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

FINAL REPORT

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1. Executive Summary

This pilot study was performed as a collaborative effort between the Central Valley Regional Water Quality Control Board (CVRWQCB), the UC Davis Aquatic Toxicology Laboratory (UCD-ATL), and 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*).

Separate experiments were conducted on June 5-12 (Experiment I) and July 17-24, 2008 (Experiment II). Both consisted of two series of increasing concentrations of total ammonia and ammonium (ammonia/um). The two sources of ammonia/um were 1) the SRWTP effluent, and 2) a stock solution of ammonium chloride. Experiment I consisted of five NH_4Cl concentrations (0.25-4 mg/L ammonia/um) and 4 SRWTP effluent concentrations (0.25-2 mg/L ammonia/um). Experiment II consisted of four NH_4Cl treatments (1.0-8.0 mg/L ammonia/um) and five SRWTP effluent treatments (0.5-8.0 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, unaltered upstream Garcia Bend Sacramento River water (field control) and delta smelt culturing facility water adjusted with deionized water to the conductivity of Sacramento River water (low-EC control). Exposure experiment I 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 controls be at least 60 percent for the test results to be considered acceptable.

Control survival of 55-d old delta smelt larvae in Experiment I was above 60%, and thus met test acceptability criteria. Mean control survival in hatchery water and low conductivity (EC) water (EC=112 µS/cm) was 91.7% and 81.3%, respectively. No significant effect on 7-d survival was detected in effluent and NH_4Cl treatments. Survival of 43-d old delta smelt larvae in Experiment II was below 60% in the low EC control treatment, and thus this test did not meet acceptability criteria. Larvae used in Experiment II were twice as sensitive to copper than larvae used in Experiment I. Additional confounding factors in Experiment II were differences between effluent and NH_4Cl treatments in EC, pH and un-ionized ammonia at high ammonia/um concentrations.

No significant reduction in 7-d survival was detected in larval fathead minnow tests performed concurrently with Experiment I. SRWTP whole effluent testing resulted in 96-h fathead minnow survival of 95-100% during the experimental period in June, and 90-95% during the experimental period in July.

The bioassay results suggest that ammonia concentrations present in the Sacramento River below the SRWTP are not acutely toxic to 55-d old delta smelt. Chronic endpoints were not tested in this study, however, based on available literature information, we conclude that ammonia/um concentrations detected below the SRWTP are of concern with respect to chronic toxicity to delta smelt and other sensitive fish species.

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^{o}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^{o}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- and temperature-dependent US EPA 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^oC (0.075 mg/L ammonia, at 150 µS/cm), to 6.57 mg/L at pH 6.6 and T=0-14^oC (0.0034 mg/L ammonia at 6.1^oC 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.

While effluents from municipal wastewater treatment plants (WWTP) are the primary point source of ammonia/um in rivers of the United States (Mitsch et al. 2001), they also contain complex mixtures of numerous other chemicals (Huang and Sedlak, 2001 and references therein). The acute effects of such chemical mixtures on delta smelt are currently unknown. This pilot study was therefore designed to investigate the potential acute toxicity of ammonia and other chemicals potentially present in SRWTP effluent to larval delta smelt. 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 study addressed the following hypotheses:

1. Larval delta smelt survival is negatively impacted by ambient ammonia/um concentrations in the Sacramento River with increasing concentrations causing increased mortality.
2. Larval delta smelt survival is negatively impacted by one or more contaminant(s) that are positively correlated with ammonia/um from SRWTP.

3. Materials and Methods

3.1 Test Design

Separate experiments were conducted on June 5-12 (Experiment I) and July 17-24, 2008 (Experiment II). Both consisted of two series of increasing concentrations of ammonia/um. Concentrations selected were based on environmental relevance and ammonia/um effect concentrations determined in a related study, where the 96-h LC50 for 50-d old delta smelt was 12 mg/L ammonia/um (0.147 mg/L ammonia). The no observed effect concentration (NOEC) was 5 mg/L ammonia/um (0.066 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 I consisted of five concentrations of ammonia/um from NH_4Cl (0.25-4 mg/L) and four concentrations (0.25-2 mg/L) of ammonia/um from SRWTP effluent (Table 1). Experiment II consisted of four NH_4Cl treatments (1.0-8.0 mg/L ammonia/um) and five SRWTP effluent treatments (0.5-8.0 mg/L ammonia/um). The dilution water used for both test series was ambient water collected from the Sacramento River at Garcia Bend, 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. Exposure experiments were conducted with larval delta smelt and larval fathead minnow (*Pimephales promelas*). *P. promelas* was used in Experiment I only.

Table 1. Treatment lists and total nominal ammonia/um concentrations for each experiment.

Experiment I **June 5-12, 2008**	**Experiment II** **July 17-24, 2008**
Sac River at Garcia Bend (SRGB)	Sac River at Garcia Bend (SRGB)
SRGB w/ 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	SRGB w/ 1.00 mg/L NH_3/NH_4^+ from NH_4Cl
SRGB w/ 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	SRGB w/ 2.00 mg/L NH_3/NH_4^+ from NH_4Cl
SRGB w/ 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	SRGB w/ 4.00 mg/L NH_3/NH_4^+ from NH_4Cl
SRGB w/ 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	SRGB w/ 8.00 mg/L NH_3/NH_4^+ from NH_4Cl
SRGB w/ 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	
SRGB w/ 0.25 mg/L NH_3/NH_4^+ from SRWTP	SRGB w/ 0.50 mg/L NH_3/NH_4^+ from SRWTP
SRGB w/ 0.50 mg/L NH_3/NH_4^+ from SRWTP	SRGB w/ 1.00 mg/L NH_3/NH_4^+ from SRWTP
SRGB w/ 1.00 mg/L NH_3/NH_4^+ from SRWTP	SRGB w/ 2.00 mg/L NH_3/NH_4^+ from SRWTP
SRGB w/ 2.00 mg/L NH_3/NH_4^+ from SRWTP	SRGB w/ 4.00 mg/L NH_3/NH_4^+ from SRWTP
	SRGB w/ 8.00 mg/L NH_3/NH_4^+ from SRWTP
Low Conductivity Control to match SRGB conductivity and turbidity (NTU)	Low Conductivity Control to match SRGB conductivity and turbidity (NTU)
Hatchery Water Control to match rearing conductivity and 11 NTU	Hatchery Water Control to match rearing conductivity and 11 NTU

3.2 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.

3.3 Measurement of Water Quality Parameters

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 Tables 2 and 3.

At test initiation, total ammonia/um, hardness, pH, DO, electrical conductivity (EC), SC, turbidity and temperature were measured in each treatment. A subsample was obtained by pooling approximately 50 mL from each of the four replicate tanks per treatment. During the test, ammonia/um, turbidity, pH, dissolved oxygen and temperature were measured twice daily at 9:00 AM and 4:00 PM. Hardness and EC were measured once daily. 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. In addition, twenty water samples (June 2008) were sent to an outside laboratory (CLS, Rancho Cordova, CA) to verify the accuracy of this method. Results showed that ammonia/um measurements obtained using the HACH AmVer™ Low Range Ammonia Test 'N Tube™ Reagent Set did not differ significantly from analytical chemistry measurements (R2=0.988-0.997). More detailed information is presented in the Appendix, Section A (Table A1, Figures A1-A3).

3.4 pH Drift Study

A 24-h test was performed to verify if pH and/or ammonia/um concentrations changed under experimental conditions. Over the 24-h period, the pH increased by an average of 0.37, and ammonia/um increased by 0.18 mg/L in both effluent and ammonium chloride solutions (nominal concentration: 2 mg/L). There were no differences between effluent and ammonium-chloride solutions (Table A2, Appendix B).

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 6/5/08 (Experiment I).

Sample	Date	Test Day	Turbidity (NTU)	Ammonia/um (mg/L)	pH	Temp (°C)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	SC (uS/cm)	DO (mg/L)
SRWTP	6/5/2008	0	5.63	31.5	6.90	6.0	120	160	931	11.1
SRWTP	6/6/2008	1	7.9	34.0	6.98	12.2	128	166	916	10.2
SRWTP	6/7/2008	2	6.12	33.0	6.91	6.9	136	166	937	10.5
SRWTP	6/8/2008	3	5.78	29.5	7.02	11.0	184	152	901	9.5
SRWTP	6/9/2008	4	4.26	28.5	6.82	6.7	128	132	844	10.6
SRWTP	6/10/2008	5	4.15	29.5	6.65	6.6	136	140	574	10.8
SRWTP	6/11/2008	6	5.02	33.0	6.89	12.1	140	146	862	9.6
Sac River at Garcia Bend	6/5/2008	0	14.3	0.03	7.92	21.1	80	74	182.3	8.6
Sac River at Garcia Bend	6/6/2008	1	10.5	0.03	7.74	10.5	64	78	198.4	9.6
Sac River at Garcia Bend	6/7/2008	2	9.61	0.00	7.93	16.5	80	78	174	9.6
Sac River at Garcia Bend	6/8/2008	3	11.5	0.04	7.84	15.2	72	70	172.4	9.1
Sac River at Garcia Bend	6/9/2008	4	12.2	0.02	7.78	17.3	64	68	175	8.7
Sac River at Garcia Bend	6/10/2008	5	14.6	0.03	7.69	16.3	56	62	122	9.5
Sac River at Garcia Bend	6/11/2008	6	15	0.02	7.96	13.1	52	60	139.3	9.8

Table 3. 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 7/17/08 (Experiment II).

Sample	Date	Test Day	Turbidity (NTU)	Ammonia/ium (mg/L)	pH	Temp (°C)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	SC (uS/cm)	DO (mg/L)
SRWTP	7/17/2008	0	4.31	31.0	7.24	6.3	124	136	850	9.8
SRWTP	7/18/2008	1	4.44	28.0	7.17	24.1	128	142	138.4	8.4
SRWTP	7/19/2008	2	5.74	35.0	6.87	7.2	120	144	866	10.6
SRWTP	7/20/2008	3	7.05	28.0	6.78	7.3	120	134	860	11.5
SRWTP	7/21/2008	4	6.68	26.0	6.79	6.8	120	127	809	11.3
SRWTP	7/22/2008	5	6.00	26.0	6.83	5.3	132	124	818	10.9
SRWTP	7/23/2008	6	4.03	25.0	6.86	5.4	120	134	820	11.8
Sac River at Garcia Bend	7/17/2008	0	6.97	0.01	7.86	13.7	52	64	154.4	9.0
Sac River at Garcia Bend	7/18/2008	1	4.73	0.00	7.91	24.0	60	64	143.5	8.3
Sac River at Garcia Bend	7/19/2008	2	5.71	0.02	7.80	17.4	32	66	147.7	9.0
Sac River at Garcia Bend	7/20/2008	3	5.90	0.11	7.85	16.4	56	64	143.1	9.8
Sac River at Garcia Bend	7/21/2008	4	5.44	0.02	7.88	13.8	56	62	139.4	10.0
Sac River at Garcia Bend	7/22/2008	5	5.60	0.02	7.87	10.9	48	62	143.5	9.9
Sac River at Garcia Bend	7/23/2008	6	7.34	0.01	7.83	10.8	56	40	138.3	9.8

3.5 Tests with Larval Delta Smelt *(H. transpacificus)*

No standard test protocols exist for delta smelt, and procedures were based on protocols developed at the UCD-ATL. According to the Ammonia Toxicity Sampling and Analysis Plan (2008), survival in both the hatchery and low EC control treatments must be at least 60% for test results to be considered acceptable.

Larval *H. transpacificus* were obtained from the UC Davis Fish Culture and Conservation Laboratory (FCCL) in Byron, CA. Fish were transported to UCD-ATL in black 2-gal buckets at a maximum density of 150 fish per bucket. The buckets were placed in coolers and 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.

3.5.1 Ammonia/um Exposures

After arrival at UCD-ATL delta smelt used in ammonia/um and low conductivity control treatments were acclimated for two days to the specific conductance of Sacramento River water. Fish age at test initiation was 55 dph (days post-hatch) and 43 dph for the June and July experiments, respectively.

Upon arrival at UCD-ATL, 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. 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. At test termination, the number of surviving fish was recorded.

3.5.2 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. Other water quality parameters were as follows: Experiment I: Turbidity, 0.70 NTU; hardness, 160 mg/L; alkalinity, 86 mg/L; ammonia/um, 0.00 mg/L; ammonia, 0.000 mg/L; Experiment II: Turbidity, 0.73 NTU; hardness, 124 mg/L; alkalinity, 68 mg/L; total ammonia/um, 0.04 mg/L; ammonia, 0.001 mg/L. 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.

3.6 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). Fish 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. 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.

3.6.1 Ammonia/um 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-hr 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.

3.6.2 Sodium Chloride Reference Toxicant

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 Larval Delta Smelt

4.1.1 Ammonia/um Exposures

<u>Experiment I - June 5, 2008:</u> Survival of delta smelt larvae after 7 d was above 60% in both the hatchery and low EC control treatments, and thus this test met acceptability criteria. Mean control survival in hatchery water and low conductivity (EC) water (EC=112 µS/cm) was 91.7% and 81.3%, respectively. There was no statistical difference between control and low EC control (Tables 4-1, 4-2). Sacramento River water from Garcia Bend significantly reduced survival to 66.3% compared to the low EC control. This difference could be due to differences in turbidity, which was lower in Sacramento River water. Turbidity has been shown to affect survival of larval delta smelt due to

negative effects on feeding behavior. However, larvae above approximately 40 d of age were not sensitive to low turbidity in previous experiments conducted at UCD-ATL (Werner et al. 2008) and fish used in this test were 55 d old. The cause of the reduced survival in Sacramento River water therefore remains unknown. Survival in ammonium-chloride and SRWTP effluent treatments was compared to Sacramento River water, and showed no statistical differences between treatments. In addition, there were no statistical differences between ammonia-chloride and SRWTP effluent treatments.

Table 4-1. Percent survival of 55-d old delta smelt larvae after a 7-d test initiated 6/05/08 (Experiment I); SRWTP = Sacramento Regional Wastewater Treatment Plant; se=standard error of the mean; shaded cells indicate significant ($p<0.05$) reduction in survival compared to the appropriate control**.**

Treatment	Survival (%)	
	mean	se
Sacramento River at Garcia Bend (SRGB)	66.3	8.8
SRGB + 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	62.5	8.0
SRGB + 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	64.1	11.4
SRGB + 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	64.2	8.3
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	72.3	5.2
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	61.2	7.1
SRGB + 0.25 mg/L NH_3/NH_4^+ from SRWTP	81.4	3.7
SRGB + 0.50 mg/L NH_3/NH_4^+ from SRWTP	45.8	4.2
SRGB + 1.00 mg/L NH_3/NH_4^+ from SRWTP	62.6	4.3
SRGB + 2.00 mg/L NH_3/NH_4^+ from SRWTP	64.9	10.1
Low EC Control[1]	81.3	7.1
Hatchery Water Control	91.7	3.4

[1] The Low EC Control consisted of hatchery water diluted with distilled water to match SRGB conductivity.

Table 4-2. Water quality parameters measured during the 7-day test initiated 6/5/08 with 55-d old delta smelt.

