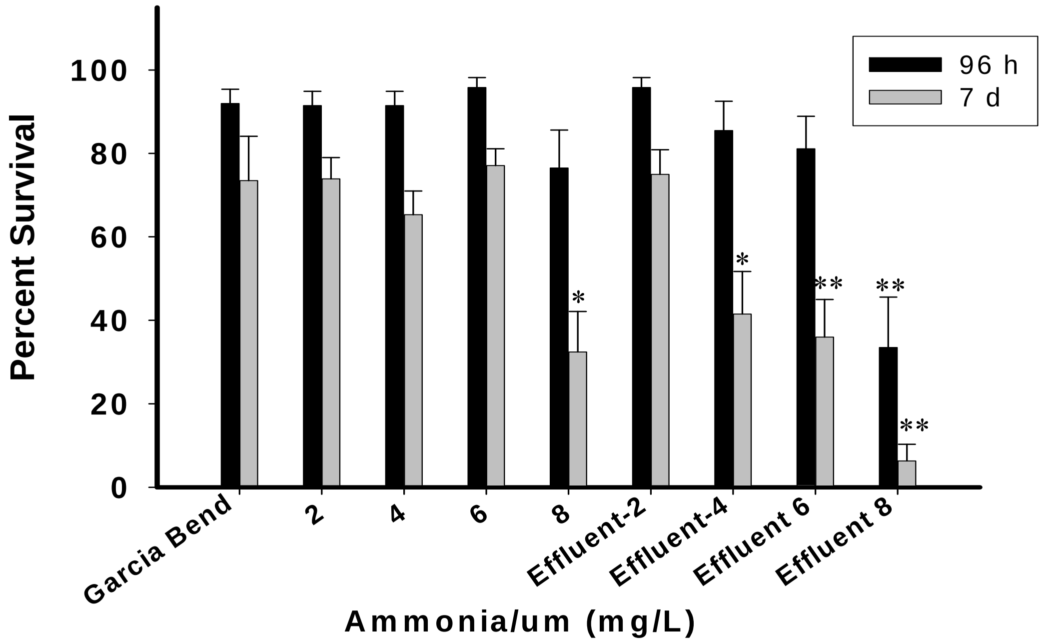

Figure 1. Percent survival (mean ± SE; n=4) of larval *H. transpacificus* (47 d old at test initiation) after exposure to different concentrations of ammonium chloride and SRWTP wastewater treatment effluent. *indicates significant ($p<0.05$) reduction in survival compared to Garcia Bend**;** *indicates significantly lower survival than in the corresponding treatment containing NH_4Cl (two-tailed test).

Table 3-1. Percent survival of 47-d old delta smelt larvae during a 7-d test initiated 6/11/09(Experiment III); SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean; shaded cells indicate significant ($p<0.05$) reduction in survival compared to SRGB**;** * indicates significantly lower survival than in the corresponding treatment containing NH_4Cl (two-tailed test).

Treatment	Mean Measured Total Ammonia/um (mg/L)	Mean Un-ionized Ammonia (mg/L)	Percent Effluent		96-hr Survival (%)		7-day Survival (%)	
			Mean	SE	Mean	SE	Mean	SE
SRGB	0.10	0.002	-	-	92.0	3.4	73.5	10.6
2.00 mg/L NH_3-N from NH_4Cl	1.90	0.038	-	-	91.5	3.4	73.9	5.1
4.00 mg/L NH_3-N from NH_4Cl	3.84	0.071	-	-	91.5	3.4	65.3	5.7
6.00 mg/L NH_3-N from NH_4Cl	5.66	0.091	-	-	95.8	2.4	77.1	4.0
8.00 mg/L NH_3-N from NH_4Cl	7.71	0.116	-	-	76.5	9.1	32.4	9.7
2.00 mg/L NH_3-N from SRWTP	1.96	0.039	9.0	0.3	95.8	2.4	75.0	5.9
4.00 mg/L NH_3-N from SRWTP	3.92	0.081	18.3	0.5	85.5	7.0	41.5	10.2
6.00 mg/L NH_3-N from SRWTP	5.85	0.092	28.1	0.8	81.1	7.8	36.0*	9.0
8.00 mg/L NH_3-N from SRWTP	7.78	0.139	37.9	1.0	33.5*	12.1	6.3*	4.0
Low EC Control	0.17	0.002	-	-	95.8	4.2	85.2	7.1
Hatchery Water Control	0.20	0.004	-	-	95.6	2.5	82.4	9.9
Hatchery Water - No Antibiotics	0.21	0.003	-	-	83.7	9.0	81.6	11.0

Table 3-2. Effect concentrations for 4 and 7 d exposures of 47-d old delta smelt larvae to ammonia/um and SRWTP effluent.

Endpoint	Ammonia/um Source	Total Ammonia/um (mg/L)							
		LC10 (mg/L)		LC25 (mg/L)		LC50 (mg/L)		NOEC	LOEC
		Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.		
96-hour Survival	NH_4Cl	6.77	5.51 - >7.71	> 7.71	-	> 7.71	-	7.71	> 7.71
	SRWTP	4.31	2.05 - 7.11	6.24	4.74 - 6.95	7.19	6.28 - >7.78	5.85	7.78
7-day Survival	NH_4Cl	5.89	<1.90 - 6.38	6.44	5.51 - 7.47	7.45	6.34 - >7.71	5.66	7.71
	SRWTP	2.32	<1.96 - 2.95	2.95	1.78 - 4.77	5.40	2.11 - 6.98	1.96	3.92

		Un-ionized Ammonia (mg/L)							
		LC10 (mg/L)		LC25 (mg/L)		LC50 (mg/L)		NOEC	LOEC
		Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.		
96-hour Survival	NH_4Cl	0.105	0.089 - > 0.116	> 0.116	-	> 0.116	-	0.116	> 0.116
	SRWTP	0.084	0.048 - 0.106	0.102	0.081 - 0.120	0.125	0.103 - > 0.139	0.092	0.139
7-day Survival	NH_4Cl	0.094	< 0.038 - 0.101	0.101	0.089 - 0.114	0.113	0.100 - > 0.116	0.091	0.116
	SRWTP	0.048	0.006 - 0.063	0.063	0.036 - 0.094	0.090	0.059 - 0.116	0.039	0.081

		Percent Effluent (%)							
		LC10 (mg/L)		LC25 (mg/L)		LC50 (mg/L)		NOEC	LOEC
		Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.		
96-hour Survival	SRWTP	20.2	9.1 - 34.5	30.1	22.4 - 33.6	34.8	30.2 - > 37.9	28.1	37.9
7-day Survival	SRWTP	10.6	< 9.0 - 13.5	13.5	8.1 - 22.5	25.7	9.2 - 33.8	9.0	18.3

Table 3-3. Water quality parameters measured during the 7-day test initiated 6/11/09 with 47-d old delta smelt.

Treatment	Temp (°C)				EC (uS/cm)				DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend	16.5	17.5	16.9	0.2	107.4	294.5	172	59	9.1	9.9	9.6	0.2	7.67	8.02	7.89	0.10
2.00 mg/L NH_3-N: NH_4Cl	16.5	17.5	17.0	0.3	152.3	339.2	221	59	8.9	9.8	9.5	0.3	7.65	7.93	7.83	0.08
4.00 mg/L NH_3-N: NH_4Cl	16.7	17.5	16.9	0.2	198.4	372.2	264	54	9.1	9.9	9.6	0.2	7.56	7.94	7.79	0.12
6.00 mg/L NH_3-N: NH_4Cl	16.7	17.5	17.0	0.2	245.4	416.2	313	53	8.9	10.0	9.6	0.3	7.42	7.89	7.74	0.14
8.00 mg/L NH_3-N: NH_4Cl	16.7	17.5	17.0	0.2	238.3	439.2	346	45	9.0	10.1	9.6	0.3	7.29	7.97	7.70	0.17
2.00 mg/L NH_3-N: SRWTP	16.1	17.5	16.9	0.4	150.4	339.5	219	60	9.0	9.9	9.6	0.3	7.58	8.00	7.83	0.12
4.00 mg/L NH_3-N: SRWTP	16.0	17.4	16.9	0.4	195.4	383.2	263	56	9.1	10.2	9.7	0.3	7.48	8.05	7.82	0.22
6.00 mg/L NH_3-N: SRWTP	16.1	17.2	16.8	0.3	250.4	426.9	314	55	8.8	10.1	9.6	0.3	7.35	7.95	7.70	0.22
8.00 mg/L NH_3-N: SRWTP	16.1	17.4	16.8	0.3	299.5	451.8	359	48	9.1	10.2	9.6	0.3	7.24	8.07	7.73	0.30
Low EC Control	16.0	17.3	16.8	0.3	116.2	282.3	177	52	8.3	10.7	9.3	0.5	7.02	8.21	7.72	0.34
Hatchery Water Control	16.0	17.5	16.7	0.4	2087	2232	2165	32	8.6	9.8	9.2	0.3	7.67	8.15	7.90	0.12
Hatchery Water - No AB	16.0	17.5	16.7	0.4	2018	2211	2151	44	8.2	9.7	8.9	0.4	7.45	8.16	7.83	0.20

Treatment	Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)				Turbidity (NTU)				Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R at Garcia Bend	0.01	0.16	0.09	0.06	0.000	0.005	0.002	0.001	2.45	26.3	6.66	5.47	51	60
2.00 mg/L NH_3-N: NH_4Cl	1.31	2.15	1.90	0.24	0.023	0.052	0.038	0.007	2.13	23.8	6.08	4.73	57	60
4.00 mg/L NH_3-N: NH_4Cl	2.86	4.32	3.84	0.38	0.044	0.104	0.071	0.017	2.28	25.4	6.18	5.04	61	60
6.00 mg/L NH_3-N: NH_4Cl	4.35	6.40	5.66	0.62	0.046	0.125	0.091	0.022	2.46	23.6	6.18	4.69	63	58
8.00 mg/L NH_3-N: NH_4Cl	6.16	8.60	7.71	0.69	0.046	0.186	0.116	0.035	2.70	23.3	6.11	4.61	67	61
2.00 mg/L NH_3-N: SRWTP	1.59	2.14	1.96	0.16	0.025	0.057	0.039	0.010	2.31	23.3	6.15	4.65	61	69
4.00 mg/L NH_3-N: SRWTP	3.34	4.28	3.92	0.22	0.033	0.122	0.081	0.032	2.46	22.0	5.97	4.34	67	80
6.00 mg/L NH_3-N: SRWTP	4.86	6.48	5.85	0.45	0.038	0.145	0.092	0.036	2.69	20.1	5.86	3.78	79	90
8.00 mg/L NH_3-N: SRWTP	6.12	8.68	7.78	0.68	0.040	0.253	0.139	0.073	2.94	18.0	5.68	3.34	85	98
Low EC Control	0.00	0.38	0.17	0.13	0.000	0.004	0.002	0.001	3.22	9.5	6.24	1.57	38	26
Hatchery Water Control	0.02	0.48	0.20	0.14	0.000	0.008	0.004	0.002	3.65	13.5	6.35	2.23	254	82
Hatchery Water - No AB	0.02	0.47	0.21	0.15	0.000	0.005	0.003	0.001	3.95	13.1	6.19	2.07	254	82

4.1.2 LC50 Tests

Larval Delta Smelt: Two LC50 tests were performed with larval (Tables 4, 5) and one with juvenile (Table 6) delta smelt. Resulting 96-h LC50 values for larval fish were 11.63 and 11.81 mg/L total ammonia/um, and 0.147 and 0.164 mg/L un-ionized ammonia. Results obtained are consistent with ammonia/um and ammonia effect concentrations established in 2008 for 50-d old larval delta smelt at UCD-ATL, where 96-h LC50 values of 12.0 mg/L total ammonia/um, and 0.147 mg/L mg/L un-ionized ammonia were determined. Water quality and exposure conditions were the same in all tests (pH 7.9, T=16^{o}C, EC=900 µS/cm).

Juvenile Delta Smelt: Juvenile fish (149 DPH) were less sensitive to ammonia/um than larval fish (Tables 6-1 to 6-3). The 96-h and 7-d LC50 values were 52.3 and 46.2 mg/L total ammonia/um, and 0.557 and 0.515 mg/L un-ionized ammonia.

Table 4-1. Results of a larval delta smelt LC50 test initiated 6/24/09 evaluating the toxicity of ammonium-chloride. Test animals were 51 days old at test initiation.

Treatment	Measured Mean Values		96-hr Survival (%)	
	Total Ammonia/um (mg/L)	**Un-ionized Ammonia (mg/L)**	**Mean**	**SE**
Filtered Hatchery Water @ 900 uS/cm	0.1	0.001	67.5	14.9
2.5 ppm Ammonia/um	1.8	0.028	57.5	21.7
5 ppm Ammonia/um	3.2	0.050	60.0	16.8
10 ppm Ammonia/um	7.1	0.091	72.5	2.5
20 ppm Ammonia/um	16.3	0.189	12.5	7.5
40 ppm Ammonia/um	32.3	0.281	0.0	0.0
80 ppm Ammonia/um	66.0	0.420	0.0	0.0
Hatchery Water no antibiotics	0.1	0.001	50.0	10.8
10 ppm Ammonia/um no antibiotics	6.8	0.088	42.5	12.5
20 ppm Ammonia/um no antiobiotics	16.0	0.173	15.0	5.0

Table 4-2. Effect concentrations for 4 d exposures of 51-d old delta smelt larvae to ammonium-chloride.