Treatment	ID	EC (uS/cm)				Temp (^oC)				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	1	118	160	141	16	16.1	17.6	16.7	0.4	8.9	9.9	9.5	0.3	7.75	8.15	7.92	0.11
SRGB + 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	2	119	160	143	16	16.1	17.6	16.8	0.4	8.8	10.3	9.6	0.3	7.88	8.10	7.96	0.06
SRGB + 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	3	121	162	146	17	16.0	17.6	16.8	0.4	9.0	10.2	9.6	0.3	7.85	8.10	7.95	0.07
SRGB + 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	4	123	168	150	17	16.2	17.5	16.8	0.3	9.0	10.3	9.6	0.3	7.79	8.07	7.92	0.06
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	5	120	176	156	20	16.2	17.6	16.7	0.3	8.7	10.3	9.6	0.3	7.83	8.01	7.93	0.06
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	6	146	192	178	16	16.0	17.4	16.6	0.3	9.0	10.3	9.7	0.3	7.77	8.04	7.92	0.07
SRWTP Effluent @ 0.25 mg/L NH_3/NH_4^+	7	118	163	145	17	16.0	17.6	16.6	0.4	8.8	10.2	9.6	0.3	7.84	8.09	7.97	0.05
SRWTP Effluent @ 0.50 mg/L NH_3/NH_4^+	8	125	168	152	17	16.0	17.6	16.6	0.4	9.1	10.3	9.7	0.3	7.75	8.06	7.91	0.08
SRWTP Effluent @ 1.00 mg/L NH_3/NH_4^+	9	135	179	162	17	15.7	17.8	16.7	0.5	9.4	10.3	9.8	0.3	7.69	8.11	7.94	0.11
SRWTP Effluent @ 2.00 mg/L NH_3/NH_4^+	10	150	202	185	20	15.3	17.7	16.7	0.6	9.3	10.3	9.7	0.3	7.56	8.14	7.89	0.18
Low EC Control	11	112	168	148	21	16.3	17.8	16.8	0.4	8.5	10.1	9.2	0.3	7.52	8.54	7.81	0.29
Hatchery Water Control	12	1480	1528	1502	19	16.1	17.6	16.8	0.4	8.9	9.9	9.3	0.3	7.78	8.17	7.92	0.09

Treatment	ID	Ammonia/um (mg/L)				Ammonia (mg/L) [1]				Turbidity (NTU)			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend	1	0.01	0.17	0.09	0.06	0.000	0.007	0.002	0.002	2.3	15.0	8.1	5.3
SRGB + 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	2	0.23	0.40	0.30	0.06	0.005	0.013	0.008	0.002	2.3	14.5	7.6	5.0
SRGB + 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	3	0.42	0.63	0.51	0.06	0.009	0.022	0.013	0.003	2.2	13.0	7.3	4.4
SRGB + 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	4	0.78	1.15	0.98	0.09	0.016	0.037	0.024	0.004	2.3	12.6	7.0	4.1
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	5	1.53	2.12	1.90	0.17	0.029	0.060	0.047	0.007	2.4	14.0	7.2	4.3
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	6	1.96	4.20	3.64	0.52	0.047	0.120	0.087	0.018	2.3	26.1	8.3	6.6
SRWTP Effluent @ 0.25 mg/L NH_3/NH_4^+	7	0.24	0.38	0.29	0.04	0.005	0.012	0.008	0.002	2.6	13.4	6.8	3.9
SRWTP Effluent @ 0.50 mg/L NH_3/NH_4^+	8	0.36	0.60	0.50	0.06	0.007	0.019	0.012	0.003	2.7	22.3	7.8	5.7
SRWTP Effluent @ 1.00 mg/L NH_3/NH_4^+	9	0.39	1.06	0.91	0.16	0.011	0.037	0.023	0.007	2.4	21.5	8.2	6.1
SRWTP Effluent @ 2.00 mg/L NH_3/NH_4^+	10	1.41	2.11	1.87	0.20	0.019	0.079	0.044	0.016	2.5	12.8	7.1	4.2
Low EC Control	11	0.03	0.69	0.24	0.19	0.000	0.008	0.004	0.002	5.3	11.2	8.1	2.5
Hatchery Water Control	12	0.02	0.41	0.19	0.12	0.000	0.010	0.004	0.003	4.2	38.2	12.7	10.3

[1] Un-ionized ammonia concentrations were calculated based on total ammonia/um, pH and water temperature measured at test initiation.

Experiment II – July 17, 2008: Survival of delta smelt larvae after 7 d was below 60% in the low EC control treatment, and thus this test did not meet acceptability criteria. Mean control survival in hatchery water and low conductivity (EC) water (EC=122 µS/cm) was 80.0% and 52.5%, respectively (Tables 5-1, 5-2).

Water quality data revealed several issues that should be taken into consideration for future exposure experiments: SRWTP effluent reduced the pH at the highest exposure concentrations thus reducing the concentration of pH-dependent ammonia, while the ammonium chloride treatment did not show this effect. Fish in the highest effluent treatment were therefore exposed to lower ammonia concentrations than fish exposed to the corresponding ammonium-chloride treatment. In addition, SRWTP effluent raised the EC of the exposure water more than ammonium chloride resulting in a difference of approximately 140 µS/cm between the highest ammonium-chloride and SRWTP effluent treatments. The mean EC in effluent treatments of 2, 4 and 8 mg/L ammonia/um (nom.) was 125, 142 and 170%, and calculated ammonia concentrations were 81, 78 and 71% of respective NH_4Cl treatments. Taking this into account the survival results cannot be directly compared between effluent and NH_4Cl treatments.

Table 5-1. Percent survival of 43-d old delta smelt larvae after a 7-d test initiated 7/17/08 (Experiment II); SRWTP = Sacramento Regional Wastewater Treatment Plant; se=standard error of the mean; shaded cells indicate significant ($p<0.05$) reduction in survival compared to the appropriate control.

Treatment	Survival (%)	
	mean	se
Sacramento River at Garcia Bend (SRGB)	65.0	8.7
SRGB + 1.0 mg/L NH_3/NH_4^+ from NH_4Cl	47.5	6.3
SRGB + 2.0 mg/L NH_3/NH_4^+ from NH_4Cl	60.0	7.1
SRGB + 4.0 mg/L NH_3/NH_4^+ from NH_4Cl	75.0	2.9
SRGB + 8.0 mg/L NH_3/NH_4^+ from NH_4Cl	40.0	12.9
SRGB + 0.5 mg/L NH_3/NH_4^+ from SRWTP	55.0	5.0
SRGB + 1.0 mg/L NH_3/NH_4^+ from SRWTP	50.0	4.1
SRGB + 2.0 mg/L NH_3/NH_4^+ from SRWTP	47.5	4.8
SRGB + 4.0 mg/L NH_3/NH_4^+ from SRWTP	60.0	5.8
SRGB + 8.0 mg/L NH_3/NH_4^+ from SRWTP	42.5	13.1
Low EC Control[1,2]	52.5	8.5
Hatchery Water Control	80.0	4.1

[1] The Low EC Control consisted of hatchery water diluted with distilled water to match SRGB conductivity.

[2] Low EC Control showed significantly lower survival compared to the hatchery water control, but not compared to SRGB.

Table 5-2. Water quality parameters measured during the 7-day test initiated 7/17/08 with 43-d old delta smelt.

Treatment	ID	EC (uS/cm)				Temp (°C)				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	1	115	125	119	3	15.0	19.3	16.6	0.9	9.3	10.1	9.7	0.3	7.70	8.14	7.93	0.11
SRGB 1.0 mg/L NH_3/NH_4^+ from NH_4Cl	2	122	131	126	3	15.2	19.3	16.5	0.9	9.4	10.2	9.9	0.2	7.71	8.07	7.90	0.10
SRGB 2.0 mg/L NH_3/NH_4^+ from NH_4Cl	3	129	149	135	7	15.0	18.7	16.5	0.9	9.4	10.4	10.0	0.3	7.84	8.11	7.95	0.08
SRGB 4.0 mg/L NH_3/NH_4^+ from NH_4Cl	4	150	158	153	3	15.0	18.0	16.5	0.7	9.3	10.4	9.9	0.3	7.66	8.07	7.90	0.10
SRGB 8.0 mg/L NH_3/NH_4^+ from NH_4Cl	5	182	198	187	5	15.0	18.3	16.5	0.8	9.4	10.5	9.9	0.3	7.76	8.07	7.92	0.08
SRGB 0.5 mg/L NH_3/NH_4^+ from SRWTP	6	129	136	132	3	15.1	18.6	16.5	0.7	9.5	10.3	9.9	0.2	7.77	8.12	7.95	0.10
SRGB 1.0 mg/L NH_3/NH_4^+ from SRWTP	7	140	171	147	11	15.2	17.6	16.4	0.6	9.4	10.3	10.0	0.3	7.76	8.13	7.94	0.11
SRGB 2.0 mg/L NH_3/NH_4^+ from SRWTP	8	166	173	169	2	15.0	18.1	16.4	0.7	9.3	10.3	9.9	0.3	7.47	8.08	7.84	0.18
SRGB 4.0 mg/L NH_3/NH_4^+ from SRWTP	9	212	227	218	5	15.1	18.4	16.5	0.8	9.1	10.7	9.8	0.4	7.48	8.02	7.76	0.19
SRGB 8.0 mg/L NH_3/NH_4^+ from SRWTP	10	245	340	318	33	15.0	18.7	16.6	0.9	9.0	10.3	9.8	0.4	7.15	8.05	7.68	0.33
Low EC Control	11	122	136	129	5	15.3	18.8	17.0	0.7	8.1	10.3	9.0	0.5	7.44	8.01	7.66	0.18
Hatchery Water Control	12	1111	1178	1156	23	15.1	18.4	16.7	0.8	8.6	9.8	9.3	0.4	7.74	8.18	7.91	0.10

Treatment	ID	Ammonia/um (mg/L)				Ammonia (mg/L)[1]				Turbidity (NTU)			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend	1	0.01	0.40	0.12	0.10	0.000	0.010	0.003	0.003	1.7	7.5	3.9	2.1
SRGB 1.0 mg/L NH_3/NH_4^+ from NH_4Cl	2	0.63	1.30	0.97	0.15	0.013	0.032	0.022	0.006	1.8	6.7	3.5	1.6
SRGB 2.0 mg/L NH_3/NH_4^+ from NH_4Cl	3	1.22	2.26	1.87	0.26	0.032	0.067	0.048	0.010	1.8	6.5	3.5	1.7
SRGB 4.0 mg/L NH_3/NH_4^+ from NH_4Cl	4	2.14	4.18	3.70	0.60	0.047	0.137	0.086	0.024	1.9	7.0	3.7	1.8
SRGB 8.0 mg/L NH_3/NH_4^+ from NH_4Cl	5	4.76	9.00	7.56	1.10	0.126	0.253	0.177	0.038	1.8	6.5	3.6	1.8
SRGB 0.5 mg/L NH_3/NH_4^+ from SRWTP	6	0.36	0.88	0.55	0.10	0.008	0.024	0.014	0.005	1.6	9.5	4.1	2.6
SRGB 1.0 mg/L NH_3/NH_4^+ from SRWTP	7	0.58	1.35	0.98	0.17	0.014	0.039	0.025	0.007	1.7	7.3	3.9	2.2
SRGB 2.0 mg/L NH_3/NH_4^+ from SRWTP	8	1.31	2.25	1.90	0.23	0.016	0.064	0.039	0.014	1.6	7.4	3.8	2.1
SRGB 4.0 mg/L NH_3/NH_4^+ from SRWTP	9	2.32	6.02	3.82	0.76	0.031	0.115	0.067	0.026	1.4	7.6	4.0	2.2
SRGB 8.0 mg/L NH_3/NH_4^+ from SRWTP	10	4.44	9.00	7.62	1.05	0.030	0.231	0.126	0.073	2.0	6.9	4.1	1.9
Low EC Control	11	0.01	1.70	0.30	0.36	0.000	0.021	0.004	0.005	3.1	10.3	5.1	1.8
Hatchery Water Control	12	0.03	0.41	0.22	0.12	0.001	0.008	0.005	0.002	3.1	12.2	7.0	3.3

[1] Un-ionized ammonia concentrations were calculated based on total ammonia/um, pH and water temperature measured at test initiation.

4.1.2 Copper Reference Toxicant Tests

Delta smelt larvae (54 d old) used in Experiment I (Tables 6-1, 6-2, 6-3) were approximately two times less sensitive to copper than 42-d old larvae used in Experiment II (Tables 7-1,7-2, 7-3). The 54-d old larvae were similar in sensitivity to larvae used previously to determine the 96-h LC50 (86.5 µg/L Cu^{2+}; dissolved) (Werner et al., unpublished data).

Table 6-1. Effect of 96-h exposure to copper on percent survival of 54-d old delta smelt larvae. This test was initiated on 6/04/08. Shaded cells indicate significant reduction in survival compared to control[1].

Treatment	Measured Cu^{2+} Concentration (ppb)		Survival (%)[1]	
	Total	Dissolved	Mean	SE
Filtered Hatchery Water (FHW)	3	2	67	17.6
FHW + 27 ppb Cu^{2+}	28	28	93	6.7
FHW + 53 ppb Cu^{2+}	54	48	73	13.3
FHW + 106 ppb Cu^{2+}	115	95	53	24.0
FHW + 213 ppb Cu^{2+}	210	178	7	6.7

[1] Data were analyzed using USEPA standard statistical protocols.

Table 6-2. Acute 96-h effect concentrations of copper for 54-d old delta smelt larvae.

Endpoint	Copper Concentration (ppb)				
	Estimate	95% C.I.	NOEC	LOEC	PMSD
Nominal - LC10	71.8	6.8 - 104.5	106	213	75.1%
LC20	86.2	14.6 - 118.6			
LC50	122.3	57 - 165			
Measured Total Copper - LC10	88	14.0 - 118.9	115	210	79.4%
LC20	101.7	25.4 - 131.8			
LC50	134.1	73 - 173			
Measured Dissolved Copper - LC10	70.8	9.4 - 97.6	95	178	79.4%
LC20	82.6	17.9 - 108.8			
LC50	110.9	57 - 145			

Table 6-3. Water quality data for the 96-hour copper test with 54-d old delta smelt larvae.

Treatment	EC (uS/cm)[2]				Temp (°C)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)[1]	-	-	779	-	16.8	17.3	17.1	0.4
FHW + 27 ppb Cu^{2+}	-	-	778	-	16.4	17.1	16.8	0.5
FHW + 53 ppb Cu^{2+}	-	-	784	-	16.4	16.9	16.7	0.4
FHW + 106 ppb Cu^{2+}	-	-	779	-	16.5	16.7	16.6	0.1
FHW + 213 ppb Cu^{2+}	-	-	773	-	16.6	17.5	17.1	0.6

Treatment	DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)[1]	9.4	10.2	9.8	0.3	7.92	8.03	7.98	0.06
FHW + 27 ppb Cu^{2+}	9.4	9.8	9.7	0.2	7.99	8.05	8.01	0.03
FHW + 53 ppb Cu^{2+}	9.8	10.1	9.9	0.1	8.02	8.06	8.03	0.02
FHW + 106 ppb Cu^{2+}	9.7	10.0	9.9	0.1	7.96	8.09	8.04	0.07
FHW + 213 ppb Cu^{2+}	9.1	9.9	9.7	0.4	7.98	8.11	8.02	0.08

[1] Matrix was water from the UC Davis Fish Conservation and Culture Laboratory, Byron, CA (Turbidity: 0.70 NTU, Hardness: 160 mg/L, Alkalinity: 86 mg/L, Ammonia/um: 0.000 mg/L, Ammonia: 0.000 mg/L).

[2] EC was measured only at test initiation.

Table 7-1. Effect of 96-h exposure to copper on percent survival of 42-d old delta smelt larvae. This test was initiated on 7/16/08. Shaded cells indicate significant reduction in survival compared to control[1].

Treatment	Measured Cu^{2+} Concentration (ppb)		Survival (%)[1]	
	Total	Dissolved	Mean	SE
Filtered Hatchery Water (FHW)	2	2	78	11.7
FHW + 27 ppb Cu^{2+}	38	37	72	6.0
FHW + 53 ppb Cu^{2+}	98	89	7	6.7
FHW + 106 ppb Cu^{2+}	149	136	7	6.7
FHW + 213 ppb Cu^{2+}	269	242	0	0.0

[1] Data were analyzed using USEPA standard statistical protocols.

Table 7-2. Acute 96-h effect concentrations of copper for 42-d old delta smelt larvae.

Endpoint	Copper Concentration (ppb)				
	Estimate	95% C.I.	NOEC	LOEC	PMSD
Nominal - LC10	20.4	1.1 - 34.3	27	53	38.0 %
LC20	25.7	2.6 - 40.2			
LC50	39.8	12.3 - 58.3			
Measured Total Copper - LC10	33.3	2.8 - 60.0	38	98	37.6%
LC20	41.3	5.4 - 64.6			
LC50	62.0	18.9 - 88.1			
Measured Dissolved Copper - LC10	32.2	3.3 - 52.4	37	89	37.6%
LC20	39.4	6.18 - 60.2			
LC50	58.3	19.8 - 81.3			

Table 7-3. Water quality data for the 96-hour copper test with 42-d old delta smelt larvae.

Treatment	EC (uS/cm)				Temp (^{o}C)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)[1]	730	751	741	15	16.5	17.8	16.9	0.6
FHW + 27 ppb Cu^{2+}	740	764	752	17	16.3	17.7	16.8	0.6
FHW + 53 ppb Cu^{2+}	744	750	747	4	16.4	17.8	16.8	0.7
FHW + 106 ppb Cu^{2+}	743	758	751	11	16.4	17.9	16.8	0.7
FHW + 213 ppb Cu^{2+}	-	-	756	-	16.4	16.4	16.4	-

Treatment	DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)[1]	9.0	9.8	9.6	0.4	7.77	8.04	7.94	0.12
FHW + 27 ppb Cu^{2+}	9.1	9.6	9.5	0.2	7.86	8.00	7.94	0.06
FHW + 53 ppb Cu^{2+}	9.4	9.8	9.6	0.2	7.86	8.04	7.98	0.08
FHW + 106 ppb Cu^{2+}	9.1	9.8	9.5	0.3	7.84	8.01	7.92	0.08
FHW + 213 ppb Cu^{2+}	9.3	9.3	9.3	-	8.00	8.00	8.00	-

Matrix was water from the UC Davis Fish Conservation and Culture Laboratory, Byron, CA (Turbidity: 0.73 NTU, Hardness: 124 mg/L, Alkalinity: 68 mg/L, Ammonia/um: 0.04 mg/L, Ammonia: 0.001 mg/L).

4.2 Tests with Larval Fathead Minnow

4.2.1 Ammonia/um Exposures

Fathead minnow tests met test acceptability criteria. No significant reduction in survival was detected (Tables 8-1, 8-2).

Table 8-1. Percent survival of fathead minnow larvae exposed for 7 d to NH_4Cl and diluted SRWTP effluent. Experiment I was initiated 6/05/08. DIEPAMH = de-ionized water amended to US EPA moderately hard standard.

Treatment	Survival (%)	
	x	se
Sacramento River at Garcia Bend (SRGB)	100.0	0.0
SRGB + 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0
SRGB + 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0
SRGB + 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	97.5	2.5
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	100.0	0.0
SRGB + 0.25 mg/L NH_3/NH_4^+ from SRWTP	100.0	0.0
SRGB + 0.50 mg/L NH_3/NH_4^+ from SRWTP	95.0	5.0
SRGB + 1.00 mg/L NH_3/NH_4^+ from SRWTP	100.0	0.0
SRGB + 2.00 mg/L NH_3/NH_4^+ from SRWTP	100.0	0.0
Low EC Control	97.5	2.5
DIEPAMH	97.5	2.5

SRWTP whole effluent testing resulted in 96-h fathead minnow survival of 95-100% during the experimental period in June, and 90-95% during the experimental period in July (Appendix, Table A27).

Table 8-2. Water quality data for the 7-day test with fathead minnow larvae initiated 6/05/08.

Treatment	ID	EC (uS/cm)				Temp (^oC)				DO (mg/L)			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend (SRGB)	1	136	185	166	21	23.8	25.2	24.4	0.3	6.6	8.5	7.7	0.7
SRGB + 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	2	144	197	173	20	23.8	25.3	24.4	0.4	6.5	8.6	7.7	0.7
SRGB + 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	3	136	191	171	21	23.7	25.9	24.3	0.6	6.4	8.6	7.7	0.8
SRGB + 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	4	142	195	174	21	23.6	26.1	24.6	0.6	6.2	8.6	7.7	0.8
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	5	148	218	187	23	23.7	25.4	24.4	0.4	6.4	8.5	7.7	0.8
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	6	156	225	200	27	23.7	26.1	24.5	0.6	6.6	8.6	7.7	0.8
SRGB + 0.25 mg/L NH_3/NH_4^+ from SRWTP	7	140	190	171	20	24.0	25.5	24.5	0.5	6.6	8.6	7.8	0.8
SRGB + 0.50 mg/L NH_3/NH_4^+ from SRWTP	8	147	201	177	18	23.2	25.6	24.4	0.6	6.4	8.6	7.7	0.8
SRGB + 1.00 mg/L NH_3/NH_4^+ from SRWTP	9	160	203	188	19	24.1	25.2	24.5	0.4	6.4	8.6	7.7	0.8
SRGB + 2.00 mg/L NH_3/NH_4^+ from SRWTP	10	185	232	215	17	23.8	25.8	24.5	0.6	6.6	8.6	7.8	0.8
Low EC Control	11	114	200	165	31	24.1	25.4	24.6	0.4	6.5	8.5	7.7	0.7
DIEPAMH	12	138	296	268	58	23.8	25.3	24.4	0.4	6.1	8.6	7.6	0.8

Treatment	ID	pH				Ammonia Nitrogen (mg/L)				Un-ionized Ammonia (mg/L)			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend (SRGB)	1	7.53	8.12	7.92	0.15	0.01	0.43	0.12	0.13	0.001	0.011	0.004	0.003
SRGB + 0.25 mg/L NH_3/NH_4^+ from NH_4Cl	2	7.69	8.10	7.95	0.11	0.24	0.62	0.33	0.11	0.010	0.018	0.014	0.002
SRGB + 0.50 mg/L NH_3/NH_4^+ from NH_4Cl	3	7.67	8.15	7.95	0.14	0.48	6.51	0.99	1.59	0.019	0.297	0.044	0.073
SRGB + 1.00 mg/L NH_3/NH_4^+ from NH_4Cl	4	7.63	8.10	7.90	0.13	0.89	1.39	1.05	0.14	0.031	0.063	0.042	0.009
SRGB + 2.00 mg/L NH_3/NH_4^+ from NH_4Cl	5	7.68	8.06	7.91	0.12	1.20	2.22	1.93	0.26	0.033	0.111	0.081	0.022
SRGB + 4.00 mg/L NH_3/NH_4^+ from NH_4Cl	6	7.68	8.02	7.87	0.09	2.10	4.20	3.71	0.56	0.050	0.200	0.139	0.034
SRGB + 0.25 mg/L NH_3/NH_4^+ from SRWTP	7	7.66	8.08	7.91	0.13	0.24	2.75	0.49	0.66	0.007	0.064	0.017	0.014
SRGB + 0.50 mg/L NH_3/NH_4^+ from SRWTP	8	7.72	8.04	7.90	0.11	0.46	0.92	0.59	0.16	0.017	0.032	0.023	0.004
SRGB + 1.00 mg/L NH_3/NH_4^+ from SRWTP	9	7.72	8.03	7.90	0.09	0.92	1.42	1.03	0.15	0.032	0.048	0.040	0.005
SRGB + 2.00 mg/L NH_3/NH_4^+ from SRWTP	10	7.67	8.03	7.85	0.10	1.39	2.23	1.96	0.20	0.045	0.095	0.070	0.015
Low EC Control	11	7.32	8.09	7.76	0.29	0.00	0.51	0.15	0.15	0.000	0.007	0.003	0.002
DIEPAMH	12	7.61	8.21	7.96	0.20	0.00	0.53	0.12	0.14	0.000	0.011	0.004	0.004

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 ensure its quality and credibility. The following is a summary of the QA/QC work completed during June and July, 2008.

5.1 Positive Control Tests with Delta Smelt

Positive control reference toxicant (RT) tests were conducted with delta smelt twice during the study period, using copper (II) 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 protocols established with the 2008-2010 POD Project. 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 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 three replicates per treatment and five fish per replicate. Concentrations were based on the copper LC_{50} for delta smelt larvae determined in May 2008 at UCD-ATL. Test results yielded a $CuCl_2$ LC_{50} between 76-95 ppb (95% CI), with a NOEC of 37.5 ppb, and a LOEC of 75 ppb. This LC_{50} test was conducted using 49 d old delta smelt larvae. Reference toxicant tests conducted for this project were initiated following protocols identical to the LC_{50} test.

The delta smelt reference toxicant test initiated on June 4, 2008, utilized fish that were 54 d old. Average control survival for this test was 70%, which met the test acceptability criterion of $\geq$60% control survival. Test results yielded an LC_{50} of 122.34 ppb, with a NOEC of 106 ppb, and a LOEC of 213 ppb. The test initiated on July 16, 2008, utilized fish that were 42 d old. Average control survival for this test was 67%, which met the test acceptability criterion. Test results yielded an LC_{50} of 39.84 ppb, with a NOEC of 27 ppb, and a LOEC of 53 ppb. As these tests met the test acceptability criterion, the delta smelt RT data for June and July, 2008, are considered reliable.

5.2 Positive Control Tests with Fathead Minnow

Reference toxicant tests with fathead minnow are performed once a month to ascertain whether organism response fell within the acceptable range as dictated by US EPA. Each reference toxicant test consisted of a dilution series made up of five different concentrations of the toxicant and a control. Sodium chloride (NaCl) was the toxicant utilized in the fathead minnow tests. A 20-month running mean control chart is continuously updated with the results of these reference toxicant test endpoints. Acceptable range for US EPA is within the 95% confidence interval of a running mean. If the LC_{50} or EC_{25} falls out of the 95% confidence interval, test organism sensitivity is considered atypical and results of tests conducted during that month are considered suspect. One data point out of 20 is expected to fall out of range by chance alone.

Organisms in control treatments tests typically do not exhibit any mortality, with overall control survival as 100%. Because the survival endpoint has a small 95% confidence interval, slight differences in control survival can cause data endpoints to fall out of the acceptable range. Control survival in tests conducted in June, 2008 was well above the 80% test acceptability criteria, so organisms are considered healthy, and there were no outliers in reference toxicant tests during June, 2008. Therefore all fathead minnow data are considered reliable.

6. Discussion and Conclusions

Results from this project provide initial information on the acute toxicity 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 or first tier investigation, 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 2008 was based, address uncertainties, and provide recommendations for future studies.

Hypothesis 1: Delta smelt survival is negatively impacted by ambient ammonia/um concentrations in the Sacramento River with increasing concentrations causing increased mortality.

The bioassay results predict that there should be no acute toxicity to delta smelt larvae (55 dph) at ammonia/um and ammonia concentrations found in the Sacramento River immediately below the SRWTP. The highest average experimental exposure concentration in the effluent and in ammonium chloride test treatments were 1.87 and 3.64 mg/L ammonia/um, and 0.044 and 0.087 mg/L ammonia, respectively (Table 4-2). 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 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 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 had ammonia/um concentrations of <0.024 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 55-d old delta smelt larvae.

Results obtained to date are consistent with ammonia/um and ammonia effect concentrations recently established for 50-d old larval delta smelt at UCD-ATL using filtered hatchery water as well as acute effect concentrations for other fish species reported in the peer-reviewed literature. The 96-h NOEC, LOEC and LC50 for ammonia/um were 5.0, 9.0, and 12.0 mg/L, respectively (at pH 7.9, T=16^{o}C, EC=900 µS/cm). The corresponding 96-h NOEC, LOEC and LC50 for ammonia were 0.066, 0.105 and 0.147 mg/L, respectively. Delta smelt larvae at the age of 50 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 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 0.090–3.350 mg/L. Average ambient ammonia/um and ammonia concentrations in the Sacramento River below SRWTP (see above) are therefore within a safety factor of >10 of acute effect concentrations reported for 50-d old delta smelt larvae and other sensitive fish species. It should be noted that this may only apply to larval fish, since Thurston and Russo (1983) demonstrated that large rainbow trout were measurably more sensitive than younger life stages.

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. SRWTP discharges treated effluent containing ammonia/um at an average concentration of 24 ± 3.4 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 (Table 9) for water bodies where early life stages of fish are present (US EPA 1999), but more detailed studies of environmental conditions are needed before the risk of effluent-associated ammonia/um toxicity to delta smelt can be accurately assessed.

Table 9. US EPA Chronic Criteria (30-day average) for Fish Early Life Stages Present (USEPA, 1999) and respective ammonia concentrations for temperature and pH extremes measured at Hood and Grand Island.

Temp. (°C)	pH	EC (µS/cm)	USEPA Chronic Ammonia/um Criterion (mg/L)	Ammonia (mg/L)
6.1	6.6	150	6.57	0.0034
25.0	6.6	150	3.13-3.56	0.0067-0.0076
6.1	8.3	150	1.52	0.038
25.0	8.3	150	0.727-0.827	0.070-0.080

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 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 ammonia effect concentrations for delta smelt larvae (ammonia 96-h LC_{50}: 0.15 mg/L) then the resulting threshold concentrations would be 0.010 and 0.006 mg/L, respectively. The average un-ionized ammonia concentration in the Sacramento River at Grand Island is 0.004 ± 0.004 mg/L (UCD-ATL, unpublished data) and would thus approach these chronic safe values for delta smelt. The chronic threshold concentrations derived above are similar to those reported by other studies. For example, Dodds and Welch (2000) suggest that chronic effects of ammonia on fish may occur at concentrations as low as 0.005 mg/L.

In conclusion, our study showed that ammonia/um at levels reported for the Sacramento River below SRWTP was not acutely toxic to 55-d old delta smelt. Chronic endpoints were not tested in this study, however, based on information available from USEPA (1999) and other related literature, we conclude that ammonia/um concentrations detected below the SRWTP are of concern with respect to chronic toxicity to delta smelt and other sensitive fish species.

Hypothesis 2: Smelt survival is negatively impacted by one or more contaminant(s) that are positively correlated with ammonia from SRWTP.

We are unable to address this hypothesis, because experiment II did not meet test acceptability criteria with respect to control survival. Delta smelt larvae used in Experiment II were about twice as sensitive to copper than larvae used in Experiment I potentially suggesting compromised health. Additional confounding factors in Experiment II caused by the addition of effluent were differences in EC, pH and un-ionized ammonia between effluent and corresponding NH_4Cl treatments. The mean EC in effluent treatments of 2, 4 and 8 mg/L ammonia/um (nom.) was 125, 142 and 170%, and calculated ammonia concentrations were 81, 78 and 71% of respective NH_4Cl treatments. Taking this into account, the survival results cannot be directly compared between effluent and NH_4Cl treatments. This test should therefore be repeated.

7. Uncertainties and Recommendations for Future Studies

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

(1) Effects of multiple stressors. 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) Effects of contaminant mixtures. - 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

- Experiment #2 was designed to evaluate the acute effects of contaminant mixtures present in SRWTP effluent on 7-d survival of delta smelt larvae, and should be repeated in 2009 to conclusively answer this question.
- Information should be generated on the influence of life-stage (larval, juvenile, adult) on the susceptibility of delta smelt to ammonia/um.

- Acute-to-chronic ratios should be established using sublethal endpoints such as 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 at lower trophic levels needs to be integrated and possibly generated to assess potential effects of reduced food availability on fish species of concern. For example, 2006-07 data for the SSJ Delta showed that ammonia/um was negatively correlated with 10-day growth of the amphipod species *Hyalella azteca*. *H. azteca* is resident in the Delta, and the most sensitive species for which Genus Mean Chronic Values (GMCV) were derived by US EPA (1999). The GMCV for this species is 1.45 mg/L ammonia/um at 25^{o}C and pH 7.94 (equal to 0.085 mg/L ammonia).
- 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 Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt

Additional Test Results and Water Quality Data

A. Method Comparison for Ammonia/um Measurements

Regressions were performed on 3 subsets of the dataset:

1) Excluding both the High Range kit values and an Anomalous reading where a nominal value of 0.5 mg/L was read on the kit as 0.48 but the analytical reading was 0.83.
2) Including the anomalous reading but excluding the High Range kit readings.
3) Including all data.

Each regression was run three ways:

A) Orthogonal Regression: gives confidence interval of the slope
B) Red/Green Fit: a normal unconstrained regression.
C) Bold Black Fit: regression constrained to Intercept = 0 and Slope = 1.

Paired T-tests were also performed to determine if the methods differed significantly in their readings of ammonia nitrogen concentration.