Point Estimate	Nominal Total Ammonia/um (mg/L)		Measured Total Ammonia/um (mg/L)		Un-ionized Ammonia (mg/L)	
	Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.
96-hr Survival LC10	10.37	< 2.5 - 11.54	7.46	< 1.8 - 8.49	0.096	< 0.001 - 0.111
96-hr Survival LC25	11.93	< 2.5 - 13.63	8.83	< 1.8 - 10.35	0.115	< 0.001 - 0.134
96-hr Survival LC50	15.03	9.18 - 18.84	11.63	6.36 - 15.2	0.147	0.085 - 0.181
96-hr NOEC	10	-	7.14	-	0.091	-
96-hr LOEC	20	-	16.31	-	0.189	-

Table 4-3. Water quality parameters measured during the 4-day test initiated 6/24/09 with 51-d old delta smelt.

Treatment	Temp (°C)			DO (mg/L)			pH			EC (uS/cm)	SC (uS/cm)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Filtered Hatchery Water @ 900 uS/cm	17.8	1.5	5	9.1	0.6	5	7.70	0.12	5	767	924
2.5 ppm Ammonia/um	18.1	1.7	5	9.2	0.7	5	7.70	0.14	5	782	956
5 ppm Ammonia/um	17.8	1.6	5	9.0	0.8	5	7.68	0.16	5	791	944
10 ppm Ammonia/um	17.7	1.2	5	8.8	1.1	5	7.60	0.18	5	832	1006
20 ppm Ammonia/um	17.7	1.2	5	9.0	0.9	5	7.58	0.10	5	898	1075
40 ppm Ammonia/um	17.1	1.0	2	8.5	1.0	2	7.49	0.08	2	1007	1204
80 ppm Ammonia/um	16.7	1.1	2	8.8	0.8	2	7.38	0.06	2	1247	1508
Filtered Hatchery Water no antibiotics	17.6	1.3	5	8.5	0.9	5	7.70	0.25	5	726	880
10 ppm Ammonia/um no antibiotics	17.7	0.9	5	8.6	0.8	5	7.62	0.15	5	816	970
20 ppm Ammonia/um no antibiotics	17.6	1.1	5	8.6	0.8	5	7.55	0.12	5	870	1047

Treatment	Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Turbidity (NTU)
	Mean	SD	*N*	Mean	SD	*N*			
Filtered Hatchery Water @ 900 uS/cm	0.09	0.06	5	0.001	0.001	5	100	79	0.74
2.5 ppm Ammonia/um	1.81	0.09	5	0.028	0.008	5	-	-	-
5 ppm Ammonia/um	3.18	0.90	5	0.050	0.026	5	-	-	-
10 ppm Ammonia/um	7.14	1.12	5	0.091	0.049	5	-	-	-
20 ppm Ammonia/um	16.31	2.21	5	0.189	0.064	5	-	-	-
40 ppm Ammonia/um	32.26	6.70	2	0.281	0.091	2	-	-	-
80 ppm Ammonia/um	65.96	11.37	2	0.420	0.098	2	-	-	-
Filtered Hatchery Water, no antibiotics	0.09	0.08	5	0.001	0.001	5	-	-	-
10 ppm Ammonia/um, no antibiotics	6.85	0.86	5	0.088	0.034	5	-	-	-
20 ppm Ammonia/um, no antibiotics	16.01	2.32	5	0.173	0.062	5	-	-	-

Table 5-1. Results of a larval delta smelt LC50 test initiated 07/08/09 evaluating the toxicity of ammonium-chloride. Test animals were 47 days old at test initiation.

Treatment	Measured Mean Values		96-hr Survival (%)	
	Total Ammonia/um (mg/L)	Un-ionized Ammonia (mg/L)	Mean	SE
Filtered Hatchery Water @ 900 uS/cm	0.1	0.002	67.5	13.1
2.5 ppm Ammonia/um	1.9	0.032	75.0	18.9
5 ppm Ammonia/um	3.7	0.064	80.0	9.1
10 ppm Ammonia/um	7.1	0.099	61.1	3.2
20 ppm Ammonia/um	14.4	0.191	27.5	8.5
40 ppm Ammonia/um	29.0	0.333	0.0	0.0
80 ppm Ammonia/um	57.8	0.645	0.0	0.0

Table 5-2. Effect concentrations for 4 d exposures of 47-d old delta smelt larvae to ammonium-chloride.

Point Estimate	Nominal Total Ammonia/um (mg/L)		Measured Total Ammonia/um (mg/L)		Un-ionized Ammonia (mg/L)	
	Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.
96-hr Survival LC10	7.44	< 2.5 - 13.25	5.38	< 1.9 - 9.38	0.084	< 0.002 - 0.127
96-hr Survival LC25	11.2	2.6 - 15.5	7.98	2.05 - 11.13	0.113	0.066 - 0.162
96-hr Survival LC50	16.45	11.35 - 25.57	11.81	8.09 - 18.47	0.164	0.119 - 0.239
NOEC	20	-	14.4	-	0.191	-
LOEC	40	-	29	-	0.333	-

Table 5-3. Water quality parameters measured during the 4-day test initiated 07/08/09 with 47-d old delta smelt.

Treatment	Temp (^oC)			DO (mg/L)			pH			EC (uS/cm)	SC (uS/cm)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Filtered Hatchery Water @ 900 uS/cm	17.3	0.5	8	8.9	0.7	8	7.84	0.18	8	758	908
2.5 ppm Ammonia/um	17.2	0.6	8	8.8	0.9	8	7.76	0.19	8	777	926
5 ppm Ammonia/um	17.3	0.8	7	8.8	0.7	8	7.75	0.19	8	792	948
10 ppm Ammonia/um	17.2	0.4	8	8.8	0.9	8	7.67	0.16	8	820	979
20 ppm Ammonia/um	17.1	0.5	7	9.0	0.7	7	7.66	0.13	7	882	1057
40 ppm Ammonia/um	17.0	0.2	4	9.3	0.6	4	7.61	0.11	4	1017	1200
80 ppm Ammonia/um	16.9	0.0	2	9.4	0.1	2	7.62	0.04	2	1264	1493

Treatment	Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*			
Filtered Hatchery Water @ 900 uS/cm	0.09	0.06	8	0.002	0.001	8	0.84	100	66
2.5 ppm Ammonia/um	1.88	0.08	8	0.032	0.013	8	-	-	-
5 ppm Ammonia/um	3.74	0.13	8	0.064	0.027	8	-	-	-
10 ppm Ammonia/um	7.08	0.72	8	0.099	0.040	8	-	-	-
20 ppm Ammonia/um	14.43	0.49	7	0.191	0.056	7	-	-	-
40 ppm Ammonia/um	28.95	2.22	4	0.333	0.100	4	-	-	-
80 ppm Ammonia/um	57.80	4.81	2	0.645	0.105	2	-	-	-

Table 6-1. Results of juvenile delta smelt LC50 test initiated 09/17/09 evaluating the toxicity of ammonium-chloride. Test animals were 149 days old at test initiation.

Treatment	**Mean Measured Total Ammonia/um (mg/L)**	**Mean Measured Un-ionized Ammonia (mg/L)**	**96-hr Survival (%)**		**7-day Survival (%)**	
			Mean	SE	Mean	SE
Hatchery Water @ 900 uS/cm	0.2	0.004	97.5	2.5	97.5	2.5
7.5 ppm Ammonia/um	6.5	0.118	100.0	0.0	97.5	2.5
15 ppm Ammonia/um	12.6	0.194	100.0	0.0	100.0	0.0
30 ppm Ammonia/um	25.3	0.356	95.0	3.3	95.0	3.3
60 ppm Ammonia/um	52.1	0.555	48.8	12.5	33.8	9.4

Table 5-2. Effect concentrations for 4 and 7-d exposures of 149-d old juvenile delta smelt to ammonium-chloride.

	Endpoint	**LC10 (mg/L)**		**LC25 (mg/L)**		**LC50 (mg/L)**		**NOEC**	**LOEC**
		Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.		
Nominal Ammonia/um	96-hour Survival	36.1	23.2 - 43.0	46.0	36.3 - 53.0	60.2	52.3 - 76.4	30	60
	7-day Survival	35.7	23.6 - 42.2	43.2	33.2 - 49.1	53.4	46.4 - 60.8	30	60
Measured Ammonia/um	96-hour Survival	30.7	19.4 - 36.8	39.5	30.9 - 45.8	52.3	45.1 - 67.0	25.3	52.1
	7-day Survival	30.3	19.7 - 36.1	37.0	28.2 - 42.3	46.2	39.9 - 52.8	25.3	52.1
Un-ionized Ammonia	96-hour Survival	0.400	0.303 - 0.447	0.468	0.403 - 0.512	0.557	0.508 - 0.649	0.356	0.555
	7-day Survival	0.398	0.305 - 0.443	0.550	0.380 - 0.488	0.515	0.471 - 0.560	0.356	0.555

Table 6-3. Water quality parameters measured during the 7-day test initiated 09/17/09 with 149-d old juvenile delta smelt.

Treatment	Temp (^oC)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Water @ 900 uS/cm	16.5	0.2	8	760	13	4	917	13	4	9.3	0.5	8
7.5 ppm Ammonia/um	16.4	0.3	8	802	15	4	972	18	4	9.5	0.5	8
15 ppm Ammonia/um	16.3	0.3	8	851	20	4	1033	21	4	9.7	0.6	8
30 ppm Ammonia/um	16.3	0.3	8	933	25	4	1132	25	4	9.7	0.6	8
60 ppm Ammonia/um	16.3	0.3	8	1108	29	4	1347	28	4	9.5	0.5	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Hatchery Water @ 900 uS/cm	7.85	0.19	8	0.24	0.19	8	0.004	0.002	8	130	72
7.5 ppm Ammonia/um	7.81	0.15	8	6.50	0.53	8	0.118	0.040	8	-	-
15 ppm Ammonia/um	7.76	0.09	8	12.63	1.30	8	0.194	0.040	8	-	-
30 ppm Ammonia/um	7.72	0.12	8	25.25	2.87	8	0.356	0.090	8	-	-
60 ppm Ammonia/um	7.61	0.10	8	52.13	6.13	8	0.555	0.087	8	-	-

4.1.3 Copper Reference Toxicant Tests

Delta smelt larvae (47-51 d old) used in Experiment III (Tables 7-1, -2) and LC50 tests (Tables 8-1, -2 and 9-1, -2) showed a range of sensitivities to copper (LC50s: 150, 133 and 85 µg/L Cu^{2+}) with larvae obtained earlier in the year being less sensitive than those obtained in July. However, sensitivity was still similar to that of larvae used in 2008 to determine the 96-h LC50 (86.5 µg/L Cu^{2+}; dissolved) for this species (Werner et al., unpublished data). Juveniles (148 d old) were similar in their sensitivity to copper (LC50: 112 µg/L Cu^{2+}; Tables 10-1, -2) as larval fish, despite being far less sensitive to ammonia/um (see Section 4.1.2).

Table 7-1. Effect of 96-h exposure to copper on percent survival of 46-d old delta smelt larvae. This test was initiated on 6/10/09.

Treatment	96-hr Survival (%)	
	Mean	SE
Filtered Hatchery Water (FHW) @ 900 uS/cm	93.3	6.7
FHW + 27 ppb Cu^{2+}	100.0	0.0
FHW + 53 ppb Cu^{2+}	86.7	6.7
FHW + 106 ppb Cu^{2+}	86.7	13.3
FHW + 213 ppb Cu^{2+}	6.7	6.7

Effect Threshold	Cu^{2+} (µg/L)	
	Estimate	95% CI
96-hr Survival: LC10	110.7	54.5 - 139.5
LC25	128.0	75.9 - 156.8
LC50	150.3	106.6 - 183.8
NOEC	106	-
LOEC	213	-

Table 7-2. Water quality data for the 96-hour copper test with 46-d old delta smelt larvae. This test was initiated on 6/10/09.

Treatment	EC (uS/cm)	SC (uS/cm)	Temp (^oC)			DO (mg/L)		
			Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water (FHW) @ 900 uS/cm	798	920	17.8	0.6	4	9.4	0.6	4
FHW + 27 ppb Cu^{2+}	787	912	17.7	0.5	4	9.4	0.7	4
FHW + 53 ppb Cu^{2+}	791	922	17.6	0.4	4	9.2	0.9	4
FHW + 106 ppb Cu^{2+}	784	914	17.6	0.5	4	9.5	0.4	4
FHW + 213 ppb Cu^{2+}	781	903	17.7	0.3	4	9.4	0.5	4

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water (FHW) @ 900 uS/cm	7.86	0.11	4	0.10	0.06	3	0.002	0.000	3
FHW + 27 ppb Cu^{2+}	7.88	0.15	4	0.09	0.04	3	0.002	0.001	3
FHW + 53 ppb Cu^{2+}	7.85	0.15	4	0.09	0.04	3	0.002	0.000	3
FHW + 106 ppb Cu^{2+}	7.88	0.09	4	0.07	0.01	3	0.001	0.000	3
FHW + 213 ppb Cu^{2+}	7.83	0.08	4	0.06	0.01	3	0.001	0.000	3

Table 8-1. Effect of 96-h exposure to copper on percent survival of 51-d old delta smelt larvae. This test was initiated on 6/24/09.