Table A1. Regression Results

Dataset	R^2	Slope	95% Confidence Interval
1	0.988	0.946	0.893 – 1.003
2	0.983	0.937	0.877 – 1.002
3	0.997	0.797	0.778 – 0.817

All regressions show predicted slopes below 1.0, indicating that the Ammonia Nitrogen Kit will tend to slightly overestimate the concentration of ammonia nitrogen, relative to the analytical result. The only dataset showing a regression slope significantly different than 1.0, was the regression including data obtained using the High Range kit measurements. The ammonia/um measurements obtained using the Low Range kit are not predicted to differ significantly from analytical chemistry measurements. In addition, paired T-tests showed no consistent difference in readings between the Low Range kit and the analytical chemistry method.

Figure A1. Regression of dataset #1

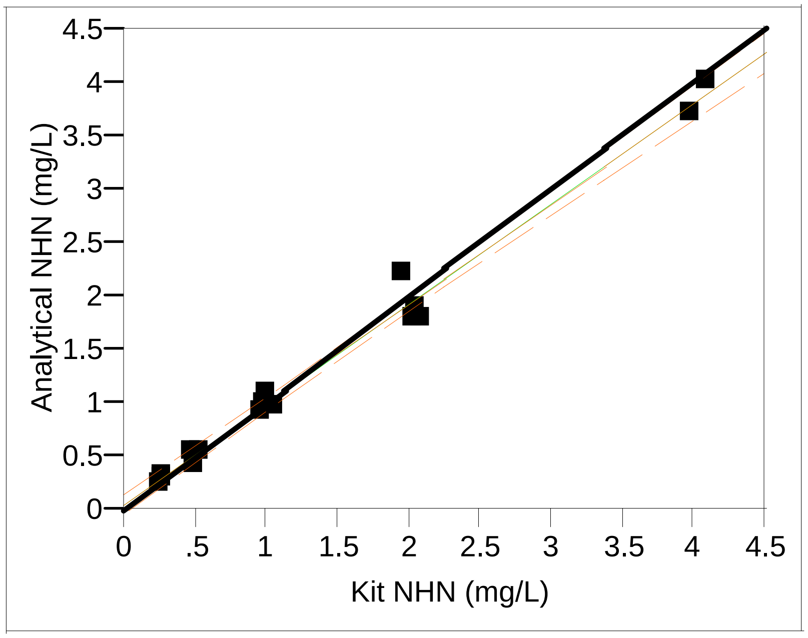


Figure A2. Regression of dataset #2

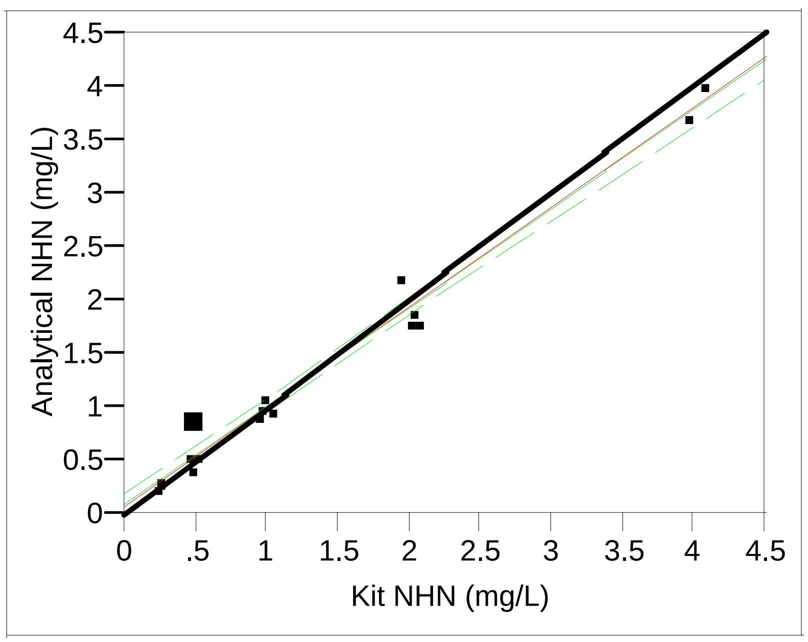
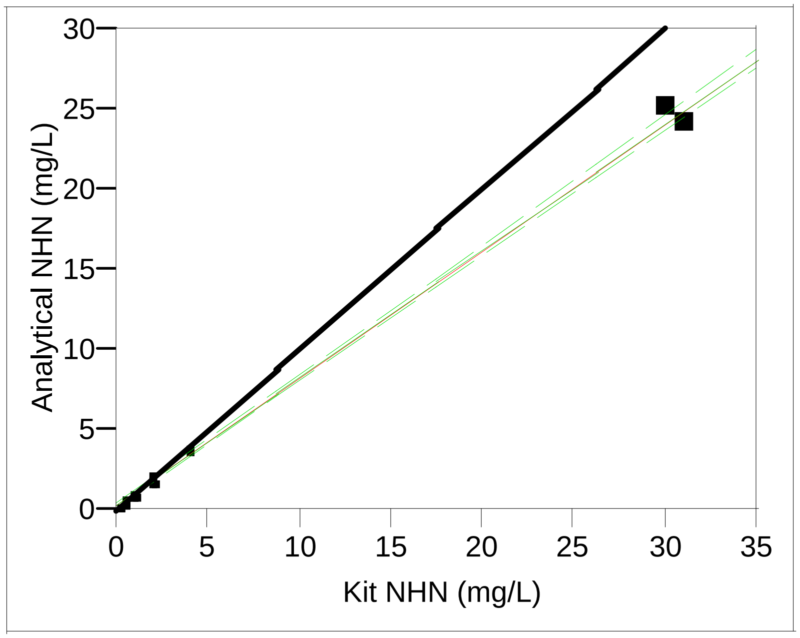


Figure A3. Regression of dataset #3



B. Results of the pH Drift Study

Table A2. Ammonia/um concentrations and pH during a 24-h study mimicking test conditions of subsequent delta smelt exposures to SRWTP effluent and ammonium chloride.

		5/13/2008 17:00		5/14/2008 9:00		5/14/2008 17:30	
ID	Treatment	pH	NH3-N	pH	NH3-N	pH	NH3-N
1	Garcia Bend w/o aeration	7.93	0.04	7.84	0	7.99	0
2	Garcia Bend	7.94	0.04	8.07	0	8.09	0
3	Dilute SRWTP @ 0.5 mg/L	7.76	0.45	8	0.39	8.1	0.42
4	Dilute SRWTP @ 2.0 mg/L	7.73	1.87	8.03	1.79	8.16	2.07
5	Garcia Bend w/ 0.5 mg/L NH4	7.81	0.48	8.08	0.43	8.13	0.42
6	Garcia Bend w/ 2.0 mg/L NH4	7.8	1.91	8.1	1.75	8.17	2.08

Notes: Treatments 2 - 6 were gently aerated to mimic test conditions. Each treatment was 1 L of water in a glass 1 L beaker (no replication). Temperature was 16^{o}C. Garcia Bend water: NH3-N = 0.04 mg/L, SRWTP effluent: NH3-N = 32 mg/L. No pH adjustments were done.

C. Water Quality Data

Table A3. Results of water quality measurements during Experiment I (June 2008) in treatment: Sacramento River at Garcia Bend.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia /um (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	154.8	16.6	8.9	7.85	72	0.01	0.000	14.2
14	Day 1 9AM Final		17.5	9.0	7.77		0.08	0.001	
21	Day 1 4PM Final		16.9	9.1	7.95		0.10	0.003	2.3
24	Day 1 Initial	160.4	16.1	9.4	7.82	64	0.03	0.000	14.2
38	Day 2 9AM Final		16.6	9.2	7.81		0.10	0.002	
45	Day 2 4PM Final		16.6	9.2	7.85		0.11	0.002	3.2
48	Day 2 Initial	147.1	16.2	9.9	7.96	64	0.01	0.000	9.6
62	Day 3 9AM Final		16.8	9.7	7.93		0.13	0.003	
69	Day 3 4PM Final		16.6	9.7	8.10		0.11	0.004	3.1
72	Day 3 Initial	146.9	16.9	9.5	7.86	72	0.03	0.001	11.5
86	Day 4 9AM Final		16.9	9.7	7.96		0.15	0.004	
93	Day 4 4PM Final		16.9	9.4	8.00		0.13	0.004	3.2
96	Day 4 Initial	136.9	16.5	9.4	7.76	64	0.02	0.000	12.2
110	Day 5 9AM Final		16.6	9.6	8.09		0.09	0.003	
117	Day 5 4PM Final		16.2	9.4	8.15		0.17	0.007	3.3
120	Day 5 Initial	123.2	16.7	9.6	7.95	56	0.03	0.001	14.6
134	Day 6 9AM Final		17.0	9.5	7.75		0.17	0.003	
141	Day 6 4PM Final		16.1	9.5	8.05		0.16	0.005	3.5
144	Day 6 Initial	117.7	17.1	9.8	7.86	52	0.02	0.000	15.0
158	Day 7 9AM Final		17.3	9.5	7.94		0.15	0.004	
162	Day 7 1PM Final		17.6	9.5	7.95		0.13	0.004	3.6

Table A4. Results of water quality measurements during Experiment I (June 2008) in treatment: Low Conductivity (EC) Control.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	162.4	16.4	9.0	8.54	40	0.07	0.006	11.1
14	Day 1 9AM Final		16.7	9.3	7.68		0.03	0.000	
21	Day 1 4PM Final		16.6	9.5	7.75		0.05	0.001	5.4
24	Day 1 Initial	168.2	16.6	9.2	8.18	48	0.10	0.004	11.1
38	Day 2 9AM Final		16.9	9.2	7.70		0.10	0.001	
45	Day 2 4PM Final		16.3	9.6	7.73		0.11	0.002	5.8
48	Day 2 Initial	158.2	16.3	10.1	7.98	44	0.10	0.003	9.9
62	Day 3 9AM Final		16.9	9.3	7.70		0.23	0.003	
69	Day 3 4PM Final		16.9	9.1	7.68		0.22	0.003	5.6
72	Day 3 Initial	151.9	16.4	9.2	8.31	40	0.07	0.004	10.9
86	Day 4 9AM Final		17.1	9.0	7.54		0.36	0.004	
93	Day 4 4PM Final		17.2	9.2	7.52		0.40	0.004	5.3
96	Day 4 Initial	156.2	16.8	8.7	8.03	40	0.11	0.003	11.2
110	Day 5 9AM Final		17.2	9.1	7.55		0.37	0.004	
117	Day 5 4PM Final		16.3	9.3	7.70		0.56	0.008	5.4
120	Day 5 Initial	111.5	16.9	8.5	7.92	32	0.10	0.002	9.0
134	Day 6 9AM Final		17.0	9.4	7.52		0.69	0.007	
141	Day 6 4PM Final		16.6	8.8	7.57		0.47	0.005	6.4
144	Day 6 Initial	124.3	16.4	9.3	8.19	32	0.09	0.004	9.8
158	Day 7 9AM Final		17.2	9.4	7.59		0.45	0.005	
162	Day 7 1PM Final		17.8	9.1	7.54		0.37	0.004	6.4

Table A5. Results of water quality measurements during Experiment I (June 2008) in treatment: Hatchery Water Control.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	1528	17.0	9.1	7.86	236	0.11	0.002	11.6
14	Day 1 9AM Final		16.6	9.2	7.78		0.02	0.000	
21	Day 1 4PM Final		16.6	9.3	7.92		0.05	0.001	5.4
24	Day 1 Initial	1481	16.9	9.4	7.88	240	0.07	0.001	11.6
38	Day 2 9AM Final		16.9	9.5	7.87		0.10	0.002	
45	Day 2 4PM Final		16.2	9.3	7.96		0.12	0.003	4.2
48	Day 2 Initial	1497	16.5	9.9	7.96	240	0.11	0.003	19.9
62	Day 3 9AM Final		16.9	9.3	7.91		0.19	0.004	
69	Day 3 4PM Final		16.8	9.2	7.96		0.18	0.004	5.2
72	Day 3 Initial	1527	16.4	9.4	8.01	239	0.09	0.003	11.5
86	Day 4 9AM Final		16.8	9.0	7.79		0.23	0.004	
93	Day 4 4PM Final		17.2	8.9	7.82		0.27	0.005	5.5
96	Day 4 Initial	1501	16.9	9.0	7.97	236	0.12	0.003	26.6
110	Day 5 9AM Final		17.1	9.0	7.85		0.33	0.006	
117	Day 5 4PM Final		16.3	9.1	7.97		0.41	0.010	4.9
120	Day 5 Initial	1500	16.8	9.0	8.05	236	0.11	0.003	22.1
134	Day 6 9AM Final		16.9	9.6	7.82		0.40	0.007	
141	Day 6 4PM Final		16.3	9.3	7.91		0.38	0.008	6.5
144	Day 6 Initial	1480	16.1	9.7	8.17	212	0.11	0.004	38.2
158	Day 7 9AM Final		17.2	9.5	7.97		0.33	0.008	
162	Day 7 1PM Final		17.6	9.1	7.99		0.31	0.008	5.3

Table A6. Results of water quality measurements during Experiment I (June 2008) in treatment: 0.25 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	159.6	16.6	8.8	7.92	64	0.24	0.006	14.0
14	Day 1 9AM Final		17.2	9.5	7.88		0.23	0.005	
21	Day 1 4PM Final		16.8	9.6	8.04		0.24	0.008	2.3
24	Day 1 Initial	156.3	16.1	10.0	7.88	60	0.26	0.005	14.0
38	Day 2 9AM Final		16.7	9.7	7.93		0.24	0.006	
45	Day 2 4PM Final		16.5	9.6	7.95		0.27	0.007	2.8
48	Day 2 Initial	149.4	16.2	9.9	7.98	60	0.25	0.007	8.9
62	Day 3 9AM Final		16.8	9.5	7.98		0.31	0.009	
69	Day 3 4PM Final		16.7	9.8	8.02		0.29	0.009	3.0
72	Day 3 Initial	152.6	17.1	9.7	7.90	60	0.24	0.006	9.2
86	Day 4 9AM Final		17.0	9.5	7.93		0.39	0.010	
93	Day 4 4PM Final		17.1	9.4	8.00		0.36	0.011	2.6
96	Day 4 Initial	140.9	16.9	9.6	7.88	60	0.25	0.006	9.8
110	Day 5 9AM Final		16.6	9.6	8.03		0.25	0.008	
117	Day 5 4PM Final		16.2	9.6	8.08		0.38	0.013	3.0
120	Day 5 Initial	125.6	16.5	9.9	7.94	56	0.26	0.006	14.5
134	Day 6 9AM Final		16.8	9.4	7.91		0.40	0.009	
141	Day 6 4PM Final		16.4	9.4	8.10		0.36	0.013	4.7
144	Day 6 Initial	119.3	17.0	10.3	7.95	52	0.26	0.007	13.7
158	Day 7 9AM Final		17.1	9.7	7.98		0.38	0.011	
162	Day 7 1PM Final		17.6	9.7	7.95		0.35	0.010	3.2