Treatment	96-hr Survival (%)	
	Mean	SE
Filtered Hatchery Water (FHW) @ 900 uS/cm	53	26.7
FHW + 27 ppb Cu^{2+}	47	24.0
FHW + 53 ppb Cu^{2+}	87	13.3
FHW + 106 ppb Cu^{2+}	40	23.1
FHW + 213 ppb Cu^{2+}	13	6.7

Effect Threshold	Cu^{2+} (µg/L)	
	Estimate	95% CI
96-hr Survival: LC10	64.4	< 27 - 175.4
LC25	86.2	< 27 - 191.7
LC50	133.8	10.3 - 241.9
NOEC	213	-
LOEC	> 213	-

Table 8-2. Water quality data for the 96-hour copper test with 51-d old delta smelt larvae. This test was initiated on 6/24/09.

Treatment	EC (uS/cm)	SC (uS/cm)	Temp (°C)			DO (mg/L)		
			Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water (FHW) @ 900 uS/cm	774	903	17.4	0.2	4	9.2	0.6	4
FHW + 27 ppb Cu^{2+}	799	931	17.2	0.1	4	8.9	1.2	4
FHW + 53 ppb Cu^{2+}	791	929	17.4	0.3	4	9.1	0.9	4
FHW + 106 ppb Cu^{2+}	790	923	17.3	0.2	4	9.1	1.2	4
FHW + 213 ppb Cu^{2+}	774	906	17.3	0.3	4	9.3	0.9	4

Treatment	pH			Ammonia/um (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*					
Filtered Hatchery Water (FHW) @ 900 uS/cm	7.71	0.15	4	0.12	0.002	0.74	100	79
FHW + 27 ppb Cu^{2+}	7.70	0.12	4	0.13	0.002	-	-	-
FHW + 53 ppb Cu^{2+}	7.70	0.09	4	0.12	0.002	-	-	-
FHW + 106 ppb Cu^{2+}	7.72	0.09	4	0.10	0.002	-	-	-
FHW + 213 ppb Cu^{2+}	7.73	0.11	4	0.08	0.002	-	-	-

Table 9-1. Effect of 96-h exposure to copper on percent survival of 47-d old delta smelt larvae. This test was initiated on 07/08/09.

Treatment	96-hr Survival (%)	
	mean	se
Filtered Hatchery Water (FHW) @ 900 uS/cm	93	6.7
FHW + 27 ppb Cu^{2+}	80	11.5
FHW + 53 ppb Cu^{2+}	67	6.7
FHW + 106 ppb Cu^{2+}	33	17.6
FHW + 213 ppb Cu^{2+}	20	20.0

Effect Threshold	Cu^{2+} (µg/L)	
	Estimate	95% CI
96-hr Survival: LC10	9.3	< 27 - 77.8
LC25	44.8	< 27 - 83.1
LC50	80.4	48.7 - 227.2
NOEC	53	-
LOEC	106	-

Table 9-2. Water quality data for the 96-hour copper test with 47-d old delta smelt larvae. This test was initiated on 07/08/09.

Treatment	EC (uS/cm)	SC (uS/cm)	Temp (^oC)			DO (mg/L)			pH		
			Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water (FHW) @ 900 uS/cm	770	931	16.8	0.8	4	9.2	0.6	4	7.86	0.15	4
FHW + 27 ppb Cu^{2+}	783	926	17.1	0.4	4	9.4	0.4	4	7.85	0.19	4
FHW + 53 ppb Cu^{2+}	755	927	16.8	0.8	4	9.4	0.4	4	7.90	0.14	4
FHW + 106 ppb Cu^{2+}	780	931	17.0	0.5	4	9.5	0.3	4	7.93	0.13	4
FHW + 213 ppb Cu^{2+}	782	931	16.9	0.5	4	9.5	0.3	4	7.90	0.13	4

Treatment	Ammonia/um (mg/L)			Un-ionized Ammonia (mg/L)			Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*			
Filtered Hatchery Water (FHW) @ 900 uS/cm	0.03	0.03	4	0.000	0.000	4	0.84	100	66
FHW + 27 ppb Cu^{2+}	0.04	0.02	3	0.001	0.000	3	-	-	-
FHW + 53 ppb Cu^{2+}	0.06	0.06	3	0.001	0.001	3	-	-	-
FHW + 106 ppb Cu^{2+}	0.05	0.05	3	0.001	0.001	3	-	-	-
FHW + 213 ppb Cu^{2+}	0.03	0.05	3	0.001	0.001	3	-	-	-

Table 10-1. Effect of 96-h exposure to copper on percent survival of 148-d old delta smelt juveniles. This test was initiated on 09/16/09.

Treatment	96-hr Survival (%)	
	mean	se
Filtered Hatchery Water (FHW) @ 900 uS/cm	100.0	0.0
75 ppb Cu^{2+}	93.3	6.7
150 ppb Cu^{2+}	13.3	13.3
300 ppb Cu^{2+}	6.7	6.7

Effect Threshold	Cu^{2+} (µg/L)	
	Estimate	95% CI
96-hr Survival: LC10	77.2	< 75 - 88.2
LC25	88.0	69.2 - 102.8
LC50	109.2	91.1 - 151.5
NOEC	75	-
LOEC	150	-

Table 10-2. Water quality data for the 96-hour copper test with 148-d old juvenile delta smelt. This test was initiated on 09/16/09.

Treatment	Temp (°C)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water (FHW) @ 900 uS/cm	16.6	0.2	4	767	9	2	913	6	2	8.1	2.4	4
75 ppb Cu^{2+}	16.7	0.3	4	757	4	2	904	6	2	8.5	2.1	4
150 ppb Cu^{2+}	16.7	0.4	4	766	17	2	911	4	2	8.6	1.9	4
300 ppb Cu^{2+}	16.5	1.0	4	754	16	2	917	3	2	9.0	1.3	4

Treatment	pH			Total Ammonia/um (mg/L)			Un-ionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water (FHW) @ 900 uS/cm	7.49	0.42	4	0.360	0.18	2	0.002	0.001	2
75 ppb Cu^{2+}	7.56	0.34	4	0.275	0.11	2	0.001	0.001	2
150 ppb Cu^{2+}	7.60	0.34	4	0.255	0.09	2	0.001	0.000	2
300 ppb Cu^{2+}	7.65	0.28	4	0.245	0.04	2	0.002	0.001	2

4.2 Tests with Larval Fathead Minnow

4.2.1 Ammonia/um and SRWTP Effluent Exposures

The fathead minnow tests met test acceptability criteria. No significant reduction in 7-d survival or 7-d growth was detected (Tables 11-1, 11-2).

Table 11-1. Percent survival of larval fathead minnow exposed for 7 d to NH_4Cl and diluted SRWTP effluent. Experiment III was initiated 6/11/09. DIEPAMH = de-ionized water amended to US EPA moderately hard standard.

Treatment	Survival (%)		Biomass (mg)	
	Mean	SE	Mean	SE
Sacramento River at Garcia Bend (SRGB)	97.5	2.5	0.634	0.014
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	97.5	2.5	0.696	0.027
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0	0.684	0.031
SRGB + 6.00 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0	0.670	0.009
SRGB + 8.00 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0	0.668	0.012
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0	0.644	0.021
SRGB + 4.00 mg/L NH_3/NH_4^+ from SRWTP	100.0	0.0	0.673	0.018
SRGB + 6.00 mg/L NH_3/NH_4^+ from SRWTP	100.0	0.0	0.664	0.017
SRGB + 8.00 mg/L NH_3/NH_4^+ from SRWTP	100.0	0.0	0.656	0.018
Low EC Control	97.5	2.5	0.582	0.020
DIEPAMH	97.5	2.5	0.652	0.026

Table 11-2. Water quality data for the 7-day test with fathead minnow larvae initiated 6/11/09.

Treatment	Temp (°C)			EC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Sac R Garcia Bend	24.2	0.3	14	139.4	7.2	7	7.5	0.9	14
2.00 mg/L NH3-N: NH4Cl	24.1	0.3	14	189.7	10.1	7	7.5	1.0	14
4.00 mg/L NH3-N: NH4Cl	24.1	0.3	14	244.7	14.4	7	7.4	1.1	14
6.00 mg/L NH3-N: NH4Cl	24.1	0.3	14	300.3	15.5	7	7.5	1.0	14
8.00 mg/L NH3-N: NH4Cl	24.2	0.2	14	357.4	19.3	7	7.4	0.9	14
2.00 mg/L NH3-N: SRWTP	24.1	0.3	14	191.9	7.2	7	7.4	1.1	14
4.00 mg/L NH3-N: SRWTP	24.2	0.3	14	250.3	11.5	7	7.4	1.1	14
6.00 mg/L NH3-N: SRWTP	24.1	0.2	14	307.9	12.5	7	7.3	1.2	14
8.00 mg/L NH3-N: SRWTP	24.2	0.2	14	359.8	17.8	7	7.4	1.2	14
Low EC Control: Dilute DIEPAMH	24.2	0.2	14	139.5	8.3	7	7.5	0.8	14
DIEPAMH	24.2	0.3	14	281.5	4.4	7	7.5	0.9	14

Treatment	pH			Total Ammonia/um (mg/L)			Un-ionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Sac R Garcia Bend	7.75	0.18	14	0.15	0.13	14	0.003	0.002	14
2.00 mg/L NH3-N: NH4Cl	7.71	0.17	14	2.07	0.14	14	0.054	0.020	14
4.00 mg/L NH3-N: NH4Cl	7.64	0.14	14	4.01	0.27	14	0.089	0.027	14
6.00 mg/L NH3-N: NH4Cl	7.59	0.11	14	6.07	0.27	14	0.118	0.030	14
8.00 mg/L NH3-N: NH4Cl	7.62	0.14	14	8.14	0.31	14	0.171	0.056	14
2.00 mg/L NH3-N: SRWTP	7.70	0.16	14	2.09	0.09	14	0.054	0.018	14
4.00 mg/L NH3-N: SRWTP	7.65	0.13	14	4.05	0.18	14	0.092	0.029	14
6.00 mg/L NH3-N: SRWTP	7.63	0.13	14	6.01	0.31	14	0.127	0.036	14
8.00 mg/L NH3-N: SRWTP	7.63	0.17	14	8.04	0.50	14	0.176	0.067	14
Low EC Control	7.67	0.41	14	0.15	0.16	14	0.002	0.001	14
DIEPAMH	7.85	0.30	14	0.16	0.14	14	0.004	0.002	14

5. Quality Assurance/Quality Control

All toxicity testing performed at UCD-ATL was supervised by the Project and Laboratory Managers to ensure quality and that testing was completed on schedule. The UCD-ATL Quality Assurance Officer has reviewed all work performed to date to assess its quality and credibility. The following is a summary of the QA/QC work completed during the 2009 project period.

5.1 Positive Control Tests with Delta Smelt

Positive control reference toxicant (RT) tests were conducted with delta smelt four times during the study period, using copper chloride ($CuCl_2$) as the toxicant, in order to track changes in organism sensitivity over time. There are currently no EPA-mandated requirements for reference toxicant testing with delta smelt; therefore test acceptability criteria were based upon historic survival in controls and were set at what was perceived to be a reasonably attainable level. Test acceptability criteria require 60% or greater control survival. These reference toxicant tests were not plotted on a control chart.

For this project, 96 h reference toxicant tests were conducted using the same batch of delta smelt used to perform the ammonia/um exposure experiments. Tests with copper chloride were initiated 24 h prior to the initiation of ammonia exposures due to the shorter period of time required to acclimate the fish from rearing water conductivity (~1500 µS/cm) to RT test conductivity (900 µS/cm). Due to the sensitive nature of the delta smelt, fish are not held in the laboratory longer than necessary to minimize stress. Reference toxicant tests consisted of a control and four concentrations of $CuCl_2$ (27, 56, 106, and 213 ppb) with larval delta smelt, and three concentrations of $CuCl_2$ (75, 150, and 300 ppb) with juvenile delta smelt. RT tests consisted of three replicates per concentration and five fish per replicate. Copper concentrations were based on the copper LC_{50} for delta smelt larvae determined in May 2008 at UCD-ATL and were increased for use with older fish. Concentration effect data are outlined below in Table 12.

Not all RT tests met test acceptability criteria. For the RT test conducted on June 24, 2009, average control survival was 53%. Of the three replicates in the control, all organisms died in one replicate. Survival in the remaining two replicates was 80%; averaging the three replicates brought the overall survival of the treatment to 53%. In the corresponding delta smelt ammonia/um exposure, average control survival was 67%, which met test acceptability criteria. As the same batch of fish were utilized for both the ammonia/um and RT tests, the differences in control survival are potentially due to contamination present in one of the replicate exposure containers.

The LC_{50} concentrations derived from these tests show that larval delta smelt used in testing earlier in the year were less sensitive to copper than fish used in July 2009. Despite the much higher tolerance of juvenile fish for ammonia/um, these fish were relatively similarly sensitive to copper as larval delta smelt.

Table 12. Copper effect concentrations derived from reference toxicant tests with larval and juvenile delta smelt used in testing during 2009.

RT Test	Smelt Age (days old)	Control Survival (%)	NOEC (μg/L)	LOEC (μg/L)	LC50 (μg/L)
Jun. 10, 2009	46	93	106	213	150.3 (106.6-183.8)*
Jun. 24, 2009[a]	51	53[1]	213	>213	133.0 (91.15-194.1)*
Jul. 8, 2009	47	93	53	106	84.97 (44.33-142.2)*
Sept. 16, 2009	148	100	75	150	111.9 (95.51-131.0)*

* 95% confidence interval

[a] This test did not meet test acceptability criteria, and was excluded from laboratory RT control charts. It is included in this table for comparison.

5.2 Positive Control Tests with Fathead Minnow

Monthly positive control RT tests were conducted with *P. promelas*, using sodium chloride (NaCl) as the toxicant, in order to ascertain whether organism response fell within the acceptable range as dictated by US EPA. For this project's study period, fathead minnows performed normally within each reference toxicant test. These data suggest that the organisms' response fell within the acceptable range of plus or minus two standard deviations around a running mean and are responding typically within that range.

6. Discussion and Conclusions

Results from this project provide information on the acute toxicity of ammonium-chloride to delta smelt at different life stages, and of SRWTP effluent to larval delta smelt. These test results need to be interpreted with caution and should not be used as a quantitative indicator of ecological health, but as one line of evidence because of obvious limitations with regard to test design and exposure duration, the relative sensitivity of different life-stages and the potential for chronic, sublethal or indirect effects. Below we discuss our results in the context of the hypotheses on which the experimental design for the tests performed in 2009 was based, address uncertainties, and provide recommendations for future studies.

<u>Hypothesis 1</u>: Larval delta smelt survival is negatively impacted by one or more contaminant(s) that are positively correlated with ammonia/um from SRWTP.

We are now able to address this hypothesis, because Experiment III met test acceptability criteria with respect to control survival, the reference toxicant tests suggest that test animals were robust, and there were no differences in EC, pH and un-ionized ammonia between effluent and corresponding NH_4Cl treatments. Therefore, the survival results can be directly compared between effluent and NH_4Cl treatments.

The highest average test concentrations in ammonium chloride and effluent treatments were 7.71±0.69 and 7.78±0.68 mg/L total ammonia/um (measured; Table 3-3). Threshold concentrations for acute toxicity to larval delta smelt were 7.45 mg/L and 5.40 mg/L ammonia/um (7-d LC50) for ammonium chloride and effluent, respectively. This demonstrated that toxicity of effluent is higher than would be expected based on ammonia/um content alone, and that additional contaminants increase effluent toxicity to delta smelt. The 7-d LOEC for ammonium chloride and effluent were 5.66 mg/L and 1.96 mg/L ammonia/um, respectively. The LOEC corresponds to 18.3% effluent in Sacramento River water. In concurrent tests with larval fathead minnow, neither ammonium-chloride nor effluent affected fish survival or growth, demonstrating that delta smelt are more sensitive to these contaminants than standard test species.

In comparison, ambient concentrations in the Sacramento River downstream of the SRWTP discharge are on average below 1 mg/L ammonia/um. For 2007-2008, SRWTP reports average weekly ammonia/um concentrations of 0.6 ± 0.3 mg/L, and ammonia concentrations of 0.004 ± 0.002 mg/L (SRWTP, unpublished data). Biweekly total ammonia/um measurements obtained by UCD-ATL for the period 2006-2008 show mean ammonia/um concentrations at Hood and Grand Island of 0.35 ± 0.15 mg/L and 0.27 ± 0.14 mg/L, respectively, with maximum concentrations of 0.59 mg/L and 0.58 mg/L, respectively. Corresponding mean un-ionized ammonia concentrations at Hood and Grand Island were 0.003 ± 0.004 mg/L and 0.004 ± 0.004 mg/L, with maximum concentrations of 0.018 mg/L and 0.021 mg/L (UCD-ATL, unpublished data). During the experimental period, Sacramento River water upstream of SRWTP at Garcia Bend contained ammonia/um at concentrations of ≤0.07 mg/L. Based on test results obtained in this study, we conclude that average as well as maximum ammonia/um and ammonia concentrations reported for the Sacramento River below SRWTP are not likely to affect 7-d survival of 47-d old delta smelt larvae.

<u>Hypothesis 2:</u> Toxic effect thresholds for ammonia/um are life-stage dependent.

Results of LC50 tests with larval and juvenile delta smelt showed that juvenile fish are significantly less sensitive to ammonia/um than larval fish. The 96-h LC50s for total ammonia/um were 52.3 mg/L for juvenile (149 DPH), and 11.63 (51 DPH) and 11.81 (47 DPH) mg/L for larval delta smelt. This appears to contradict findings by Thurston and Russo (1983) who demonstrated that large rainbow trout were measurably more sensitive than younger life stages. The data obtained for larval fish are consistent with effect concentrations previously established for 50-d old delta smelt (12.0 mg/L) at UCD-ATL using filtered hatchery water as well as acute effect concentrations for other fish species reported in the peer-reviewed literature.

Delta smelt larvae at the age of 47-51 DPH are more sensitive to ammonia/um than larval fathead minnow (Werner et al., 2009), and about as sensitive as salmonid species, which are considered the most sensitive fish species with species mean acute values of 11.23, 17.34 and 20.26 mg/L ammonia/um (at pH 8.0) for rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*O. tshawytscha*) and Coho salmon (*O. kisutch*) (US EPA, 1999). For un-ionized ammonia, Eddy (2005) reports toxic concentrations (96-h LC50) to freshwater fish in the range 0.068–2.0 mg/L and for marine species in the range 0.09–3.35 mg/L, while the 7-d LOEC for larval delta smelt were 0.116 (NH_4Cl) and 0.081 (SRWTP effluent) mg/L ammonia.

Fate and transport of SRWTP effluent, as well as seasonal variation of environmental conditions likely affect concentrations and potential toxicity of ammonia/um discharged into the Sacramento River. During the 2009 experimental period, SRWTP discharged treated effluent containing ammonia/um at an average concentration of 26.9 mg/L (2007-2008) approximately 30 miles upstream of important spawning and nursery areas for delta smelt and other pelagic fish species. While the pH of river water at Hood is relatively low (7.0-7.6; Werner et al. 2008), it can reach 8.3 about 30 miles downstream at Grand Island with water temperatures ranging from 6.1-25^{o}C (Werner et al. 2008). Reported long-term average ammonia/um concentrations downstream of the point of discharge (0.6 mg/L) are below pH- and temperature-dependent US EPA chronic water quality criteria for water bodies where early life stages of fish are present (US EPA 1999; Tables 13 a, b), and 6.6 and 3.3-fold lower than the effluent LOEC (3.92 mg/L) and NOEC (1.96 mg/L), respectively, for 7-d survival of larval delta smelt determined in this study. The US EPA acute criterion is approximately twice as high as the effluent LOEC of larval delta smelt. For effluents where ammonia/um is present as part of a contaminant mixture the US EPA acute criterion therefore may not be protective of this species.

Table 13 a. US EPA Acute and Chronic Criteria for total ammonia/um for salmonids and fish early life stages present (USEPA, 1999), and acute NOEC/LOEC values (7-d survival) of 47-d old delta smelt for water quality conditions of Experiment III.

T (^{o}C)	pH	EC (µS/cm)	Acute Criterion	30-d Chronic Criterion	NOEC/LOEC Effluent
16.0-18.0	7.8	221	8.11 mg/L	2.89-2.54 mg/L	1.96/3.92 mg/L

Table 13 b. US EPA Acute and Chronic Criteria for un-ionized ammonia equivalents for salmonids and fish early life stages present (USEPA, 1999), and acute NOEC/LOEC values (7-d survival) of 47-d old delta smelt for water quality conditions of Experiment III.

T (^{o}C)	pH	EC (µS/cm)	Acute Criterion	30-d Chronic Criterion	NOEC/LOEC Effluent
16.0-18.0	7.8	221	0.138-160 mg/L	0.049-0.050 mg/L	0.039/0.081 mg/L

Exposure duration is an important factor influencing the toxicity of ammonia. Seven-day toxicity tests, as performed in this study, are unable to detect the potential chronic effects of ammonia/um exposure on delta smelt. Acute-to chronic ratios (ACR) are one method that has traditionally been used to extrapolate between acute and chronic toxicity when procedures for chronic testing are not available. For fish, the US EPA (1999) reports mean acute-to-chronic ammonia/um ratios for warm water fish that range between 2.7 (channel catfish, *Ictalurus punctatus*) and 10.9 (fathead minnow, *P. promelas*). Cold water species such as rainbow trout, with acute ammonia/um sensitivity similar to delta smelt, have a ratio between 14.6 and 23.5, respectively (US EPA, 1999; Passell et al., 2007). If these safety factors were applied to acute effect concentrations for effluent and delta smelt larvae (7-d LC_{50}: 3.92 mg/L) then the resulting threshold concentrations for total ammonia/um would be 0.27 and 0.17 mg/L for the above safety ratios of 14.6 and 23.6, respectively. Using a more conservative approach, with an ACR of 12.4 as applied by TenBrook et al. (2009), results in a threshold value of 0.32 mg/L. These chronic effect thresholds are below long-term average concentrations in the Sacramento River below SRWTP.

In conclusion, our study showed that ammonia/um at levels reported for the Sacramento River below SRWTP are not acutely toxic to larval (47-51 DPH) and juvenile (149 DPH) delta smelt. However, toxicity of SRWTP effluent was higher than would be expected based on ammonia/um content alone, and we conclude that additional contaminants increase effluent toxicity to larval delta smelt. Chronic endpoints were not tested in this study, but based on information available from US EPA (1999) and other related literature we conclude that ammonia/um concentrations detected in the Sacramento River below the SRWTP are of concern with respect to chronic toxicity to delta smelt and other sensitive species.

7. Uncertainties and Recommendations for Future Studies

Significant uncertainties remain with respect to the potential for deleterious effects of ammonia/um and SRWTP effluent in the Sacramento-San Joaquin Delta:

(1) <u>Effects of multiple stressors</u>. Many environmental factors can modify the toxicity of a single contaminant such as ammonia/um. Pre-exposure or simultaneous exposure to multiple contaminants, disease, or other stressful environmental conditions may considerably alter the physiological condition and therefore susceptibility of the organism, as well as modify the toxicity of ammonia. For example, parasitism increased ammonia susceptibility of amphipods (Prenter et al., 2004) five-fold.

(2) <u>Effects of contaminant mixtures</u>. - Our experiment showed that unknown effluent-associated contaminants caused acute toxicity to larval delta smelt, albeit at concentrations above those that occur in the Sacramento River. Contaminants in the Delta occur dominantly as complex mixtures and come from a variety of sources. The toxicity of contaminant mixtures may be significantly different than that of individual chemicals. For example, a study on the effects of wastewater treatment effluent on silvery minnow in the Rio Grande, found that copper and un-ionized ammonia were the primary toxic

components in the mixture, with copper contributing 49–62% and ammonia contributing 36–50% of the mixture's toxicity (Buhl 2002). A mixture of five toxicants, aluminum, ammonia, arsenic, copper, and nitrate, produced a toxicity that was more toxic than any of the five chemicals tested alone. Based on their results, Buhl (2002) estimated an appropriate chronic criterion for silvery minnow, a species similar in sensitivity to the fathead minnow, in the Rio Grande could be as low as 0.001 mg/L ammonia. For the lower Sacramento River, the effects of contaminant mixtures with and without multiple stressors present (e.g. temperature, pathogens, food availability), and their influence on the susceptibility of fish species of concern are little understood.

(3) Sublethal toxic effects. - Sublethal toxic effects can occur at exposure levels far below the concentrations that cause lethality, and can have severe consequences for the fitness, reproductive success and survival of aquatic organisms, especially where organisms are exposed to many different stressors. Exposure of fish to sublethal concentrations of ammonia/um can cause loss of equilibrium, hyperexcitability, increased respiratory activity and oxygen uptake, and increased heart rate. Increased ammonia/um levels in the water have been shown to result in impairment of swimming performance, reduced feeding and slower growth (Eddy, 2005 and references therein). For example, in rainbow trout and coho salmon there was a decrease in critical swimming velocity with increasing water ammonia levels, and the LC50 in resting fish was 6.5-fold higher than that in swimming fish. Exposure to ammonia concentrations as low as 0.002 mg/L for six weeks caused hyperplasia of gill lining in salmon fingerlings (Eddy, 2005).

Recommendations for Future Research

- Information should be generated on the susceptibility of early larval stages of delta smelt (<47 DPH) to ammonia/um.
- To obtain information on the variability of effluent toxicity Experiment III should be repeated.
- Acute-to-chronic ratios should be established using sublethal endpoints such as swimming ability and histopathologic lesions.
- More detailed information is needed with respect to river conditions, in particular pH and temperature, during times when delta smelt are spawning and larval delta smelt are found in the Cache Slough, Deep Water Shipping Channel and Lower Sacramento River in order to assess the risk of ammonia/um toxicity to POD species spawning in these areas.
- Source analysis: Information on sources of ammonia/um (agricultural, residential, atmospheric) in the Delta, in particular in the vicinity of important fish habitat should be generated.
- Information on toxic effects of ammonia/um and SRWTP effluent at lower trophic levels needs to be integrated and possibly generated to assess potential effects of reduced food availability on fish species of concern.

- Sources and concentrations of ammonia determined from characterizing spatial and temporal trends should be used to develop a fate and transport model for ammonia/um (see Passell et al., 2007).
- More information is needed on the toxicity of ammonia/um when other stressors are present, in particular under conditions of food deprivation, and in mixture with other contaminants of concern in the Delta such as copper and pesticides.
- Every attempt should be made to use ecologically significant, sublethal toxicity endpoints, such as growth, reproductive success, and swimming ability to evaluate the effects of ammonia/um on Delta fish species.
- Biomarkers (histopathologic, biochemical, molecular) can provide important information on biologically active toxicants present at extremely low concentrations or as mixtures, and therefore difficult to detect by analytical chemistry. Well characterized biomarkers should be integrated into monitoring efforts, especially where other sublethal endpoints (growth, behavior) are difficult to obtain.
- Where possible, *in situ* methods should be used to monitor ambient toxicity.