Table A7. Results of water quality measurements during Experiment I (June 2008) in treatment: 0.50 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	162.2	16.5	9.0	8.01	60	0.51	0.015	12.6
14	Day 1 9AM Final		16.9	9.4	7.85		0.42	0.009	
21	Day 1 4PM Final		17.0	9.3	7.90		0.46	0.011	2.2
24	Day 1 Initial	162.2	16.4	10.2	7.90	60	0.51	0.011	12.6
38	Day 2 9AM Final		16.6	9.7	7.92		0.49	0.012	
45	Day 2 4PM Final		16.6	9.4	7.97		0.52	0.014	3.2
48	Day 2 Initial	152.6	16.0	9.9	8.03	64	0.49	0.014	8.7
62	Day 3 9AM Final		16.8	9.4	7.98		0.43	0.012	
69	Day 3 4PM Final		16.7	9.6	8.04		0.49	0.015	3.3
72	Day 3 Initial	153.6	16.9	10.0	7.92	60	0.49	0.012	9.3
86	Day 4 9AM Final		16.9	9.5	7.89		0.53	0.012	
93	Day 4 4PM Final		17.1	9.2	7.96		0.52	0.014	3.4
96	Day 4 Initial	145.3	16.8	9.7	7.85	60	0.51	0.010	10.4
110	Day 5 9AM Final		16.7	9.5	7.99		0.42	0.012	
117	Day 5 4PM Final		16.2	9.5	8.10		0.63	0.022	3.3
120	Day 5 Initial	125.8	16.8	9.8	7.99	52	0.50	0.014	13.0
134	Day 6 9AM Final		16.9	9.6	7.87		0.61	0.013	
141	Day 6 4PM Final		16.4	9.4	8.01		0.59	0.017	4.1
144	Day 6 Initial	121.4	17.2	10.2	7.93	48	0.48	0.012	12.6
158	Day 7 9AM Final		17.1	9.7	7.96		0.60	0.016	
162	Day 7 1PM Final		17.6	9.7	7.94		0.53	0.014	3.4

Table A8. Results of water quality measurements during Experiment I (June 2008) in treatment: 1.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	167.4	16.8	9.0	7.94	68	1.01	0.025	11.1
14	Day 1 9AM Final		16.9	9.4	7.85		0.78	0.016	
21	Day 1 4PM Final		16.9	9.4	7.97		0.81	0.022	2.3
24	Day 1 Initial	167.5	16.5	10.1	7.85	60	1.03	0.021	11.1
38	Day 2 9AM Final		16.6	9.5	7.89		0.86	0.019	
45	Day 2 4PM Final		16.3	9.3	7.91		0.98	0.022	2.9
48	Day 2 Initial	157.2	16.3	10.0	7.95	60	1.01	0.025	8.3
62	Day 3 9AM Final		16.8	9.6	7.96		0.92	0.024	
69	Day 3 4PM Final		16.8	9.6	8.00		0.99	0.028	3.1
72	Day 3 Initial	156.8	17.1	10.0	7.92	60	0.98	0.024	9.9
86	Day 4 9AM Final		16.9	9.3	7.84		0.95	0.019	
93	Day 4 4PM Final		17.0	9.3	7.97		0.96	0.026	3.9
96	Day 4 Initial	148.9	16.8	9.8	7.79	56	1.04	0.019	10.4
110	Day 5 9AM Final		16.9	9.6	7.96		0.84	0.022	
117	Day 5 4PM Final		16.2	9.8	8.07		1.15	0.037	3.3
120	Day 5 Initial	130.4	16.8	9.8	7.87	52	1.02	0.022	12.3
134	Day 6 9AM Final		16.8	9.6	7.87		1.08	0.023	
141	Day 6 4PM Final		16.7	9.5	7.99		1.06	0.030	3.0
144	Day 6 Initial	123.1	16.9	10.3	7.88	48	1.05	0.023	12.6
158	Day 7 9AM Final		17.2	9.6	7.90		1.07	0.025	
162	Day 7 1PM Final		17.5	9.8	7.91		1.05	0.026	3.3

Table A9. Results of water quality measurements during Experiment I (June 2008) in treatment: 2.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	169.9	16.2	9.1	7.98	60	2.07	0.054	11.9
14	Day 1 9AM Final		16.6	9.3	7.83		1.53	0.029	
21	Day 1 4PM Final		16.5	9.4	8.01		1.56	0.045	2.4
24	Day 1 Initial	175.9	16.7	10.3	7.87	60	2.00	0.042	11.9
38	Day 2 9AM Final		16.7	9.7	7.89		1.70	0.038	
45	Day 2 4PM Final		16.4	9.6	7.92		1.81	0.042	3.0
48	Day 2 Initial	167.3	16.7	9.9	8.00	64	2.03	0.058	8.3
62	Day 3 9AM Final		16.7	9.6	7.96		1.76	0.046	
69	Day 3 4PM Final		16.7	9.8	8.01		1.83	0.053	2.9
72	Day 3 Initial	168.3	17.3	9.9	7.98	60	2.04	0.058	10.1
86	Day 4 9AM Final		16.8	9.3	7.89		1.91	0.043	
93	Day 4 4PM Final		17.0	9.3	7.90		1.85	0.043	3.5
96	Day 4 Initial	155.8	16.6	9.9	7.86	60	1.99	0.041	10.6
110	Day 5 9AM Final		16.9	9.4	7.96		1.67	0.044	
117	Day 5 4PM Final		16.2	9.6	8.01		2.12	0.060	3.4
120	Day 5 Initial	137.9	16.9	10.0	7.94	56	2.02	0.051	14.0
134	Day 6 9AM Final		16.8	9.6	7.83		2.05	0.040	
141	Day 6 4PM Final		16.4	9.5	7.94		1.99	0.049	4.2
144	Day 6 Initial	119.8	16.3	8.7	7.90	52	2.09	0.047	12.0
158	Day 7 9AM Final		17.0	9.7	7.86		2.00	0.043	
162	Day 7 1PM Final		17.6	9.5	7.94		1.97	0.053	3.4

Table A10. Results of water quality measurements during Experiment I (June 2008) in treatment: 4.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	192.3	16.8	9.0	8.00	64	4.12	0.118	11.8
14	Day 1 9AM Final		16.6	9.4	7.86		3.08	0.063	
21	Day 1 4PM Final		16.7	9.6	7.99		3.10	0.086	2.3
24	Day 1 Initial	185.2	16.1	9.9	7.80	60	4.02	0.070	11.8
38	Day 2 9AM Final		16.5	9.8	7.92		3.30	0.077	
45	Day 2 4PM Final		16.4	9.6	7.94		3.56	0.086	3.0
48	Day 2 Initial	180.7	16.6	10.0	7.88	64	3.94	0.085	8.6
62	Day 3 9AM Final		16.7	9.7	7.97		3.02	0.080	
69	Day 3 4PM Final		16.7	9.7	8.04		3.88	0.120	3.0
72	Day 3 Initial	185.7	16.0	9.9	7.91	60	4.08	0.090	9.1
86	Day 4 9AM Final		16.8	9.5	7.88		3.66	0.080	
93	Day 4 4PM Final		17.0	9.4	7.93		3.72	0.092	3.6
96	Day 4 Initial	172.1	16.5	9.8	7.77	60	3.84	0.064	12.6
110	Day 5 9AM Final		16.8	9.5	7.94		3.78	0.095	
117	Day 5 4PM Final		16.3	9.7	8.04		3.90	0.118	3.4
120	Day 5 Initial	184.5	16.9	10.1	7.84	56	4.20	0.084	13.1
134	Day 6 9AM Final		16.8	9.6	7.90		3.92	0.090	
141	Day 6 4PM Final		16.4	9.5	7.93		1.96	0.047	3.9
144	Day 6 Initial	145.5	16.3	10.3	7.86	52	3.96	0.080	26.1
158	Day 7 9AM Final		17.0	9.6	7.97		3.50	0.095	
162	Day 7 1PM Final		17.4	9.7	7.93		3.82	0.098	3.6

Table A11. Results of water quality measurements during Experiment I (June 2008) in treatment: 0.25 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	162.8	16.7	9.1	7.99	60	0.26	0.007	11.3
14	Day 1 9AM Final		16.7	9.5	7.88		0.24	0.005	
21	Day 1 4PM Final		16.5	9.5	8.02		0.26	0.008	2.6
24	Day 1 Initial	158.5	16.4	10.2	7.84	68	0.24	0.005	11.3
38	Day 2 9AM Final		16.6	9.6	7.94		0.26	0.006	
45	Day 2 4PM Final		16.3	9.8	7.98		0.27	0.007	3.3
48	Day 2 Initial	153	16.3	10.1	7.96	60	0.24	0.006	8.1
62	Day 3 9AM Final		16.7	9.6	8.01		0.30	0.009	
69	Day 3 4PM Final		16.7	9.6	8.03		0.29	0.009	3.1
72	Day 3 Initial	155.1	16.6	10.0	7.99	64	0.26	0.007	9.8
86	Day 4 9AM Final		16.8	9.5	7.95		0.30	0.008	
93	Day 4 4PM Final		17.0	9.5	7.97		0.34	0.009	3.6
96	Day 4 Initial	142	16.0	9.7	7.93	60	0.24	0.006	9.2
110	Day 5 9AM Final		16.8	9.5	8.01		0.30	0.009	
117	Day 5 4PM Final		16.2	9.4	8.09		0.36	0.012	3.1
120	Day 5 Initial	128.6	17.0	10.0	7.96	56	0.25	0.007	13.4
134	Day 6 9AM Final		16.7	9.7	7.94		0.38	0.010	
141	Day 6 4PM Final		16.7	9.8	7.96		0.32	0.008	3.4
144	Day 6 Initial	118.2	16.1	8.8	7.90	52	0.24	0.005	9.5
158	Day 7 9AM Final		17.1	9.8	8.00		0.34	0.010	
162	Day 7 1PM Final		17.6	9.7	7.98		0.30	0.009	3.0

Table A12. Results of water quality measurements during Experiment I (June 2008) in treatment: 0.50 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	168.3	16.8	9.1	7.90	64	0.49	0.011	10.4
14	Day 1 9AM Final		16.6	9.4	7.86		0.36	0.007	
21	Day 1 4PM Final		16.6	9.5	7.98		0.41	0.011	2.7
24	Day 1 Initial	165.5	16.5	10.1	7.77	72	0.52	0.009	10.4
38	Day 2 9AM Final		16.7	9.7	7.93		0.44	0.011	
45	Day 2 4PM Final		16.2	9.8	7.96		0.47	0.012	3.2
48	Day 2 Initial	161.2	16.6	10.0	7.88	64	0.50	0.011	8.4
62	Day 3 9AM Final		16.8	9.5	7.99		0.47	0.013	
69	Day 3 4PM Final		16.7	9.6	8.04		0.52	0.016	3.4
72	Day 3 Initial	160.7	16.2	10.0	7.89	64	0.46	0.010	9.7
86	Day 4 9AM Final		16.7	9.5	7.92		0.54	0.013	
93	Day 4 4PM Final		17.1	9.3	7.95		0.53	0.014	3.7
96	Day 4 Initial	149.8	16.0	9.9	7.75	60	0.48	0.007	11.4
110	Day 5 9AM Final		16.9	9.4	7.95		0.52	0.013	
117	Day 5 4PM Final		16.2	9.5	8.06		0.60	0.019	3.2
120	Day 5 Initial	132.5	16.4	10.3	7.82	52	0.48	0.009	13.7
134	Day 6 9AM Final		16.7	9.6	7.89		0.58	0.013	
141	Day 6 4PM Final		16.6	9.4	7.94		0.56	0.014	3.5
144	Day 6 Initial	124.5	16.1	10.2	7.81	52	0.50	0.009	22.3
158	Day 7 9AM Final		17.4	9.6	7.95		0.57	0.015	
162	Day 7 1PM Final		17.6	9.7	7.92		0.54	0.014	3.3

Table A13. Results of water quality measurements during Experiment I (June 2008) in treatment: 1.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	178.8	16.5	9.5	7.83	68	0.96	0.018	15.7
14	Day 1 9AM Final		17.0	9.5	7.93		0.66	0.016	
21	Day 1 4PM Final		16.6	9.5	8.02		0.72	0.021	2.4
24	Day 1 Initial	174.8	16.3	10.3	7.78	68	0.95	0.016	15.7
38	Day 2 9AM Final		16.7	9.8	7.98		0.39	0.011	
45	Day 2 4PM Final		16.2	9.8	8.03		0.84	0.025	3.2
48	Day 2 Initial	170.5	16.4	10.2	7.82	64	0.98	0.018	8.1
62	Day 3 9AM Final		16.9	9.5	8.02		0.87	0.026	
69	Day 3 4PM Final		16.9	9.7	8.08		0.84	0.029	3.6
72	Day 3 Initial	169	16.4	10.1	7.86	64	0.97	0.020	8.7
86	Day 4 9AM Final		16.8	9.6	8.01		0.98	0.029	
93	Day 4 4PM Final		17.2	9.5	8.05		0.94	0.031	4.0
96	Day 4 Initial	162.4	16.2	10.0	7.81	60	1.04	0.019	11.6
110	Day 5 9AM Final		17.1	9.4	8.03		0.95	0.030	
117	Day 5 4PM Final		16.2	9.7	8.11		1.06	0.037	3.1
120	Day 5 Initial	142	16.0	10.2	7.87	60	0.96	0.019	10.2
134	Day 6 9AM Final		16.8	9.7	7.95		1.04	0.027	
141	Day 6 4PM Final		16.6	9.5	7.96		1.01	0.026	3.3
144	Day 6 Initial	135.3	15.7	10.3	7.69	56	0.95	0.013	21.5
158	Day 7 9AM Final		17.4	9.8	8.00		0.97	0.029	
162	Day 7 1PM Final		17.8	9.7	7.97		1.00	0.029	3.1

Table A14. Results of water quality measurements during Experiment I (June 2008) in treatment: 2.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	200.1	16.5	9.3	7.65	72	2.01	0.025	12.4
14	Day 1 9AM Final		16.9	9.6	7.87		1.41	0.030	
21	Day 1 4PM Final		16.5	9.6	8.04		1.48	0.045	2.5
24	Day 1 Initial	201.6	16.7	10.0	7.60	72	1.95	0.022	12.4
38	Day 2 9AM Final		16.7	9.8	7.95		1.62	0.041	
45	Day 2 4PM Final		16.2	9.8	8.08		1.71	0.056	3.1
48	Day 2 Initial	192.8	16.8	9.9	7.72	64	1.94	0.029	8.4
62	Day 3 9AM Final		16.8	9.6	8.02		1.73	0.052	
69	Day 3 4PM Final		16.9	9.6	8.06		1.62	0.053	3.5
72	Day 3 Initial	199.6	16.4	10.2	7.75	68	1.99	0.031	9.6
86	Day 4 9AM Final		17.0	9.5	7.96		1.98	0.052	
93	Day 4 4PM Final		17.3	9.4	7.98		1.91	0.054	3.2
96	Day 4 Initial	183	15.3	10.1	7.56	64	2.03	0.019	10.0
110	Day 5 9AM Final		17.1	9.6	8.02		1.92	0.059	
117	Day 5 4PM Final		16.2	9.6	8.14		2.11	0.079	3.1
120	Day 5 Initial	165.6	16.0	10.1	7.65	60	1.95	0.024	11.3
134	Day 6 9AM Final		16.9	9.7	7.95		2.01	0.052	
141	Day 6 4PM Final		16.7	9.5	7.92		2.05	0.049	3.4
144	Day 6 Initial	149.5	15.7	10.3	7.68	56	2.08	0.027	12.8
158	Day 7 9AM Final		17.4	9.8	8.02		1.94	0.061	
162	Day 7 1PM Final		17.7	9.6	7.99		1.82	0.055	3.2

Table A15. Results of water quality measurements during Experiment II (July 2008) in treatment: Sacramento River at Garcia Bend.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	121.3	15.5	9.3	7.70	52	0.01	0.000	7.0
14	Day 1 9AM Final		17.9	9.6	7.92		0.14	0.004	
21	Day 1 4PM Final		19.3	9.3	7.96		0.14	0.004	2.3
24	Day 1 Initial	117.9	15.0	9.5	7.83	60	0.01	0.000	4.4
38	Day 2 9AM Final		16.7	10.1	7.94		0.22	0.006	
45	Day 2 4PM Final		17.3	9.5	8.14		0.16	0.007	1.9
48	Day 2 Initial	125.1	15.3	9.8	7.85	56	0.02	0.000	4.6
62	Day 3 9AM Final		16.5	10.1	8.02		0.21	0.006	
69	Day 3 4PM Final		16.7	9.5	8.01		0.15	0.004	2.0
72	Day 3 Initial	119.2	16.0	9.4	7.89	56	0.11	0.002	5.9
86	Day 4 9AM Final		16.4	10.0	8.06		0.18	0.006	
93	Day 4 4PM Final		16.4	10.0	8.13		0.06	0.002	2.0
96	Day 4 Initial	115	16.0	9.5	7.86	56	0.03	0.000	5.4
110	Day 5 9AM Final		16.3	9.9	8.03		0.18	0.005	
117	Day 5 4PM Final		16.7	9.5	7.95		0.40	0.010	1.9
120	Day 5 Initial	115.5	16.2	9.5	7.87	48	0.01	0.000	5.6
134	Day 6 9AM Final		16.6	9.7	7.81		0.20	0.004	
141	Day 6 4PM Final		17.1	9.6	7.91		0.15	0.004	1.7
144	Day 6 Initial	119.9	16.9	9.7	7.84		0.01	0.000	7.5
158	Day 7 9AM Final		16.5	10.1	7.83		0.13	0.003	
162	Day 7 1PM Final		17.4	9.3	7.96		0.08	0.002	1.9