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Appendix

The Effects of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt

Water Quality Data and Results of NPDES Testing

A. Water Quality Data

Table A1. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: Sacramento River at Garcia Bend.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	157.4	187.1	16.6	9.6	7.67	0.025	0.000	8.44	36	66
4	Day 1 9AM Final	293.4		16.9	9.8	7.97	0.02	0.001	8.24		
21	Day 1 4PM Final	294.5		17.1	9.4	8.00	0.09	0.003	4.53		
24	Day 1 Initial	116.8	140.0	16.8	9.6	7.80	0.035	0.001	7.93	56	62
28	Day 2 9AM Final	289.1		17.0	9.2	7.88	0.15	0.003	4.2		
45	Day 2 4PM Final	218.1		16.8	9.6	8.01	0.09	0.003	3.26		
48	Day 2 Initial	107.4	135.7	17.5	9.5	7.79	0.015	0.000	7.76	52	57
52	Day 3 9AM Final	220.6		17.0	9.4	7.98	0.16	0.004	3.02		
69	Day 3 4PM Final	185.7		16.9	9.6	8.02	0.14	0.004	3.03		
72	Day 3 Initial	123.6	145.3	17.2	9.8	7.79	0.05	0.001	11.6	52	60
76	Day 4 9AM Final	180.8		16.6	9.9	7.99	0.10	0.003	2.45		
93	Day 4 4PM Final	170.2		17.2	9.7	7.98	0.16	0.005	3.02		
96	Day 4 Initial	110.7	131.4	16.8	9.7	7.80	0.01	0.000	11.1	52	56
100	Day 5 9AM Final	166.3		16.9	9.8	7.94	0.16	0.004	2.45		
117	Day 5 4PM Final	146.1		16.8	9.6	7.87	0.15	0.003	3.68		
120	Day 5 Initial	123.1	144.3	16.5	9.7	7.87	0.025	0.001	11.3	56	58
124	Day 6 9AM Final	143.8		16.8	9.5	7.94	0.08	0.002	3.36		
141	Day 6 4PM Final	148.6		16.7	9.7	7.87	0.15	0.003	3.91		
144	Day 6 Initial	125.9	147.1	17.2	9.3	7.81	0.06	0.001	26.3	56	62
148	Day 7 9AM Final	146.2		17.2	9.1	7.85	0.15	0.003	3.1		
165	Day 7 1PM Final	151.4		17.0	9.4	7.78	0.11	0.002	7.14		

Table A2. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: Low Conductivity (EC) Control.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	164.5	196.3	16.3	8.3	8.16	0.07	0.003	3.22	36	28
14	Day 1 9AM Final	278.5		16.9	9.3	7.98	0.05	0.001	8.01		
21	Day 1 4PM Final	279.6		17.0	9.5	7.72	0.13	0.002	7.14		
24	Day 1 Initial	120.0	142.9	16.8	8.9	7.02	0.07	0.000	5.05	24	12
38	Day 2 9AM Final	282.3		17.1	9.0	7.65	0.14	0.002	6.64		
45	Day 2 4PM Final	214.2		16.7	9.5	7.52	0.10	0.001	5.43		
48	Day 2 Initial	120.7	141.4	17.3	10.7	8.16	0.01	0.000	9.16	36	26
62	Day 3 9AM Final	217.3		17.0	9.2	7.47	0.19	0.002	4.78		
69	Day 3 4PM Final	188.5		16.8	9.4	7.42	0.21	0.002	5.82		
72	Day 3 Initial	124.7	148.1	16.7	10.2	8.19	0.06	0.003	6.54	40	32
86	Day 4 9AM Final	187.9		16.6	9.5	7.51	0.17	0.002	5.22		
93	Day 4 4PM Final	173.1		16.9	9.4	7.52	0.24	0.002	4.68		
96	Day 4 Initial	116.2	140.9	16.0	9.1	8.19	0.02	0.001	7.09	36	30
110	Day 5 9AM Final	174.8		16.9	9.7	7.56	0.30	0.003	4.11		
117	Day 5 4PM Final	160.5		16.5	9.0	7.68	0.30	0.004	6.11		
120	Day 5 Initial	133.3	158.5	16.7	9.0	8.10	0.03	0.001	8.01	48	32
134	Day 6 9AM Final	158.4		16.7	9.0	7.55	0.33	0.003	5.85		
141	Day 6 4PM Final	167.4		16.8	9.0	7.53	0.38	0.004	5.89		
144	Day 6 Initial	132.2	158.9	16.2	9.0	8.21	0.00	0.000	9.49	44	24
158	Day 7 9AM Final	157.3		17.0	8.7	7.52	0.35	0.003	6.09		
162	Day 7 1PM Final	166.2		16.8	9.0	7.42	0.34	0.003	6.68		

Table A3. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: Hatchery Water Control.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	2161	2611	16.1	9.5	8.15	0.11	0.004	4.41	324	84
14	Day 1 9AM Final	2205		16.8	9.7	8.07	0.08	0.002	10.1		
21	Day 1 4PM Final	2170		16.7	9.0	7.82	0.14	0.003	7.51		
24	Day 1 Initial	2087	2564	16.2	9.0	7.93	0.11	0.002	7.54	324	84
38	Day 2 9AM Final	2185		17.4	9.0	7.90	0.20	0.004	7.86		
45	Day 2 4PM Final	2161		16.8	9.4	7.81	0.14	0.002	6.54		
48	Day 2 Initial	2195	2559	17.5	8.6	7.96	0.02	0.000	13.53	160	78
62	Day 3 9AM Final	2159		16.8	9.4	7.85	0.21	0.004	6.41		
69	Day 3 4PM Final	2171		17.0	9.4	7.82	0.21	0.004	6.33		
72	Day 3 Initial	2150	2548	16.8	9.2	8.01	0.06	0.002	5.78	160	78
86	Day 4 9AM Final	2117		16.6	9.5	7.92	0.20	0.004	5.24		
93	Day 4 4PM Final	2154		16.8	9.2	7.77	0.24	0.004	4.33		
96	Day 4 Initial	2148	2559	16.4	9.0	8.02	0.06	0.002	7.72	160	78
110	Day 5 9AM Final	2151		16.7	9.8	7.78	0.30	0.005	3.65		
117	Day 5 4PM Final	2232		16.7	9.1	7.84	0.34	0.006	5.64		
120	Day 5 Initial	2149	2557	16.8	8.8	8.03	0.06	0.002	5.44	320	84
134	Day 6 9AM Final	2145		16.7	9.1	7.81	0.38	0.006	5.17		
141	Day 6 4PM Final	2221		16.7	9.2	7.77	0.48	0.007	5.25		
144	Day 6 Initial	2153	2602	16.0	8.9	8.06	0.04	0.001	5.32	332	88
158	Day 7 9AM Final	2175		16.9	8.8	7.87	0.40	0.008	5.07		
162	Day 7 1PM Final	2176		17.1	9.0	7.67	0.37	0.005	4.56		

Table A4. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: Hatchery Water Control without antibiotics.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	2158	2591	16.3	9.4	8.16	0.11	0.004	4.35	324	84
14	Day 1 9AM Final	2188		16.8	9.3	8.06	0.08	0.002	9.04		
21	Day 1 4PM Final	2092		16.8	9.3	7.81	0.12	0.002	6.61		
24	Day 1 Initial	2018	2470	16.1	9.7	7.96	0.11	0.002	6.98	324	84
38	Day 2 9AM Final	2166		17.3	8.4	7.79	0.15	0.002	6.38		
45	Day 2 4PM Final	2154		16.8	9.0	7.77	0.14	0.002	5.57		
48	Day 2 Initial	2184	2574	17.5	8.5	7.97	0.02	0.000	8.19	160	78
62	Day 3 9AM Final	2124		17.0	8.8	7.79	0.21	0.003	5.23		
69	Day 3 4PM Final	2182		17.1	8.9	7.75	0.19	0.003	5.80		
72	Day 3 Initial	2139	2532	16.9	9.1	8.03	0.07	0.002	7.14	160	78
86	Day 4 9AM Final	2154		16.7	9.0	7.75	0.27	0.004	4.73		
93	Day 4 4PM Final	2148		16.7	8.6	7.58	0.37	0.004	4.84		
96	Day 4 Initial	2098	2499	16.0	8.9	8.01	0.06	0.001	7.20	160	78
110	Day 5 9AM Final	2200		16.7	9.7	7.63	0.42	0.005	3.95		
117	Day 5 4PM Final	2211		16.6	8.5	7.74	0.37	0.005	5.38		
120	Day 5 Initial	2162	2579	16.5	8.7	8.08	0.06	0.002	6.43	320	84
134	Day 6 9AM Final	2147		16.7	8.6	7.68	0.40	0.005	4.77		
141	Day 6 4PM Final	2200		16.7	8.4	7.65	0.47	0.005	4.90		
144	Day 6 Initial	2121	2557	16.0	8.9	8.10	0.04	0.001	13.1	332	88
158	Day 7 9AM Final	2155		17.1	8.2	7.66	0.41	0.005	4.35		
162	Day 7 1PM Final	2171		16.9	8.4	7.45	0.40	0.003	4.96		

Table A5. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 2.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	203.3	242.3	16.5	9.8	7.86	1.87	0.038	6.99	56	66
14	Day 1 9AM Final	332.1		16.8	9.7	7.88	1.41	0.030	8.03		
21	Day 1 4PM Final	339.2		17.0	9.4	7.93	1.31	0.032	4.65		
24	Day 1 Initial	165.9	193.3	17.2	9.5	7.75	2.04	0.034	6.57	56	58
38	Day 2 9AM Final	336.1		17.3	9.0	7.82	1.50	0.029	4.48		
45	Day 2 4PM Final	275.3		16.7	9.6	7.89	1.78	0.039	3.3		
48	Day 2 Initial	153.6	181	17.0	9.8	7.65	2.10	0.028	7.4	52	60
62	Day 3 9AM Final	267.2		17.1	9.3	7.92	1.96	0.047	3.16		
69	Day 3 4PM Final	239.1		16.8	9.7	7.92	1.92	0.046	2.97		
72	Day 3 Initial	172.9	204.2	17.2	9.8	7.91	2.15	0.052	8.86	56	62
86	Day 4 9AM Final	236.9		16.8	9.6	7.88	1.98	0.043	2.39		
93	Day 4 4PM Final	218.3		17.1	9.3	7.83	2.03	0.040	3.1		
96	Day 4 Initial	152.3	180.2	16.8	9.7	7.81	2.08	0.039	9.06	56	58
110	Day 5 9AM Final	223.7		17.0	9.8	7.80	1.92	0.035	2.13		
117	Day 5 4PM Final	196.3		16.9	9.4	7.90	2.03	0.047	3.68		
120	Day 5 Initial	163.3	194.6	16.5	9.7	7.86	2.12	0.043	9.85	60	60
134	Day 6 9AM Final	191.3		17.0	9.3	7.86	2.01	0.043	3.61		
141	Day 6 4PM Final	196.6		16.9	9.3	7.76	1.99	0.033	3.43		
144	Day 6 Initial	174.5	204.5	17.3	9.4	7.83	1.96	0.040	23.8	60	58
158	Day 7 9AM Final	194.9		17.5	8.9	7.71	2.02	0.032	3.2		
162	Day 7 1PM Final	199.7		17.0	9.3	7.68	1.65	0.023	6.95		

Table A6. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 4.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	257.9	305.7	16.7	9.7	7.83	4.02	0.077	7.56	60	60
14	Day 1 9AM Final	372.2		16.9	9.8	7.87	3.04	0.064	7.89		
21	Day 1 4PM Final	372.1		16.9	9.6	7.94	2.86	0.070	4.55		
24	Day 1 Initial	215.5	254.8	16.9	9.6	7.77	4.00	0.068	6.14	56	62
38	Day 2 9AM Final	371.3		17.2	9.1	7.92	4.32	0.104	4.22		
45	Day 2 4PM Final	309.3		16.9	9.7	7.84	3.44	0.068	3.37		
48	Day 2 Initial	198.4	233.5	17.1	9.8	7.65	3.9	0.052	7.73	56	58
62	Day 3 9AM Final	306.8		17.1	9.4	7.91	3.8	0.089	3.03		
69	Day 3 4PM Final	273.2		16.7	9.9	7.89	3.66	0.080	2.97		
72	Day 3 Initial	216.8	257.6	16.7	9.9	7.56	4.22	0.044	9.73	64	58
86	Day 4 9AM Final	271.7		16.7	9.8	7.91	3.94	0.090	2.54		
93	Day 4 4PM Final	262.0		16.9	9.7	7.78	3.98	0.069	2.87		
96	Day 4 Initial	198.9	235.2	16.9	9.4	7.62	4.06	0.049	9.23	60	56
110	Day 5 9AM Final	266.8		16.9	9.7	7.85	3.92	0.080	2.28		
117	Day 5 4PM Final	239.3		16.8	9.6	7.92	3.86	0.092	3.66		
120	Day 5 Initial	213.5	252.3	16.7	9.8	7.66	4.30	0.056	9.03	60	58
134	Day 6 9AM Final	231.7		16.8	9.5	7.91	4.00	0.093	3.46		
141	Day 6 4PM Final	240.4		16.9	9.7	7.77	3.98	0.068	3.91		
144	Day 6 Initial	233.8	272.6	17.5	9.4	7.57	4.00	0.045	25.4	68	70
158	Day 7 9AM Final	240.9		17.3	9.1	7.76	4.02	0.069	3.45		
162	Day 7 1PM Final	250.8		17.0	9.5	7.75	3.32	0.054	6.84		