Table A16. Results of water quality measurements during Experiment II (July 2008) in treatment: Low Conductivity (EC) Control.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	131.1	15.3	8.9	7.87	44	0.08	0.002	10.3
14	Day 1 9AM Final		17.9	9.3	7.79		0.17	0.003	
21	Day 1 4PM Final		18.8	8.7	7.66		0.20	0.003	4.1
24	Day 1 Initial	135.5	17.2	8.1	7.55	20	0.01	0.000	4.7
38	Day 2 9AM Final		17.4	9.1	7.61		1.70	0.021	
45	Day 2 4PM Final		17.5	8.9	7.61		0.26	0.003	3.8
48	Day 2 Initial	128.1	17.1	9.0	7.85	40	0.06	0.001	5.6
62	Day 3 9AM Final		16.8	8.9	7.51		0.38	0.004	
69	Day 3 4PM Final		16.5	8.7	7.44		0.28	0.002	4.2
72	Day 3 Initial	129.6	16.5	8.3	7.99	36	0.65	0.018	5.8
86	Day 4 9AM Final		16.3	9.7	7.46		0.37	0.003	
93	Day 4 4PM Final		16.9	9.0	7.66		0.23	0.003	4.0
96	Day 4 Initial	121.8	16.8	10.3	8.01	36	0.08	0.002	5.4
110	Day 5 9AM Final		16.5	9.4	7.56		0.35	0.004	
117	Day 5 4PM Final		16.9	9.3	7.52		0.35	0.003	3.1
120	Day 5 Initial	129.7	17.1	9.2	7.85	40	0.03	0.001	5.6
134	Day 6 9AM Final		16.5	9.4	7.53		0.34	0.003	
141	Day 6 4PM Final		17.5	8.9	7.52		0.27	0.003	3.3
144	Day 6 Initial	124	16.9	9.0	7.88		0.04	0.001	7.0
158	Day 7 9AM Final		16.4	9.3	7.48		0.28	0.002	
162	Day 7 1PM Final		17.8	8.4	7.46		0.20	0.002	4.1

Table A17. Results of water quality measurements during Experiment II (July 2008) in treatment: Hatchery Water Control.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	1111	15.1	8.6	7.89	152	0.09	0.002	5.2
14	Day 1 9AM Final		17.8	9.2	7.88		0.14	0.003	
21	Day 1 4PM Final		18.4	9.2	7.91		0.17	0.004	4.4
24	Day 1 Initial	1162	17.6	8.8	8.00	144	0.03	0.001	3.1
38	Day 2 9AM Final		16.9	9.7	7.85		0.28	0.005	
45	Day 2 4PM Final		17.2	9.5	7.85		0.24	0.005	4.8
48	Day 2 Initial	1170	17.1	8.7	8.06	144	0.11	0.003	11.2
62	Day 3 9AM Final		16.8	9.7	7.92		0.31	0.007	
69	Day 3 4PM Final		16.2	9.5	7.87		0.28	0.005	4.1
72	Day 3 Initial	1146	15.9	9.0	8.05	144	0.11	0.003	10.8
86	Day 4 9AM Final		16.0	9.8	7.88		0.31	0.006	
93	Day 4 4PM Final		16.4	9.8	7.93		0.21	0.005	5.5
96	Day 4 Initial	1151	16.5	8.8	8.18	144	0.15	0.006	11.3
110	Day 5 9AM Final		16.2	9.7	7.94		0.37	0.008	
117	Day 5 4PM Final		17.1	9.3	7.80		0.36	0.006	5.6
120	Day 5 Initial	1178	16.1	9.2	7.98	144	0.11	0.003	12.2
134	Day 6 9AM Final		16.4	9.7	7.85		0.39	0.007	
141	Day 6 4PM Final		17.3	9.2	7.83		0.31	0.006	4.9
144	Day 6 Initial	1175	16.8	8.9	7.94		0.04	0.001	11.0
158	Day 7 9AM Final		16.3	9.8	7.79		0.41	0.007	
162	Day 7 1PM Final		17.5	9.0	7.74		0.29	0.004	4.7

Table A18. Results of water quality measurements during Experiment II (July 2008) in treatment: 1.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	127.2	15.2	9.8	7.71	76	0.95	0.013	4.5
14	Day 1 9AM Final		17.5	9.7	7.90		0.63	0.015	
21	Day 1 4PM Final		19.3	9.4	7.95		0.64	0.020	2.5
24	Day 1 Initial	126.7	15.2	10.0	7.89	56	1.02	0.020	4.6
38	Day 2 9AM Final		16.3	10.1	7.78		0.83	0.014	
45	Day 2 4PM Final		16.8	9.8	8.06		0.97	0.032	1.9
48	Day 2 Initial	130.6	15.5	9.7	7.71	52	0.94	0.013	4.7
62	Day 3 9AM Final		16.6	10.0	8.01		0.96	0.028	
69	Day 3 4PM Final		16.5	9.8	7.98		0.96	0.026	1.8
72	Day 3 Initial	125.3	16.0	9.9	7.90	52	1.04	0.023	4.7
86	Day 4 9AM Final		16.4	10.1	7.99		0.99	0.027	
93	Day 4 4PM Final		16.5	10.2	8.07		0.90	0.030	2.3
96	Day 4 Initial	123.7	15.8	10.2	7.78	52	1.06	0.017	4.8
110	Day 5 9AM Final		16.2	10.0	8.02		1.04	0.030	
117	Day 5 4PM Final		16.5	9.7	7.91		1.30	0.030	1.9
120	Day 5 Initial	121.5	15.8	10.1	7.79	48	0.99	0.017	5.1
134	Day 6 9AM Final		16.5	9.8	7.87		1.13	0.024	
141	Day 6 4PM Final		17.4	9.4	7.92		1.01	0.025	2.0
144	Day 6 Initial	126.8	16.3	10.0	7.82		0.98	0.018	6.7
158	Day 7 9AM Final		16.2	10.1	7.88		1.11	0.023	
162	Day 7 1PM Final		17.6	9.6	7.93		0.90	0.024	1.8

Table A19. Results of water quality measurements during Experiment II (July 2008) in treatment: 2.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	134.9	15.0	10.0	7.92	40	1.93	0.041	4.9
14	Day 1 9AM Final		17.8	9.5	7.93		1.22	0.032	
21	Day 1 4PM Final		18.7	9.4	7.92		1.25	0.034	2.3
24	Day 1 Initial	133.2	15.0	10.1	7.87	60	1.97	0.037	4.4
38	Day 2 9AM Final		16.8	10.0	7.98		1.52	0.042	
45	Day 2 4PM Final		17.2	9.8	8.05		1.77	0.059	1.9
48	Day 2 Initial	149.3	16.5	10.0	7.92	52	1.90	0.045	4.7
62	Day 3 9AM Final		16.3	9.9	8.03		1.80	0.053	
69	Day 3 4PM Final		16.7	10.0	8.06		1.78	0.058	1.8
72	Day 3 Initial	133.6	15.4	10.2	7.93	56	2.00	0.044	4.3
86	Day 4 9AM Final		16.5	10.1	8.06		1.87	0.060	
93	Day 4 4PM Final		16.5	10.4	8.11		1.85	0.067	2.0
96	Day 4 Initial	128.9	15.5	10.2	7.85	56	2.06	0.039	5.5
110	Day 5 9AM Final		16.2	10.0	8.03		2.09	0.062	
117	Day 5 4PM Final		16.4	9.9	7.99		2.26	0.062	1.8
120	Day 5 Initial	131.6	15.9	10.3	7.84	52	1.92	0.036	5.1
134	Day 6 9AM Final		16.4	9.8	7.85		2.09	0.042	
141	Day 6 4PM Final		17.5	9.5	7.94		1.95	0.052	2.0
144	Day 6 Initial	134.9	16.3	10.1	7.85		1.98	0.039	6.5
158	Day 7 9AM Final		16.2	10.2	7.93		2.14	0.050	
162	Day 7 1PM Final		17.2	10.0	7.94		1.85	0.048	1.8

Table A20. Results of water quality measurements during Experiment II (July 2008) in treatment: 4.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	154.6	15.0	9.8	7.84	56	3.84	0.067	4.7
14	Day 1 9AM Final		17.4	9.6	7.94		2.14	0.056	
21	Day 1 4PM Final		18.0	9.3	7.94		2.44	0.067	2.5
24	Day 1 Initial	152.9	15.7	9.9	7.66	56	3.82	0.047	5.1
38	Day 2 9AM Final		16.5	10.1	7.88		3.04	0.065	
45	Day 2 4PM Final		16.7	9.8	7.99		4.08	0.114	2.0
48	Day 2 Initial	157.6	16.8	10.0	7.83	64	3.86	0.076	5.1
62	Day 3 9AM Final		16.6	9.9	8.00		4.00	0.113	
69	Day 3 4PM Final		16.7	9.8	8.00		3.54	0.101	1.9
72	Day 3 Initial	153	16.2	9.6	7.87	52	4.08	0.084	5.4
86	Day 4 9AM Final		16.5	10.1	8.01		3.92	0.113	
93	Day 4 4PM Final		16.5	10.4	8.07		4.18	0.137	2.0
96	Day 4 Initial	149.6	16.2	10.2	7.85	52	4.00	0.079	5.0
110	Day 5 9AM Final		16.4	10.0	8.00		4.06	0.113	
117	Day 5 4PM Final		16.7	9.6	7.91		2.75	0.064	1.9
120	Day 5 Initial	152	16.1	10.1	7.78	52	4.12	0.069	5.4
134	Day 6 9AM Final		16.4	9.8	7.88		4.18	0.089	
141	Day 6 4PM Final		17.5	9.5	7.97		4.00	0.113	1.9
144	Day 6 Initial	150.1	15.7	10.2	7.76		4.08	0.063	7.0
158	Day 7 9AM Final		16.2	10.2	7.88		4.14	0.087	
162	Day 7 1PM Final		17.4	9.9	7.89		3.52	0.082	2.0

Table A21. Results of water quality measurements during Experiment II (July 2008) in treatment: 8.00 mg/L Ammonia/um from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	186.5	15.0	9.8	7.83	56	8.20	0.140	5.2
14	Day 1 9AM Final		17.6	9.5	7.94		4.76	0.126	
21	Day 1 4PM Final		18.3	9.5	7.96		4.80	0.140	2.2
24	Day 1 Initial	187.1	15.7	9.7	7.85	56	7.72	0.145	5.0
38	Day 2 9AM Final		16.8	9.9	7.95		6.48	0.165	
45	Day 2 4PM Final		16.9	9.4	8.04		6.48	0.204	1.9
48	Day 2 Initial	197.5	16.0	9.9	7.93	52	8.16	0.188	5.6
62	Day 3 9AM Final		16.7	9.8	7.99		9.00	0.250	
69	Day 3 4PM Final		16.4	9.8	8.01		7.52	0.213	1.8
72	Day 3 Initial	185.3	15.4	10.1	7.87	52	8.32	0.160	4.9
86	Day 4 9AM Final		16.3	10.2	8.00		7.88	0.217	
93	Day 4 4PM Final		16.7	10.3	8.07		7.64	0.253	2.1
96	Day 4 Initial	186.8	16.4	10.0	7.83	52	8.16	0.155	4.8
110	Day 5 9AM Final		16.3	10.1	7.99		8.04	0.217	
117	Day 5 4PM Final		16.6	9.9	7.91		8.32	0.191	1.9
120	Day 5 Initial	181.9	16.2	10.5	7.76	52	7.88	0.126	5.0
134	Day 6 9AM Final		16.3	9.9	7.89		8.32	0.179	
141	Day 6 4PM Final		17.1	9.6	7.89		7.68	0.175	1.8
144	Day 6 Initial	185.2	15.7	10.5	7.79		8.36	0.137	6.5
158	Day 7 9AM Final		16.1	10.1	7.90		8.04	0.174	
162	Day 7 1PM Final		17.4	9.9	7.88		7.08	0.161	2.0

Table A22. Results of water quality measurements during Experiment II (July 2008) in treatment: 0.50 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	131.0	15.1	10.0	7.81	60	0.48	0.008	9.5
14	Day 1 9AM Final		17.5	9.6	7.93		0.36	0.009	
21	Day 1 4PM Final		18.6	9.5	7.99		0.42	0.013	2.2
24	Day 1 Initial	132.1	15.7	9.9	7.82	56	0.54	0.010	7.1
38	Day 2 9AM Final		17.0	9.8	7.94		0.55	0.014	
45	Day 2 4PM Final		16.9	9.5	8.08		0.53	0.018	2.0
48	Day 2 Initial	134.5	16.4	10.1	7.88	56	0.52	0.011	6.0
62	Day 3 9AM Final		16.8	10.0	8.05		0.63	0.020	
69	Day 3 4PM Final		16.2	9.9	8.07		0.58	0.019	1.8
72	Day 3 Initial	130.4	16.7	9.7	7.89	56	0.52	0.012	4.9
86	Day 4 9AM Final		16.2	10.1	8.07		0.56	0.018	
93	Day 4 4PM Final		16.3	10.3	8.12		0.46	0.017	1.9
96	Day 4 Initial	131.5	16.3	9.9	7.81	56	0.51	0.009	4.9
110	Day 5 9AM Final		16.1	10.1	8.04		0.61	0.018	
117	Day 5 4PM Final		16.2	10.1	7.99		0.88	0.024	1.8
120	Day 5 Initial	128.9	15.8	10.1	7.79	56	0.50	0.008	5.9
134	Day 6 9AM Final		16.3	9.9	7.95		0.62	0.015	
141	Day 6 4PM Final		16.8	9.7	7.96		0.60	0.016	1.6
144	Day 6 Initial	136.4	16.6	10.1	7.77		0.50	0.008	6.7
158	Day 7 9AM Final		16.0	10.2	7.97		0.66	0.017	
162	Day 7 1PM Final		17.2	9.7	7.95		0.54	0.014	1.6

Table A23. Results of water quality measurements during Experiment II (July 2008) in treatment: 1.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	139.7	15.2	10.1	7.76	60	0.95	0.014	7.3
14	Day 1 9AM Final		17.6	9.6	8.00		0.61	0.019	
21	Day 1 4PM Final		17.6	9.5	8.00		0.75	0.023	2.4
24	Day 1 Initial	144.3	15.6	10.1	7.80	60	1.00	0.017	6.6
38	Day 2 9AM Final		16.4	10.0	7.94		0.58	0.014	
45	Day 2 4PM Final		16.5	9.4	8.00		1.02	0.029	1.8
48	Day 2 Initial	145	16.5	9.8	7.87	56	0.96	0.020	4.5
62	Day 3 9AM Final		16.6	10.0	8.03		1.07	0.032	
69	Day 3 4PM Final		16.2	9.9	8.03		1.00	0.029	1.8
72	Day 3 Initial	143.2	16.1	9.8	7.86	60	0.97	0.019	5.2
86	Day 4 9AM Final		16.3	10.1	8.08		1.02	0.034	
93	Day 4 4PM Final		16.3	10.3	8.13		0.91	0.034	1.9
96	Day 4 Initial	141	16.3	10.3	7.84	60	0.98	0.019	5.8
110	Day 5 9AM Final		16.1	9.9	8.05		1.08	0.033	
117	Day 5 4PM Final		16.4	9.9	8.01		1.35	0.039	1.9
120	Day 5 Initial	170.7	16.1	10.3	7.78	60	0.97	0.016	5.7
134	Day 6 9AM Final		16.2	9.9	7.95		1.10	0.027	
141	Day 6 4PM Final		17.2	9.8	7.94		1.07	0.028	1.7
144	Day 6 Initial	147.2	16.5	10.3	7.77		0.97	0.016	6.4
158	Day 7 9AM Final		16.0	10.2	7.98		1.13	0.029	
162	Day 7 1PM Final		16.8	9.8	7.95		1.02	0.026	1.7