Table A7. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 6.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	303.7	359.9	16.7	9.8	7.76	6.32	0.103	7.69	68	60
14	Day 1 9AM Final	416.2		16.9	9.7	7.80	4.68	0.084	8.06		
21	Day 1 4PM Final	415.4		16.9	9.8	7.85	4.35	0.087	4.43		
24	Day 1 Initial	265.4	316.3	17.5	9.6	7.68	5.84	0.084	5.95	64	60
38	Day 2 9AM Final	414.6		17.1	9.2	7.86	4.72	0.085	4.21		
45	Day 2 4PM Final	349.5		17.0	9.7	7.83	4.68	0.091	3.74		
48	Day 2 Initial	245.4	288	17.2	9.8	7.68	6.32	0.090	7.49	60	58
62	Day 3 9AM Final	348.2		17.0	9.3	7.85	5.12	0.104	3.14		
69	Day 3 4PM Final	362.5		16.8	9.8	7.77	5.72	0.095	3.06		
72	Day 3 Initial	264.0	313.8	16.8	10.0	7.51	5.92	0.055	9.78	60	58
86	Day 4 9AM Final	315.8		16.7	9.9	7.89	5.64	0.123	2.56		
93	Day 4 4PM Final	303.4		16.9	9.7	7.76	6.24	0.103	3.11		
96	Day 4 Initial	260.1	307	16.9	10.0	7.42	6.04	0.046	9.67	64	56
110	Day 5 9AM Final	312.1		17.0	9.7	7.81	5.64	0.105	2.46		
117	Day 5 4PM Final	295.0		16.8	9.5	7.88	5.80	0.125	3.77		
120	Day 5 Initial	253.6	299.5	16.9	9.8	7.59	6.12	0.069	9.16	68	56
134	Day 6 9AM Final	286.5		16.9	9.4	7.86	5.80	0.120	3.46		
141	Day 6 4PM Final	293.8		16.9	9.6	7.75	6.32	0.102	3.86		
144	Day 6 Initial	279.9	326.7	17.4	9.4	7.42	6.40	0.051	23.6	60	60
158	Day 7 9AM Final	290.2		17.3	8.9	7.74	5.96	0.097	3.49		
162	Day 7 1PM Final	294.7		17.0	9.3	7.73	5.32	0.083	7.08		

Table A8. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 8.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	343.9	407.6	16.8	9.9	7.70	8.2	0.117	7.46	68	61
14	Day 1 9AM Final	439.2		16.9	9.8	7.78	6.16	0.105	8.19		
21	Day 1 4PM Final	340.0		17.0	9.3	7.84	6.28	0.125	5.14		
24	Day 1 Initial	341.5	394.6	17.5	9.5	7.45	8.6	0.073	5.92	68	60
38	Day 2 9AM Final	427.1		17.1	9.1	7.82	6.68	0.127	4.22		
45	Day 2 4PM Final	399.9		16.7	9.7	7.82	7.36	0.136	3.47		
48	Day 2 Initial	292.4	342.3	17.3	9.8	7.47	8.28	0.073	6.66	64	58
62	Day 3 9AM Final	399.5		17.1	9.3	7.85	7.8	0.159	3.14		
69	Day 3 4PM Final	238.3		16.9	9.8	7.97	6.96	0.186	3.09		
72	Day 3 Initial	315.6	374.5	16.7	10.0	7.52	7.88	0.074	9.95	72	56
86	Day 4 9AM Final	360.2		16.8	9.8	7.87	7.64	0.160	2.70		
93	Day 4 4PM Final	356.2		16.9	9.6	7.79	8.16	0.143	3.29		
96	Day 4 Initial	307.5	364.2	17.0	10.1	7.29	8.08	0.046	9.76	60	56
110	Day 5 9AM Final	358.8		17.0	9.8	7.77	7.8	0.132	2.71		
117	Day 5 4PM Final	345.9		16.8	9.4	7.76	8.04	0.131	3.70		
120	Day 5 Initial	304.0	357.6	17.0	9.7	7.54	8.0	0.081	8.70	68	56
134	Day 6 9AM Final	334.5		17.0	9.3	7.79	7.64	0.136	3.39		
141	Day 6 4PM Final	343.3		16.9	9.6	7.69	8.44	0.118	3.49		
144	Day 6 Initial	331.7	388.6	17.4	9.4	7.47	8.6	0.076	23.3	68	78
158	Day 7 9AM Final	335.5		17.5	9.0	7.77	7.96	0.140	3.32		
162	Day 7 1PM Final	344.1		16.8	9.4	7.72	7.4	0.110	6.63		

Table A9. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 2.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	170.8	202.9	16.7	9.9	7.72	2.13	0.032	8.28	60	73
14	Day 1 9AM Final	336.7		16.9	9.7	7.94	1.60	0.040	7.38		
21	Day 1 4PM Final	339.5		17.0	9.3	7.92	1.59	0.038	4.37		
24	Day 1 Initial	166.8	195.0	17.5	9.9	7.58	2.12	0.025	6.31	64	72
38	Day 2 9AM Final	333.2		17.1	9.0	7.81	1.68	0.031	5.05		
45	Day 2 4PM Final	274.3		16.5	9.7	7.84	1.87	0.036	3.52		
48	Day 2 Initial	156.5	183.8	17.2	9.7	7.64	2.03	0.027	7.83	60	64
62	Day 3 9AM Final	274.7		17.1	9.2	7.97	1.94	0.052	3.37		
69	Day 3 4PM Final	233.3		17.1	9.5	7.85	1.95	0.040	3.00		
72	Day 3 Initial	173.4	203.2	17.2	9.9	7.80	2.14	0.040	9.66	64	68
86	Day 4 9AM Final	231.2		16.7	9.7	7.92	2.04	0.048	2.67		
93	Day 4 4PM Final	215.9		16.9	9.5	7.81	2.06	0.039	3.08		
96	Day 4 Initial	150.4	181.2	16.2	9.9	7.68	2.08	0.028	9.77	52	66
110	Day 5 9AM Final	223.0		17.0	9.7	7.96	1.94	0.051	2.31		
117	Day 5 4PM Final	196.2		16.8	9.6	7.99	2.03	0.057	3.76		
120	Day 5 Initial	161.7	194.4	16.1	9.9	7.72	2.13	0.031	9.02	64	68
134	Day 6 9AM Final	188.4		16.8	9.5	8.00	1.97	0.056	3.23		
141	Day 6 4PM Final	197.6		16.9	9.7	7.89	1.99	0.045	3.55		
144	Day 6 Initial	176.8	207.2	17.4	9.3	7.67	1.94	0.028	23.3	64	74
158	Day 7 9AM Final	195.1		17.3	9.0	7.85	1.97	0.042	3.18		
162	Day 7 1PM Final	198.8		16.8	9.5	7.88	1.86	0.041	6.54		

Table A10. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 4.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	221.3	264.2	16.3	10.1	7.53	4.28	0.040	7.74	68	82
14	Day 1 9AM Final	371.0		16.9	9.8	7.84	3.50	0.069	7.17		
21	Day 1 4PM Final	370.6		16.9	9.6	7.87	3.34	0.070	4.53		
24	Day 1 Initial	204.8	235.5	17.4	9.8	7.48	4.20	0.039	6.52	64	86
38	Day 2 9AM Final	383.2		17.0	9.1	7.98	3.96	0.107	4.10		
45	Day 2 4PM Final	313.8		16.6	9.7	7.95	3.76	0.093	3.46		
48	Day 2 Initial	205.4	241.3	17.3	9.8	7.50	4.00	0.038	7.91	64	78
62	Day 3 9AM Final	303.1		17.0	9.5	8.02	4.04	0.121	3.34		
69	Day 3 4PM Final	277.5		16.9	9.9	7.99	3.78	0.105	3.43		
72	Day 3 Initial	224.7	263.6	17.2	10.0	7.63	4.14	0.053	9.24	68	78
86	Day 4 9AM Final	274.3		16.7	9.9	8.05	3.90	0.122	2.69		
93	Day 4 4PM Final	261.8		16.8	9.7	7.98	4.02	0.109	2.98		
96	Day 4 Initial	195.4	235.8	16.0	10.2	7.48	4.00	0.033	9.34	68	76
110	Day 5 9AM Final	268.0		17.0	9.9	7.99	3.94	0.110	2.46		
117	Day 5 4PM Final	243.2		16.7	9.6	7.99	3.94	0.108	3.74		
120	Day 5 Initial	209.6	251.0	16.4	10.0	7.58	4.22	0.045	8.39	68	76
134	Day 6 9AM Final	234.9		16.9	9.5	8.04	3.82	0.119	3.22		
141	Day 6 4PM Final	243.6		16.9	9.7	7.90	3.82	0.087	3.56		
144	Day 6 Initial	230.4	270.7	17.3	9.4	7.52	3.86	0.038	22	68	86
158	Day 7 9AM Final	239.4		17.2	9.2	7.95	3.86	0.101	3.31		
162	Day 7 1PM Final	248.1		16.8	9.5	7.93	3.96	0.096	6.24		

Table A11. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 6.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	265.3	317.2	16.1	10.1	7.47	6.36	0.051	7.29	80	94
14	Day 1 9AM Final	418.4		16.9	9.7	7.68	5.04	0.069	7.02		
21	Day 1 4PM Final	416.6		17.0	9.6	7.85	4.86	0.098	4.56		
24	Day 1 Initial	269.1	316.5	16.7	10.0	7.35	5.96	0.038	6.13	84	94
38	Day 2 9AM Final	426.9		17.1	9.1	7.93	5.28	0.128	4.67		
45	Day 2 4PM Final	363		16.6	9.6	7.83	5.64	0.106	3.89		
48	Day 2 Initial	250.4	293.8	17.2	9.8	7.37	5.96	0.042	7.77	72	86
62	Day 3 9AM Final	363.8		17.1	9.2	7.89	5.88	0.131	3.58		
69	Day 3 4PM Final	330.9		17.0	9.6	7.85	5.56	0.113	3.37		
72	Day 3 Initial	269.6	317.6	17.2	10.1	7.52	6.00	0.059	8.34	80	86
86	Day 4 9AM Final	328.1		16.7	9.7	7.91	5.72	0.130	2.95		
93	Day 4 4PM Final	313.6		16.9	9.4	7.84	5.80	0.115	3.42		
96	Day 4 Initial	256.3	308.6	16.1	9.9	7.36	6.40	0.040	8.06	72	86
110	Day 5 9AM Final	309.1		17.0	9.6	7.85	5.32	0.108	2.69		
117	Day 5 4PM Final	302.1		16.7	9.4	7.90	6.48	0.145	4.00		
120	Day 5 Initial	250.4	297.7	16.6	9.9	7.46	6.20	0.051	7.73	76	88
134	Day 6 9AM Final	294.5		16.7	9.3	7.95	5.76	0.144	3.61		
141	Day 6 4PM Final	299.3		16.9	9.4	7.78	6.08	0.105	4.17		
144	Day 6 Initial	284	334.5	17.0	9.4	7.41	6.44	0.048	20.1	88	94
158	Day 7 9AM Final	290.3		17.1	8.8	7.75	6.00	0.098	3.24		
162	Day 7 1PM Final	302.3		16.7	9.2	7.78	6.12	0.104	6.42		

Table A12. Results of water quality measurements during Experiment III (June 11-18, 2009) in treatment: 8.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	SC (uS/cm)	Temp (°C)	DO (mg/L)	pH	NH_3	NH_4^+	Turbidity (NTU)	Hardness	Alkalinity
0	Day 0 Initial	304.8	364.6	16.1	10.2	7.42	8.68	0.062	7.66	84	106
14	Day 1 9AM Final	445.9		16.7	9.8	7.67	6.4	0.084	6.83		
21	Day 1 4PM Final	451.7		16.9	9.4	7.97	6.12	0.160	4.63		
24	Day 1 Initial	334.0	390.9	17.4	10.0	7.24	8.36	0.044	6.44	92	104
38	Day 2 9AM Final	451.8		17.1	9.1	7.95	6.88	0.174	4.60		
45	Day 2 4PM Final	400.7		16.9	9.7	7.91	7.6	0.174	4.11		
48	Day 2 Initial	299.5	351.9	17.3	9.9	7.33	7.74	0.049	7.34	84	96
62	Day 3 9AM Final	399.9		16.9	9.2	7.96	8.16	0.209	3.46		
69	Day 3 4PM Final	376.3		16.8	9.7	7.97	7.56	0.197	3.35		
72	Day 3 Initial	321.1	378.5	17.0	10.1	7.39	8.44	0.060	7.99	84	96
86	Day 4 9AM Final	364.1		16.7	9.7	8.07	7.8	0.253	3.10		
93	Day 4 4PM Final	362.8		16.9	9.6	7.86	7.76	0.160	3.46		
96	Day 4 Initial	303.3	364.2	16.2	10.1	7.24	8.28	0.040	7.79	84	94
110	Day 5 9AM Final	360.4		16.8	9.7	7.91	7.4	0.169	2.94		
117	Day 5 4PM Final	345.4		16.6	9.6	8.01	8.48	0.239	3.96		
120	Day 5 Initial	300.6	358.0	16.7	10.0	7.38	8.2	0.056	7.04	76	92
134	Day 6 9AM Final	334.9		16.7	9.4	8.04	7.44	0.227	3.32		
141	Day 6 4PM Final	346.9		16.8	9.4	7.82	8.0	0.149	3.96		
144	Day 6 Initial	337.7	398.0	17.0	9.5	7.32	8.52	0.052	18.0	92	100
158	Day 7 9AM Final	335.6		17.0	9.1	7.96	8.0	0.208	3.50		
162	Day 7 1PM Final	353.2		16.7	9.4	7.87	7.56	0.157	5.89		

Comments and Responses

Comments received from SRCSD, letter attached as separate file.