Table A24. Results of water quality measurements during Experiment II (July 2008) in treatment: 2.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	165.6	15.0	10.0	7.62	68	2.07	0.022	7.4
14	Day 1 9AM Final		17.2	9.5	7.86		1.31	0.028	
21	Day 1 4PM Final		18.1	9.3	7.95		1.39	0.039	2.2
24	Day 1 Initial	170.2	15.8	9.8	7.65	64	1.89	0.023	5.7
38	Day 2 9AM Final		16.7	9.8	7.89		1.63	0.036	
45	Day 2 4PM Final		16.8	9.7	8.00		1.94	0.056	1.9
48	Day 2 Initial	169.3	15.3	10.0	7.68	60	1.85	0.023	4.5
62	Day 3 9AM Final		16.7	9.8	7.95		1.84	0.047	
69	Day 3 4PM Final		16.1	9.8	7.97		1.85	0.047	1.8
72	Day 3 Initial	167.6	16.7	9.9	7.75	52	1.95	0.032	4.8
86	Day 4 9AM Final		16.3	10.1	8.05		1.87	0.058	
93	Day 4 4PM Final		16.3	10.3	8.08		1.94	0.064	2.0
96	Day 4 Initial	169.1	16.3	10.0	7.47	52	1.92	0.016	5.6
110	Day 5 9AM Final		16.2	9.9	8.03		1.89	0.055	
117	Day 5 4PM Final		16.4	9.8	7.90		2.25	0.050	1.9
120	Day 5 Initial	169.5	15.6	10.3	7.59	60	2.03	0.021	5.6
134	Day 6 9AM Final		16.2	9.9	7.93		2.07	0.049	
141	Day 6 4PM Final		17.0	9.6	7.87		2.01	0.044	1.6
144	Day 6 Initial	172.7	16.2	10.2	7.56		2.09	0.021	6.4
158	Day 7 9AM Final		16.0	10.2	7.93		2.17	0.050	
162	Day 7 1PM Final		16.9	9.8	7.86		2.00	0.042	1.7

Table A25. Results of water quality measurements during Experiment II (July 2008) in treatment: 4.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	212.2	15.1	10.2	7.54	68	3.86	0.034	7.6
14	Day 1 9AM Final		17.7	9.3	7.86		2.32	0.051	
21	Day 1 4PM Final		18.4	9.1	7.89		2.62	0.065	2.4
24	Day 1 Initial	217.7	15.8	10.1	7.48	64	3.80	0.031	6.2
38	Day 2 9AM Final		16.8	9.7	7.78		3.26	0.057	
45	Day 2 4PM Final		16.8	9.5	7.88		3.30	0.072	2.1
48	Day 2 Initial	214	16.0	10.0	7.57	64	3.70	0.038	5.0
62	Day 3 9AM Final		16.7	9.7	7.91		5.00	0.115	
69	Day 3 4PM Final		16.3	9.7	7.95		3.60	0.088	2.0
72	Day 3 Initial	216	16.2	9.8	7.51	64	3.80	0.034	5.5
86	Day 4 9AM Final		16.4	10.1	7.95		3.76	0.093	
93	Day 4 4PM Final		16.5	10.2	8.02		3.24	0.094	1.9
96	Day 4 Initial	220.9	15.9	10.0	7.54	64	3.88	0.036	5.8
110	Day 5 9AM Final		16.4	9.8	7.94		3.84	0.093	
117	Day 5 4PM Final		16.5	9.7	7.84		4.14	0.080	2.1
120	Day 5 Initial	219.5	15.1	10.7	7.48	64	6.02	0.046	5.9
134	Day 6 9AM Final		16.6	9.8	7.91		4.20	0.096	
141	Day 6 4PM Final		17.2	9.6	7.92		3.84	0.094	1.4
144	Day 6 Initial	226.5	16.3	10.4	7.49		4.06	0.035	6.3
158	Day 7 9AM Final		16.3	9.9	7.80		4.28	0.075	
162	Day 7 1PM Final		17.2	9.5	7.79		3.76	0.069	1.9

Table A26. Results of water quality measurements during Experiment II (July 2008) in treatment: 8.00 mg/L Ammonia/um from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Un-ionized Ammonia (mg/L)	Turbidity (NTU)
0	Day 0 Initial	245.0	15.0	10.0	7.26	80	7.92	0.037	6.9
14	Day 1 9AM Final		17.3	9.3	7.88		4.44	0.099	
21	Day 1 4PM Final		18.7	9.0	7.90		5.12	0.132	2.5
24	Day 1 Initial	315.5	15.1	10.1	7.25	80	8.16	0.037	6.1
38	Day 2 9AM Final		16.9	9.9	7.83		6.80	0.131	
45	Day 2 4PM Final		17.3	9.4	8.05		7.00	0.228	2.1
48	Day 2 Initial	339.1	17.0	10.1	7.34	76	7.88	0.050	5.3
62	Day 3 9AM Final		16.5	9.8	7.95		9.00	0.221	
69	Day 3 4PM Final		16.4	9.7	7.98		7.88	0.205	2.2
72	Day 3 Initial	320.2	15.1	10.1	7.29	80	8.44	0.042	6.1
86	Day 4 9AM Final		16.4	9.9	7.96		8.00	0.199	
93	Day 4 4PM Final		16.7	10.0	8.04		7.60	0.231	2.5
96	Day 4 Initial	331.5	15.8	10.3	7.15	80	7.96	0.030	5.8
110	Day 5 9AM Final		16.6	9.9	7.98		7.68	0.203	
117	Day 5 4PM Final		16.9	9.3	7.73		7.96	0.122	2.5
120	Day 5 Initial	333.5	16.5	10.1	7.23	76	8.20	0.039	5.3
134	Day 6 9AM Final		16.7	9.6	7.81		8.20	0.149	
141	Day 6 4PM Final		17.3	9.6	7.86		7.72	0.164	2.1
144	Day 6 Initial	340.4	16.6	10.3	7.22		7.96	0.037	5.5
158	Day 7 9AM Final		16.5	9.9	7.82		8.28	0.152	
162	Day 7 1PM Final		17.3	9.5	7.80		7.76	0.144	2.0

D. SRWTP Results of NPDES Testing

Table A27. Water quality data and results of WET testing performed by SRWTP.

Test Point	EFFLUENT FLOW-ACC 9	EFF NH3-N COMP	DFE pH AVG (Discharged)	DFE composite	DFE composite Temp	EFFLUENT TEMPERATURE	TSS	Turbidity (Average EOS)	Fathead WET (IC25)	96-hr FHM Flow-through Survival
Units	MGD	MG/L	pH	pH	Deg C	DEGF	MG/L	NTU	TUc	%
6/4/2008	143		6.4			75.0	8	5.9		
6/5/2008	144	26	6.4			75.3	9	7.6		100
6/6/2008	146		6.4			75.5	8	7.4		
6/7/2008	142.5		6.4			75.3	7	5.2		
6/8/2008	129.9	23	6.2			75.3	8	4.5		
6/9/2008	140.4		6.4			75.6	7	4.9		95
6/10/2008	143.4	26	6.4			75.9	8	5.1		
7/15/2008	149.3	24	6.2			78.7	8	4.7		
7/16/2008	144.2		6.3	6.6	7.9	78.8	9	5.3		95
7/17/2008	144.3		6.2	6.6	7.5	79.0		6.1		
7/18/2008	143		6.2	6.6	8.7	79.0	11	7.2	1.2	
7/19/2008	141.7		6.2	6.5	7.8	79.0	11	8.5		
7/20/2008	137.4	20	6.2	6.5	7.3	78.6	10	8.4		
7/21/2008	108.6		6.2	6.5	7.0	78.4	11	7.1		90
7/22/2008	168.7	22	6.2	6.5	6.5	78.2	7	4.7		

Comments and Responses

Comments received from Frances Brewster (SCVWD)

Thank you for the opportunity to comment on the UCD ATL ammonia report (Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009. The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt: Draft Final Report, dated January 28, 2009). In general, I think it is a very well written report. It clearly describes the test hypotheses, methods, results and conclusions without trying to conclude more than the data allow. Based on the study design and reported results, I agree with the conclusion that ammonia/um levels detected in the Sacramento River immediately downstream of the treatment plant are not likely to affect 7-d survival of 55-d old delta smelt. While the study did not evaluate chronic effects on Delta smelt, given the traditional use of acute-to-chronic ratios, including within the USEPA 1999 Update of Ambient Water Quality Criteria for Ammonia, I think the presentation of acute-to-chronic ratios is appropriate when presented with a clear description of the uncertainties. The report clearly describes those uncertainties and only suggests that measured concentrations may be chronically toxic to Delta smelt - a potential effect recommended for future study.

Importantly, I think the report does a good job describing the limitations of the study and what conclusions cannot be drawn from the results. The report clearly describes the many uncertainties that still exist including how chronic and sublethal effects, effects of multiple stressors and contaminant mixtures, and effects on different life stages could still be a concern. These uncertainties are presented with reference to findings in the published literature and on reasonable extrapolation to measured concentrations in the Delta.

Unfortunately, experiment II did not meet acceptability criteria. The report appropriately does not try to draw any conclusions from this test.

Finally, I think the report does a good job listing potential follow-up studies that could be conducted to address the many remaining uncertainties described in the report.

Sincerely,

Frances Brewster

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Comments received from Brian Finlayson (Ca DFG)

The study investigated the acute toxicity of ammonia to Delta smelt and other constituents in the Sacramento Regional Water Treatment Plant. The study's data suggests that ammonia concentrations present in the Sacramento River below the SRWTP were likely not acutely toxic to Delta smelt. Chronic toxicity of ammonia to Delta smelt was not investigated.

My comments fall into several categories: (1) Test protocol, (2) Results, (3) Quality Control/Quality Assurance, and (4) Discussion

1. Test Protocol

A. There is no toxicity test protocol in the report so it is hard to discern exactly how the tests were performed. A detailed protocol should be provided so others can reproduce the results.

Response: A detailed test protocol provided in Werner et al. (2008) was referenced in the text. This report is posted on the website of the Calfed Bay-Delta Program, http://www.science.calwater.ca.gov/pod/pod_reports.html

B. It is unclear as to why fish 40- to 60- day old fish were used when younger larvae are likely more susceptible to stressors, including ammonia? Riley and Finlayson (2004) used 9- to 14-old smelt successfully with > 90% survival of control groups.

Response: Dr. Finlayson did not specify the water quality parameters at which their young larvae were exposed. The water of the Sacramento River is characterized by very low conductivity (EC <200 µS/cm) and low turbidity in the summer. Prior to this study the ATL had determined that older larvae were more tolerant of these conditions than younger (<40 d) larvae, and we therefore chose to use older larvae to increase the likelihood of good control survival. Test duration in Dr. Finlayson's study was 96 h, while our tests run for 7 days, which likely makes a difference. In addition, the availability of delta smelt larvae at the time these tests were performed dictated the age group used. The available literature indicates that older fish may be more susceptible to ammonia than larvae, and we will be establishing age-dependent effect concentrations in 2009 using larval and juvenile fish.

C. It is unclear why the test duration was 7 days instead of 4 which is normal for acute tests. Seven-day duration chronic tests are used with other species to measure larval growth, but that was not done here.

Response: The original intent was to measure 7-d growth in addition to survival, but the size variability of the available delta smelt larvae was too great to detect significant differences in growth. We therefore abandoned this endpoint, but we are currently *working on establishing protocols for measuring abnormal swimming as a sublethal endpoint.*

D. It is unclear as to whether the smelt were fed during the tests, and if so, what were they fed and how much?

Response: During acclimation and testing, fish were fed three times a day with 1 mL of artemia and 1 mL of rotifers. A detailed test protocol is provided in Werner et al. (2008). This report is posted on the website of the Calfed Bay-Delta Program, http://www.science.calwater.ca.gov/pod/pod_reports.html

E. It is unclear why 60% survival in control groups was acceptable when ASTM (Designation E 729) and other acute test protocols normally require 90% survival of controls for test acceptability?

Response: Based on the sensitivity of delta smelt to handling stress, and the relatively unfavorable conditions (low EC, low turbitiy) in Sacramento River water, it was unrealistic to use test acceptability criteria for standard test species. The fish did well in hatchery water (higher EC) in both tests.

F. Was control mortality in the controls compensated for in the calculation of the LC50 values, and if so, how?

Response: The LC50 tests on ammonia/um were determined as part of our ongoing IEP investigations and results are presented in detail in our progress report submitted in September 2008 to the Department of Water Resources and IEP. Lethal 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). LC50s and EC50s were calculated using linear regression, non-linear regression, or linear interpolation methods. In addition each treatment was compared to control. For each analysis, the threshold for statistical significance was $p < 0.05$.

G. In section 3.5.1, why was a concentrated Nannochloropsis algae solution was added during acclimation? Was it added during the tests?

Response: It was added to increase turbidity (thus reducing stress) during acclimation to low E. During the exposure period, turbidity of hatchery control water was adjusted daily to 12 NTU using Nanno 3600™ to match delta smelt rearing conditions. Turbidity of the Low EC Control water was adjusted to match Garcia Bend water. This was not clearly stated in the original text, and we have included this information in the Final Report.

H. In section 3.5.2, Artemia were fed to smelt during the acclimation and tests on copper. Was this just for the copper reference tests or for all tests?

Response: Please see response to Question D.

2. Results

A. Section 4.1.1 – Normally, a control survival of 60% would suggest that test organisms are under stress. See comment on Section 4.1.1.

Response: Please see response to Question E.

B. Section 4.1.2 – Both reference tests had control survival less than 90%. Is it possible that the low survival of the controls influenced the sensitivity values?

Response: No, since all data is compared to the control values (see above).

3. Quality Assurance/Quality Control

A. Although there are no EPA-mandated requirements, it is generally accepted that conditions listed in ASTM E 729 and other EPA testing documents should be followed.

Response: We agree and have done so to the extent possible for delta smelt.

B. Although it is generally accepted that younger fish are more sensitive to most toxicants including copper, it is unclear from your reference tests that this is true for the small age span of the tested smelt; there is considerable overlap in 95% confidence intervals for the LC50 values of 54- and 44-day old fish. Generally, LC50 values within a factor of two can be attributed to variability and not necessarily differences in sensitivity.

Response: We agree, and have removed the paragraph discussing age-dependent toxicity of Cu.

4. Discussion and Conclusion

In discussing Hypothesis 1, the case is made that a chronic MATC value for Delta smelt larvae (using the maximum ACR for salmonids of 23.5 and Delta smelt larvae LC50 value of 0.15 mg/L) might be as low as 0.0064 ppm ammonia. This is certainly possible given this worse-case scenario, but is there any evidence of chronic ammonia exposure to Delta smelt in the Sacramento-San Joaquin Delta?

Response: This information is not available at this time. However, delta smelt hatch and rear in Cache Slough/Deep Water Channel, where elevated ammonia/um concentrations have been documented over the past 3 years.

Brian

Riley, F., and S. Finlayson. 1984. Acute toxicities of herbicides used to control water hyacinth and Brazilian elodea on larval Delta smelt and Sacramento splitail. Office of Spill Prevention and Reponse Admin Rept. 04-003. June 8, 2004.