General Comment 1. Please confirm that you are presenting the correct number of significant figures. Fewer significant figures are probably appropriate.

Response: We are not sure what the correct number of significant figures would be.

General Comment 2. This report holds a very different interpretation of reference toxicity data and toxicity data in relation to ammonia concentrations in paired treatments than was provided for the 2008 delta smelt effluent-ammonia bioassays (Werner *et al*. 2009). Results that were considered extreme enough to invalidate test results in 2008 are apparently acceptable for the 2009 test results (e.g., also see specific comments 7, 14, and 29). While we seem unable to agree on whether or not the 2008 data met the test acceptability criteria defined in the formal sampling and analysis plan (Werner *et al*. 2008), we contend that it would be appropriate to re-evaluate these data based on the acceptability criteria applied to the 2009 test reported here, which were agreed upon at a meeting of the POD-CWT ammonia subcommittee (April 8th, 2009). This is especially relevant since the 2009 test is a repeat of the July 2008 test. There is a great deal of value in all of the smelt toxicity testing completed by the ATL and it is a shame to see an entire tests invalidated when at least some of the test hypotheses evaluated can be interpreted. If July 2008 test data are not re-evaluated for a comparison between the results from two years of this study, then we strongly recommend a comprehensive report summarizing all ammonia-effluent delta smelt toxicity data at the completion of these evaluations.

Response: Please see our responses to with regard to the validity of one of the tests performed in 2008 in Werner et al. 2009 "Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt", Final Report to the Central Valley Regional Water Quality Control Board. In addition, please see below our responses to specific comments 7, 14 and 29.

SPECIFIC COMMENTS
SECTION 1 – EXECUTIVE SUMMARY

Comment 1: **page 4, third paragraph**. It is a bit confusing to mention three control samples in the preceding paragraph, but only results provided for two of them in the following paragraph. Reporting survival in the Garcia Bend Field Control – as written on page 13, paragraph 3, second sentence would alleviate this confusion.

Response: We have included information on Garcia Bend in the Executive Summary.

Comment 2: **page 4, third paragraph**. Please consider describing the environmental relevance of these concentrations in the executive summary. The concentration of effluent in river water that caused significant mortality (18%) was approximate 10 times the effluent concentration in the Sacramento River during sampling.

Response: The authors did not have information on river concentrations during the experimental period at the time this report was written. It would take more than one sentence to truly compare those concentrations to exposure concentrations therefore we decided against including this information in the Executive Summary. We compare effect concentrations to environmental concentrations in the body of the report. However, we are glad to include the additional information if SRCSD will provide the data.

Comment 3: **page 5, fourth paragraph**. This would be an appropriate section to describe the percent of SRWTP effluent that is typically present in the Sacramento River (~2%), which is important for determining the environmental relevance of this study and the effect concentrations determined.

Response: Again, this information was not available to the authors. If SRCSD will provide the supporting data for this statement, we are willing to include it in this report.

Comment 4: **page 5, fourth paragraph**. Please include the EPA acute ammonia criteria range. This number is even more relevant that the chronic benchmarks provided, since this is an acute toxicity study.

Response: We used the equation provided in US EPA 1999 to calculate the pH-dependent acute water quality criterion (criterion maximum concentration, CMC) for ammonia/um when salmonids are present and included that information on page 5.

Comment 5: **page 5, fourth paragraph**. In addition to describing water quality criteria for ammonia associated with the maximum pH and temperature conditions in the Delta, please also describe the average concentrations in the Sacramento River and their associated water quality criteria benchmarks. It would also be very helpful to describe how frequently, when, and where you expect these maximum temperature and pH conditions to occur. RMP data show that a pH over 8.3 has been reported in less than 10% of ambient delta samples. These are important considerations for determining environmental relevance of the study data.

Response: We included actual concentrations (average and standard dev.) in this paragraph, but providing information on how often these values or pH values are exceeded is beyond the scope of this report. If SRCSD has this information and will provide the supporting data for the statements above, we will gladly include it in this report.

Comment 6: **page 5, second paragraph**. It is encouraging to see the 2008 study referenced to provide context to the current study. However, the District believes 7/17/08 effluent-ammonia study did yield some conclusive data. Please consider reporting that these tests showed that high concentrations of effluent (up to 32%) did not cause significantly lower survival than controls. This finding is worthy of mention, even if we cannot agree if it is appropriate to compare the data in terms of ammonia concentrations.

Response: We agree to some extent, and have included the following information: "42.5% of fish survived the 7-d exposure to 7.95 mg/L ammonia/um from SRWTP effluent (~26.9%)." - The percent number in the comment was incorrect.

Comment 7: **page 5, second fifth paragraph**. While we are unable to agree on whether or not the ammonia-effluent-smelt toxicity data in 2008 met the test acceptability criteria defined in the formal sampling and analysis plan (Werner *et al*. 2008), we contend that it would be appropriate to evaluate these 2008 data based on the acceptability criteria presented in the 2009 test reported here. This is especially relevant since the 2009 test is a repeat of the July 2008 test and the present draft report does provide a comparison with many past results. None of the reasons previously given for invalidating these 2008 data stand up to review.

1) The statement that "*Test protocol specified that delta smelt survival in both culture facility and low-EC control water be at least 60 percent for the test results to be considered acceptable*." is inconsistent with the sampling and analysis plan (SAP). The SAP states only that "*Hatchery controls <60% would invalidate the test*", but does not specifically state anything about the low-EC control. Reconsideration of test acceptability criteria and data analysis for these tests by the POD-CWT ammonia subcommittee that were adopted for interpreting 2009 bioassays (April 8^{th}, 2009) supported the conclusion that low EC controls are of secondary importance to Garcia Bend Field Controls (water used for ammonia and effluent treatments) and poor performance of the low-EC controls are insufficient to invalidate test results.

Response: To the best of our knowledge, we concluded that the condition of larval delta smelt used in Experiment II conducted in 2008 was compromised, and that data obtained in that test are inconclusive. Please refer to detailed responses to this series of comments in Werner et al. 2009 "Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt", Final Report to the Central Valley Regional Water Quality Control Board. This conclusion was not only based on test acceptability criteria alone, but also on water quality issues as well as results of concurrent reference toxicant test. Luckily, fish survival was very good in all control treatments of Experiment III.

2) Concerns that "*SRWTP effluent reduced the pH at the highest exposure concentration thus reducing the concentration of pH-dependent ammonia, while the ammonium chloride treatment did not show this effect*" are unfounded. The maximum difference in pH was 0.24 pH units, only 3 percent less in effluent-ammonia treatments than in Garcia Bend-ammonia treatments. Further, differences in unionized ammonia were equally different between treatments in 2009 as they were in 2008. 2008 test results were considered invalid because of 9-16% lower ammonia concentration (maximum measured) in effluent-ammonia treatments. However, in 2009 there is no discussion of a 16-36% greater ammonia concentration (maximum measured) in effluent-ammonia treatments than in paired Garcia Bend-ammonia treatments in 2009. Similarly, mean ammonia concentrations in 2008 were 20-29% lower in effluent-ammonia than in Garcia Bend-ammonia, but mean ammonia concentrations up to 20% greater in effluent-ammonia than in Garcia Bend-ammonia received no discussion in 2009. Please provide a discussion addressing these issues.

Response: We disagree with these statements, and believe that the reviewers are mistaken. For the two most important variables the maximum differences between corresponding treatments were 3.8% for EC and 0.5% for pH. In addition, prior to this *test all parties agreed that comparison between the treatment series should be based on total ammonia/um concentrations..*

3) The rationale that sensitive smelt, as indicated by reference toxicity testing in 2008, compromised the test results is unsubstantiated. Reference toxicity data show that Delta smelt tested in July 2008 were indeed the most sensitive tested, but this means they would be most likely to show adverse effects, and they did not. These smelt sensitivities were also well within 2 standard deviations of the running-mean reference toxicity result and meet EPA test acceptability criteria for reference toxicity tests. Likewise, some of the 2009 reference toxicity data presented in this report lie just outside the first standard deviation of the mean, just like the 2008 test in question, but the sensitivity of those fish are called "similar" to the larvae sensitivities in 2008. This would be apparent if reference toxicity data were plotted, as are typically done in toxicity reports. Please provide plots of the reference toxicity data.

Response: We hope that the information we included on delta smelt survival in Experiment II (see Comment 6) will satisfy the reviewers.

4) Any suggestion that low EC affected larval delta smelt should be considered in light of the high survival shown in Garcia Bend (Field Control) water with only 115-119 uS/cm.

Response: We hope that the information we included on delta smelt survival in Experiment II (see Comment 6) will satisfy the reviewers.

SECTION 3 MATERIALS AND METHODS
SECTION 3.2 AMMONIA/UM – SRWTP EFFLUENT TEST

Comment 8: **page 7, first paragraph**. Please include the LOEC in addition to the NOEC determined in 2008 in this description of the basis for test concentrations. The relevance of the NOEC is diminished when presented as an unbounded no-effect concentration.

Response: The LOEC was 9 mg/L ammonia/um and 0.105 mg/L ammonia. We included LOEC information in the text.

SECTION 3.3 TESTS WITH DELTA SMELT
Comment 9: **Page 9, first paragraph**. The heading numbers seem to be out of place. Section 3.3.4 should not precede Section 3.3.2.

Response: We corrected this typo.

Comment 10: **page 11, first paragraph.** "Four to seven-day flow-through tests…" Please clarify which tests were 4 days and which were 7 days. I first thought this might mean that seven-day tests were conducted with survival counts made on the fourth and seventh day. Later it became clear that only one LC50 test was continued to 7 days.

Response: We modified the text for improved clarity: Four-day tests consisting of a control treatment and five concentrations of ammonia/um (5, 10, 20, 40, 80 mg/L total ammonia/um) were performed. One test with juvenile delta smelt was carried out for 7 d.

Comment 11: **page 13, fourth paragraph**. It may be helpful to present these data in a figure, rather than just in a table. An example is provided below.

Response: We added Figure 1 on page 14. We prefer to limit the data presented to the information that is pertinent to this report.

SECTION 4.1.3 COPPER REFERENCE TOXICANT TESTS

Comment 12: **page 23**, **first paragraph**. It is difficult to follow the history of reference toxicity test data for delta smelt as written, and some data are missing. Please present all historical delta smelt reference toxicity data for comparison with the current results. Reference toxicity testing is meant to allow a comparison of organism sensitivity among current and past bioassays. It would be very helpful to present these data visually as is typically done in toxicity reports (example shown below for delta smelt reference toxicity data).

Response: Please see response to Comment 14 below.

Comment 13: **page 23, first paragraph**. Delta smelt age at testing has been repeatedly stated to be an important factor in delta smelt sensitivity. Please consider presenting the age of delta smelt used for reference toxicity tests when discussing test endpoints.

Response: Fish age for each test is provided in table legends and we now provide the juvenile fish age in the first paragraph of page 23.

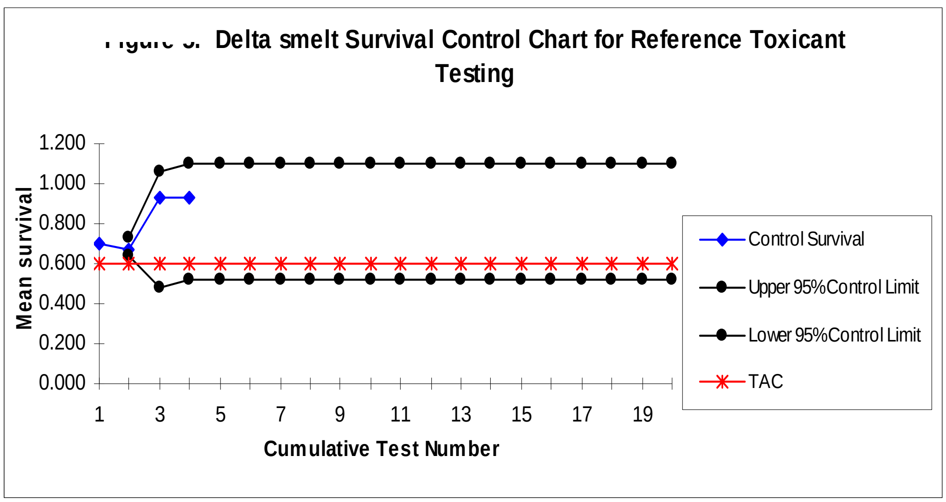
SECTION 5 – QUALITY ASSUARANCE/QUALITY CONTROL
SECTION 5.1 POSTIVIVE CONTROL TESTS WITH DELTA SMELT

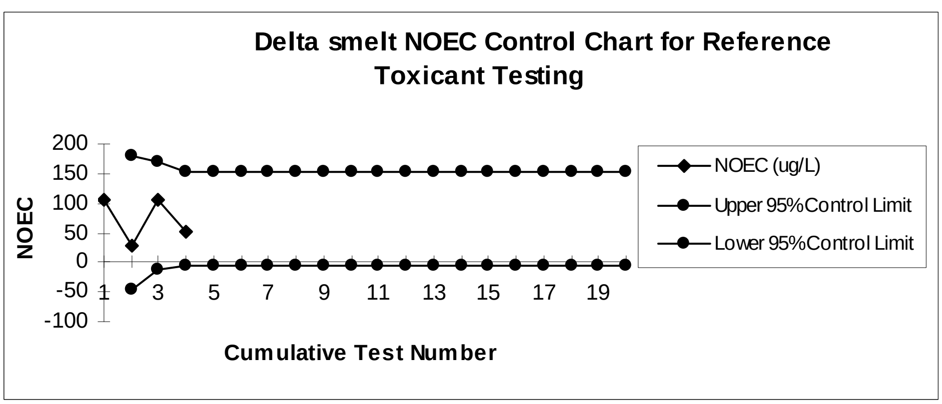
Comment 14: **page 30**, **fifth paragraph**. It would be appropriate and consistent with USEPA recommendations to interpret delta smelt reference toxicity data in the context of two standard deviations of the running mean, as is done for fathead minnow reference toxicity tests. Some of the 2009 reference toxicity data presented in this report lie just outside the first standard deviation of the mean of all reference toxicity test results (i.e., 150 mg/L), just like the 2008 test data (40 mg/L) that was invalidated for this very reason. However, in 2009 these fish sensitivities are called "similar" to the initial reference toxicity results in 2008 (87 mg/L). Please explain the rationale for these disparate interpretations of reference toxicity data and their validation of these bioassays.

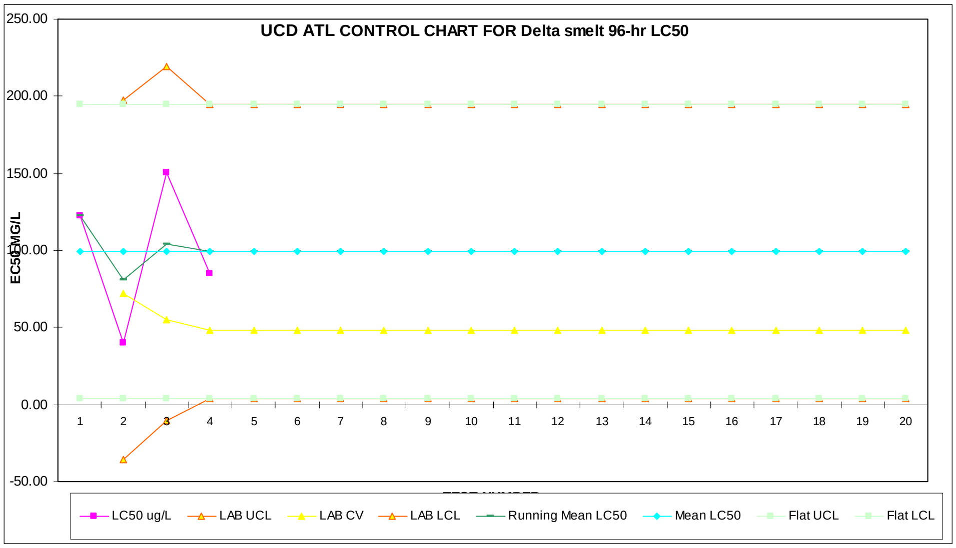
Response: Reference toxicant data have been plotted on a control chart, evaluating both larval and juvenile delta smelt concentration effect data. With the exception of the larval delta smelt RT test conducted on June 24, 2009 (as qualified in the text on page 31, paragraph 4) all larval data fell within the 95% confidence interval of the running mean, and are therefore considered valid. As there is only one juvenile delta smelt data point to plot, comparison within the 95% CI is not possible. However, data met all test acceptability criteria and the test is considered valid. Control charts are generally not included in reports but serve as quality control for laboratory performance documentation. However, we are presenting the graphs below.

USEPA (2002) chronic manual recommends setting a P=0.05 probability level when calculating point estimates. This creates a 95% confidence interval, which is plus/minus two standard deviations around a running mean. Data falling within these control limits are considered acceptable, or animals are acting normally within a specific range. As our delta smelt RT data fell well within the 95% CI, as recommended by USEPA, the data are considered valid. The 2008 RT data also fell within this 95% CI, and all 2008 RT tests were considered valid. The only reasons that a RT test would be considered invalid would be if the control did not meet survival criterion, or if no dose response curve could be generated. There were multiple reasons for invalidating data from Experiment II (2008), please see related comments above.

The "similarity" stated in paragraph 5 of page 30 refers to the similarity between larval and juvenile delta smelts' sensitivity to copper, even though there is a difference between larval and juvenile sensitivity to ammonia/um (e.g., while juvenile delta smelt are much less sensitive to ammonia/um than larval delta smelt, both juvenile and larval delta smelt exhibit similar sensitivity to copper).







SECTION 6 – DISCUSSION AND CONCLUSIONS

Comment 15: **page 31, second paragraph**. It is not clear if or how a "first tier" investigation differs from a screening study, or what significance this term implies. Please consider using language describing this test that is consistent with that used in the past to avoid confusion. This was called a "screening study" when performed in 2008 and when discussed in meetings.

Response: We removed the term "first tier".

Comment 16: **page 31, third paragraph**. This study began in 2008 with two objectives. The second of these hypotheses is presented in the report. Please consider mentioning the other hypothesis that is also addressed by this study, especially since it is mentioned as a conclusion on the following page

Response: Hypothesis I was addressed in the 2008 study. It is not pertinent for this report.

Comment 17: **page 31, third paragraph**. The District believes it is incorrect to state that the hypothesis listed "*…delta smelt survival is negatively impacted by one of more contaminant(s) that are positively correlated with ammonia/um from SRWTP*" could not be addressed to any degree by previous tests. Neither Test I n test II (2008) found significantly reduced delta smelt survival at environmentally relevant effluent concentrations bracketing those that occur in the Sacramento River. This is a significant finding of the 2008 tests (Tests I and II) that cannot be disputed. Please include these important findings and provide a comparison with the current results.

Response: Please see responses to this comment above and in Werner et al. 2009 "Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt", Final Report to the Central Valley Regional Water Quality Control Board.

Comment 18: page 32, second paragraph. The text states a LOEC of 9% effluent. This is inconsistent with the results presented in Table 3-2 where the NOEC was 9% effluent and the LOEC was 18% effluent. Please correct this statement.

Response: This typo was corrected.

Comment 19: page 33, second paragraph. Please describe the environmental relevance of effluent concentrations at the NOEC and LOEC. For example, the concentration of effluent in river water that caused significant mortality (18%) was approximately 10 times the average effluent concentration in the Sacramento River (~2%). The NOEC for effluent (7-day) was determined to be 9% and effects did not occur until effluent comprised 18% of the test solution. This is relevant information that is ignored when considering the potential for adverse effects in the environment on page 33. *"These criteria may not be protective of larval delta smelt especially when considering the additional toxicity of unknown effluent-associated contaminants (Table 13)."* The potential for adverse acute effects from effluent is very low when considering the NOEC and LOEC effect levels from effluent.

Response: We deleted the cited sentence on criteria, and expanded the following sentence: "Reported long-term average ammonia/um concentrations downstream of the point of discharge (0.6 mg/L) are below pH- and temperature-dependent US EPA chronic water quality criteria for water bodies where early life stages of fish are present (US EPA 1999; Table 13), and 6.6 and 3.3-fold lower than the effluent LOEC (3.92 mg/L) and NOEC (1.96 mg/L), respectively, for 7-d survival of larval delta smelt determined in this study. The US EPA acute criterion is approximately twice as high as the LOEC of larval delta smelt, and therefore not protective of this species."

Because we do not have access to the information on percent effluent in the Sacramento River, we refrain from including the suggested statement.

Comment 20: page 33, Table 13. Please add the USEPA acute ammonia criteria to this table, since these are the relevant benchmarks for comparing acute toxicity test results from this study.

Response: We have included this information in the table legends, and divided the table into two. We also detected a mistake in the calculation of the un-ionized ammonia concentration of the chronic criterion. The correct number is 0.050 mg/L ammonia.

Comment 21: page 33, Table 13. Please explain why a range of 30-day Chronic Criteria are presented for single temperature and pH values, rather than a single chronic criterion.

Response: USEPA (1999) presents a table for chronic criteria for ammonia/um for different temperature and pH conditions. We used the numbers for 16.0 and 18.0^{o}C, because our test was conducted at 17^{o}C. We changed the temperature to the appropriate range to make this clear, and calculated all ammonia concentrations for both temperatures, pH 7.8 and EC of 221 uS/cm. The water quality conditions reflect conditions of Experiment III from which the effect concentrations were derived.

Comment 22: **page 33**, **Table 13**. The District suggests changing the fifth column heading form "30-d Chronic Criterion Ammonia" to "30-d Chronic Criterion Ammonia *Equivalent*". The USEPA ammonia/ium criteria are based on total ammonia and it is misleading to state these criteria are unionized ammonia concentrations.

Response: We have made the suggested changes.

Comment 23: **page 33**, **third paragraph**. The District disagrees with the assertions that chronic toxicity may be occurring in the Sacramento River. This was based on extrapolating worst case acute-to-chronic ratios (ACRs) to chronic values for Delta smelt and then comparing these hypothetical benchmarks with ambient data. Instead, an analysis of available paired pH, temperature and ammonia data should be performed to make the most credible assessment of chronic toxicity conditions in the river. At a minimum, please qualify this discussion as an extremely conservative (e.g., worst possible case) exercise and not as a likely situation.

Response: We have added the following sentence: "Using a more conservative approach with an ACR of 12.4 as applied by TenBrook et al. (2009) results in a threshold value of 0.32 mg/L.", and believe that we are sufficiently careful in expressing "concern" rather than making assertions regarding the potential for chronic toxicity.

Comment 24: **page 33**, **third paragraph**. Please consider increasing the credibility and utility of the ACR discussion by including recent results from pilot Delta smelt chronic testing (i.e., swimming impairment). These tests showed threshold chronic effects to delta smelt at concentrations similar to those that caused acute effects. This translates into a species specific ACR closer to 1.

Response: As this was the very first time we used this tool for delta smelt, we consider this to be preliminary data. The work plan did not include chronic endpoints. – For other fish species that are easier to work with than delta smelt, effects on swimming are generally observed far below concentrations that affect survival.

Comment 25: **page 33**, **third paragraph**. Please comment on the fact that RBT LC50s are typically in the 10's to 20's mg/L NH3-N and use of the ACRs cited (15-24) would drive these values below the EPA chronic criterion.

Response: We consider a discussion of effects on rainbow trout to be outside the scope of this report. Also, please see response to comment 23 above.

SECTION 7 – UNCERTAINTIES AND RECOMMENDATIONS FOR FUTURE STUDIES

Comment 26: **page 34**, **third paragraph**. Please consider qualifying this sentence. For example *"Significant uncertainties remain with respect to the* [potential for] *deleterious effects of ammonia/um and SRWTP effluent in the Sacramento-San Joaquin Delta:"*

Response: We made the change.

Comment 27: **page 34**, **fifth paragraph**. Please consider qualifying the first statement by adding that these effects occurred at effluent concentrations well above than those that occur in the Sacramento River.

Response: We added this information to the sentence.

Comment 28: **page 34**, **fifth paragraph**. Please comment on the potential for adverse effects in the Sacramento-San Joaquin Delta from ammonia concentrations near background (0.001 mg/L), as referenced by Buhl (2002).

Response: This goes beyond the scope of this report.

Comment 29: **page 34**, **third paragraph**. Please expand the uncertainty discussion to include how effects from differences in pH/unionized ammonia in test concentrations may have affected the test results. As an example, consider commenting on how there is no discussion of up to 20% greater ammonia concentrations (mean) in effluent-ammonia treatments than in paired Garcia Bend-ammonia treatments in 2009; whereas, differences of 20-30% lower ammonia concentrations were one of the reasons for invalidating test results in 2008. Likewise, maximum measured ammonia concentrations up to 36% greater in the effluent-ammonia treatments were not mentioned in 2009 tests. Mean ammonia differences less than 30% at the effect concentration invalidated test results in 2008.

Response: Please see our response to comment 7.2, above.

Comment 30: Please consider adding to the uncertainty descriptions that the 2009 results represent only a single toxicity test (up to three if the results from 2008 are portrayed). Therefore, these results do not account for variability in smelt, effluent quality, or river water quality among other things.

Response: We agree and added a recommendation to this effect.

Comment 31: page 35. Please consider adding the recommendation to repeat this study. It is very surprising to see the reproducibility of this study omitted from the listed recommendations, especially when there have already been discussions of when these repeated tests could occur.

Response: We agree and added a recommendation to this effect.

Comment 32: **page 35**. Please consider adding the recommendation to conduct 7-day LC50 data for larvae delta smelt exposed to ammonia/ium. This is a critical data gap to allow comparison with the 7-day effluent-ammonia and Garcia Bend-ammonia bioassays. Especially since LC50s for the 4-day effluent-ammonia and Garcia Bend-ammonia bioassays (7.2 and >7.7 mg/L) were both very different from the LC50 test results of 11.6 mg/L in lab water. Only one 7-day LC50 study has been conducted, and this was with juvenile smelt (149 dph) which were shown to be less sensitive to ammonia than larvae used in effluent-ammonia bioassays.

Response: We recommend that the reviewer(s) themselves present this recommendation to the Regional Board.