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Comments received from Debra Denton (EPA)

Here are my brief summary comments on the UCD ammonia report (Werner, I., et al., 2009), "The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt: Draft Final Report, dated January 28, 2009". The report is very well written and describes the test methods, results and conclusions, along with areas of research to be considered for future work. Such future work includes the need to examine the chronic toxicity to Delta Smelt both as a single toxicant to ammonia and in combination with other toxicants from the Sacramento wastewater and other toxicants in the Sacramento River. It is important to ascertain the sublethal effects (such as growth, behavioral endpoints, swimming performance, etc) to sensitive fish life stages to ammonia/um. Swimming is a measure of performance in fishes, is a key factor in linking an organism's phenotypic character (e.g., genetic makeup, anatomy) with its use of environmenatl resources (e.g., food, oxyygen) for the overall reproductive output and survival of the individual and population (Wainwright, 1994). Predator prey studies are an important behavorial endpoint to assess, as concentrations above 0.34 mg/L with the predator (large mouth bass) and prey (mosquitofish) to ammonia, it was found that there was a lower rate of predation (Woltering et al., 1978). Additionally, the use of in-situ methods would be ideal along with traditional laboratory exposures to evaluate the ambient toxicity along with understanding the fate and transport of ammonia/um from the wastewater treatment plant. I support additional research along these lines and support the research efforts conducted by Dr Werner et al., as they are well suited to continue the some of these future research needs as the study proposes.

Wainwright PC. 1994. Functional morphology as a tool in ecological research. In Wainwright PC, Reilly SM, eds, Ecological Morphology. The University of Chicago Press. Chicago, IL, USA.

Woltering D., J Hedtke., L. Weber. 1978. Predator-prey interactions of fishes under the influence of ammonia. Trans. Am. Fish. Soc. 107, 500.

regards,
Debra

Disclaimer: This message was written with voice activated software. It may contain errors. Some of them might be interesting. Observe the context and the meaning will, hopefully, be obvious.

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General comments received from Stephanie Fong (CVRWQCB)

I found the conclusions well organized and clear. I appreciate the authors' attempts to stay within the bounds of the study hypotheses when reporting the results, and thought they clearly stated the limitations of interpreting their data in the discussion and conclusions. The future studies recommended were appropriate. Specific comments are attached and embedded in tracked changes.

Response: We appreciate the thorough review and incorporated Ms. Fong's comments and suggestions into the final report.

Comments received from Linda Dorn (SRCSD), letter attached as separate file.

Detailed SRCSD Comments on Draft ATL Report: The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt, Draft Final Report, January 28, 2009

TITLE
Comment 1: The title would be more accurate and informative by adding reference to **ammonia**, since it is the most significant component of this study. For example,

"*The Effects of Ammonia and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*"

rather than

"*The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*"

Response: We have changed the title to include SRCSD's version and further reflect the exact nature of the study to: "Acute toxicity of Ammonia/um and Wastewater ….."

SECTION 1 – EXECUTIVE SUMMARY

Comment 2: Second paragraph, last sentence. 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. Why is the field control/treatment control test acceptability criteria (TAC) not mentioned and how is it considered. This serious question of scientific validation has implications for future testing and the appropriate interpretation of these and other possible conditions for test acceptability need to be addressed.

Response: The "Low-EC Control" consists of hatchery water diluted to an EC that matches Sacramento River water, and is therefore the appropriate control to verify if delta smelt larvae were healthy enough to adjust to the water quality conditions of the test. It is true that the field control, which consists of Garcia Bend water used to prepare all other treatment solutions, is an appropriate control and should be considered. However, we have been very cautious in interpreting the test results and consider the high sensitivity of delta smelt larvae to low EC in Experiment II as a confounding factor.

The fact that these larvae were also considerably more sensitive to the reference toxicant, copper, confirms that they were more sensitive to stress(ors) than larvae used in Experiment I.

Additional confounding factors in Experiment II are that the addition of effluent caused differences in EC, pH and un-ionized ammonia at high ammonia/um concentrations. The mean EC in effluent treatments of 2, 4 and 8 mg/L ammonia/um (nom.) was 125, 142 and 170%, and calculated ammonia concentrations were 81, 78 and 71% of respective NH_4Cl *treatments. While statistical analysis could likely account for differences in ammonia, the effects of differences in EC are unknown. Taking this into account the survival results cannot be directly compared between effluent and* NH_4Cl *treatments, and thus the experiment should be repeated taking precautions to avoid most confounding factors. We included this information in the final report to present all reasons for rejecting this test.*

Comment 3: **Third paragraph, last sentence.** As stated above. I think you need to really consider the most appropriate scientific interpretation and test validation criteria. The treatment control (Garcia Bend) met TAC and is an appropriate surrogate for the low-EC control. This is the environmentally relevant control, and the appropriate experimental control. It is of secondary importance that the low-EC control failed TAC, because it is not really that important that delta smelt were sensitive to low EC in culture water, when they met TAC in the low EC treatment controls. A qualification may be necessary, but there is a great deal of good information in this test that can still be discussed. There also seems to be inconsistent weight on the TAC between this report and other reports from the ATL where smelt bioassay results are presented.

Response: Please see our response to Comment 2. This study is the first that uses TAC for delta smelt testing.

Comment 4: **Last paragraph, second sentence**. Either eliminate this sentence entirely or modify it to state that chronic testing of Delta smelt was not performed in this study. This study investigated the potential for acute ammonia and effluent toxicity near Freeport, not other parts of the Delta and not chronic toxicity. Is it really appropriate to make a concluding statement in this Executive Summary that was not part of the investigation?

Response: We included a statement that chronic testing was not performed in this study. We also changed the report title to reflect this information.

SECTION 2 – BACKGROUND

Comment 5: **First paragraph, last sentence**. Eliminate this sentence since it is not related to Delta smelt toxicity and is a controversial conclusion that should be evaluated by the CWT. The vague reference to toxicity is further misleading because 1) you couldn't be referring to acute toxicity when ambient concentrations are below acutely toxic concentrations, and 2) no Toxicity Identification Evaluations confirmed ammonia toxicity in the ambient samples you are referring.

Response: We have removed this statement, but to clarify: This information was derived from analysis of a 2-year data set and refers to chronic toxicity. TIEs can only be conducted if acute toxicity is detected.

Comment 6: **Fourth paragraph, third sentence**. Please present at mean ± error in addition to or instead of min-max. Grand Island has a large range in pH, but the central tendency is a more relevant descriptor.

Response: This information has been added.

SECTION 3.4 – PH DRIFT STUDY

Comment 7: Clarification should be stated whether the upward drift in pH was accounted for in the reported results, or was this pH drift neglected in the determination of LC50 and/or other endpoints. If pH drift was neglected, this section should state that the results presented in this report are therefore conservative, i.e. overstate the actual acute impacts of ammonia on Delta smelt.

Response: The determination of the LC50 and other endpoints was based on ammonia/um concentrations measured daily over the course of the experiment, therefore drift was accounted for and effect concentrations do not overstate actual acute impacts of ammonia.

SECTION 3.5 – TESTS WITH LARVAL DELTA SMELT

Comment 8: **First paragraph, second sentence**. "*According to the Ammonia Toxicity Sampling and Analysis Plan (2008), survival in both the hatchery and low EC control treatments must be at least 60% for test results to be considered acceptable*" As stated above, this is not correct or scientifically appropriate. Why isn't the Garcia Bend (treatment control) mentioned? What would be concluded if Garcia Bend controls fail the TAC in future tests? This needs to be resolved. Deviations and novel QA/QC situations should have also been discussed among the study team, since this represents a significant change from the conclusions written in the September 26, 2008 Draft Final report.

Response: Please see our response to Comment 2.

SECTION 4.1.1 – AMMONIA EXPOSURES

Comment 9: **Experiment II, first sentence**. Discounting the second test results in its entirety is not appropriate. See comments #2, 3, and 8. Please provide interpretation and qualification, if necessary, for these results. Consider that EC was lower in Garcia Bend controls than in low-EC controls, yet the smelt survived fine.

Response: We have added additional information on our reasons for rejecting this test to "conclusions" and "executive summary" sections. As previously pointed out in the results section, several confounding factors (see response to Comment 2) rendered the results of this test inconclusive.

Comment 10: **Experiment II, second paragraph**, **first sentence**. "*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.* " Please provide explanation as to why experimental results would be different from the pH drift study results? Note that pH was only reduced by 0.24 pH units. At a test temp of 17°C, this could change the NH3 proportion from 0.245 to 0.156.

Response: SRWTP effluent has a low pH and a relatively high salt content/conductivity (see Table 2), which at the higher ammonia/um concentrations altered these parameters in treatment waters. The drift study is independent of this and served the sole purpose of making sure there were no major changes in water quality parameters during exposure.

Comment 11: **Experiment II, second paragraph, second sentence**. "*Fish in the highest effluent treatment were therefore exposed to lower ammonia concentrations than fish exposed to the corresponding ammonium-chloride treatment.*" Max ammonia concentrations were still quite comparable and the mean only differed by 0.05. Yes this is worth commenting on, but please elaborate on the significance of this underestimation of ammonia. How do the other dilutions compare?

Response: We have provided this information in our response to Comment 2.

Comment 12: **Experiment II, second paragraph, last sentence**. Please comment on the extent of EC changes in treatments in addition to the highest and whether this could have affected test results.

Response: We have provided this information in our response to Comment 2, and included it in the report.

SECTION 4.1.2 – COPPER REFERENCE TOXICANT TESTS

Comment 13: First paragraph, first sentence. Please comment on the fact that copper reference test 2 didn't show significant differences from controls (at least up to 4 mg/L) despite the assumption that smelt were more sensitive.

Response: We are not sure we understand this comment, but to clarify: Our statement that delta smelt used in Experiment II were about twice as sensitive to copper than larvae used in Experiment I is based on effect concentrations calculated with measured copper concentrations (Table 6-2 and 7-2), which were derived using regression analysis. Our results show that the LC50 of larvae used in Experiment I was 110.9 ug/L Cu (diss.). For larvae used in Experiment II it was 58.3 ug/L Cu (diss.).

SECTION 4.2.1 – TESTS WITH LARVAL FATHEAD MINNOWS / AMMONIA/IUM EXPOSURES

Comment 14: First paragraph, first sentence. Please confirm and provide explanation why fathead minnow were not tested concurrently with the July smelt study?

Response: The decision to include a concurrent fathead minnow test was made on very short notice, and was not part of the work plan and budget for this study. It was not included with the second experiment partly for work-load and budgetary reasons.

SECTION 6 – DISCUSSION AND CONCLUSIONS

Comment 15: **Second paragraph, third sentence**. The actual ambient ammonia concentrations, pH and temperature conditions in the Sacramento River immediately downstream of the SRCSD discharge should be more clearly and accurately stated. A graphic showing those levels should be presented.

Response: We have replaced these numbers with the new information provided by SRCSD, and found and corrected the erroneous ammonia/um concentrations stated for Garcia Bend water as follows: "During the experimental period, Sacramento River water upstream of SRWTP at Garcia Bend had ammonia/um concentrations of <0.024 mg/L." We also added additional information from a 3 year monitoring effort conducted by UCD-ATL.

Comment 16: **Third paragraph.** The discussion should be clarified to avoid confusion between levels of total and un-ionized ammonia. A table should be created to categorize the values presented. Temperature and pH values should always be presented in tandem with ammonia LC50, NOEC or LOEC results. For instance, the range of LC50 values attributed to Eddy do not have associated pH and temperature values. You could also present a more balanced perspective by discussing the 0.21 mg/L un-ionized ammonia criteria mentioned by Eddy. But, for this study, relevant pH and temperature levels are those in the Sacramento River near the SRCSD discharge, since those are the levels that were tested.

Response: References for all numbers have been provided. In addition, we have simplified the text slightly to avoid confusion between ammonia and ammonia/um.

Comment 17: **Fourth, fifth and sixth paragraphs**. These paragraphs are not germain to the acute toxicity study which was designed and performed and should arguably not be included in this report at all. If included, they should be moved to Section 7 and modified.

Response: The CVRWQCB specifically requested a discussion of how results of this study relate to information in the relevant literature.

Comment 18: **Fourth paragraph, third sentence**. Do these pH and temperature extremes co-occur? What are the matching ambient pH and temp? These data should be available from the ATL 2006-present ambient sampling and testing, but I have not seen an evaluation of them compared to the EPA ammonia criteria.

Response: These pH and temperature extremes do not necessarily co-occur. We have used the range information provided in Werner et al. (2008) to cover the full range of possible conditions. However, a more detailed study of ammonia concentrations over diurnal and seasonal cycles is clearly needed.

Comment 19: Fourth paragraph. A discussion of the possibility that ammonia could exceed USEPA chronic criteria levels near Grand Island is presented. This statement assumes ammonia is conserved from Hood to Grand Is. Paired data are available to verify this possibility, as noted

in Comment 18. Further, it is not appropriate to use maximum observed data points and maximum observed pH values in combination when the USEPA chronic criterion is a 30-day average value and the preponderance of the data is below the cited maximums.

Response: We agree and have removed these sentences.

Comment 20: **Fifth paragraph.** It is not appropriate to use speculative, worst case acute-to-chronic ratios to then calculate chronic values for comparison with ambient data as the basis for assertions that chronic toxicity may be occurring in the Sacramento River. Again, the 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. Are you saying that EPA criteria are not adequately protective of fish in the delta? Again, a modified version of this discussion belongs in the uncertainty section.

Response: We have modified this paragraph to include the lower acute-to-chronic ratio, and to exclude ammonia concentration maxima reported for the Sacramento River.

Comment 21: **Fifth paragraph, fourth sentence**. Please comment on the fact that RBT LC50s are typically in the 10's to 20's mg/L NH3-N.

Response: We have already provided this information in paragraphs above and stated that the 96-h LC50 of 12 mg/L ammonia/um falls within the same range as salmonid (among them RBT) LC50s. This paragraph refers to unionized ammonia, and acute-to-chronic ratios.

Comment 22: **Fifth paragraph, fifth sentence**. I don't think this is correct. There is no ACR for delta smelt, so the values must be referring to a range or ACRs for rainbow trout. EPA does not report ACRs for Rainbow trout.

Response: That is correct. We combined information provided by EPA (1999) and Passell et al., which are both referenced in this paragraph.

Comment 23: **Fifth paragraph, eighth sentence**. Please clarify which chronic values "*above*" are being referenced?

Response: This refers to the discussion in the same paragraph. For clarity, we have changed the words "chronic values" to "chronic threshold".

Comment 24: **In the sixth paragraph.** This is a report on a fish toxicity study, not on invertebrates. Please remove this paragraph or move to the second on uncertainties and recommendations for further study. Statements regarding the toxicity to *Hyallela azteca* are also misleading. The USEPA chronic criteria considers invertebrates (including *H. azteca*) in the development of protective values. The negative correlation referenced in the paragraph is inconsistent with the ambient comparison to USEPA criteria near Hood. *H. azteca* data used in the EPA (1999) criteria are also based on 10 week life cycle test testing in low-ion water and the relevance of these results to shorter-term testing in Sacramento River water should be explained.

Response: Although this information was requested previously, we have moved it to the "Recommendations" section.

Comment 25: **Hypothesis 2.** The following statement "*We are unable to address this hypothesis, because experiment II did not meet test acceptability criteria*." is not true. Test 1 was designed to address both Hypothesis 1 and 2 even before test 2 was conceived. The first test unconditionally showed that there are no complicating effects from effluent/ammonia toxicity at and exceeding environmentally relevant concentrations. A major part of the planning discussions resulted in the agreement that these tests would be intended to answer questions about ambient conditions. The concentrations tested in test 1 already exceeded those that are relevant. Test 2 validation does need to be discussed and clarified. But, you can still interprets the results from test 2 in light of hypothesis 2, in concentrations up to 4 mg/L. Below which pH and EC effects from added effluent didn't confound the results.

Response: See our responses to several previous comments.

SECTION 7 – UNCERTAINTIES AND RECOMMENDATIONS FOR FUTURE STUDIES

Comment 26: Ammonia effects data presented in this section should be clarified by noting the pH and temperature conditions that were measured in the referenced studies. In the sections pertaining to multiple stressors or mixtures of water quality constituents, the relevance to conditions existing in the Delta should be addressed. For instance, the magnitude of the concentrations of different constituents plays an important role in effects cited in this discussion.

Response: This section addresses uncertainties, and the recommendations are clearly those of the authors. We do not believe that greater detail is required at this time.