



**Aquatic Toxicology Laboratory  
School of Veterinary Medicine  
University of California  
Davis, California**

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

**DRAFT FINAL  
REPORT**

I. Werner, L.A. Deanovic, M. Stillway, D. Markiewicz

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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 Toxicity 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.

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/ium). The two sources of ammonia/ium were 1) the SRWTP effluent, and 2) a concentrated stock solution of ammonium chloride (4,000 ppm). Experiment I consisted of five concentrations of ammonia/ium from  $\text{NH}_4\text{Cl}$  (0.25-4 mg/L) and 4 concentrations (0.25-2 mg/L) of ammonia/ium from SRWTP effluent. Experiment II consisted of four  $\text{NH}_4\text{Cl}$  treatments (1.0-8.0 mg/L ammonia/ium) and five SRWTP effluent treatments (0.5-8.0 mg/L ammonia/ium). The dilution water used for both test series was ambient water collected from the Sacramento River at Garcia Bend upstream from 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 distilled water to the conductivity of Sacramento River water (low-EC control). Exposure experiment I was conducted concurrently with larval delta smelt (*Hypomesus transpacificus*) 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 both culture facility and low-EC control water 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  $\mu\text{S}/\text{cm}$ ) was 91.7% and 81.3%, respectively. No significant effect on 7-d survival was detected in effluent and  $\text{NH}_4\text{Cl}$  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.

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. However, based on information provided by USEPA (1999) and other related studies, it is possible that concentrations below the SRWTP may be chronically toxic to delta smelt and other sensitive fish species.

## 2. Background

Potential sources of contaminants and their 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. Results of a recent study performed in the Sacramento-San Joaquin (SSJ) Delta suggest, that ammonia/ium may be contributing to aquatic invertebrate toxicity observed during 2006-2007, but to date there is no conclusive evidence for ammonia-related toxicity on delta smelt (Werner et al. 2008).

The term ammonia/ium refers to two chemical species which are in equilibrium in water ( $\text{NH}_3$ , un-ionized and  $\text{NH}_4^+$ , ionized) according to  $\text{NH}_3 + \text{H}^+ \rightleftharpoons \text{NH}_4^+$ . Tests for ammonia/ium usually measure total ammonia plus ammonium, while the toxicity is primarily attributable to the un-ionized form. In general, more ammonia and greater toxicity exist at higher pH, because its relative proportion increases with increasing pH according to the following equations (US EPA, 1985):

$$1 / 1 + 10^{\text{pKa}-\text{pH}} = \% \text{NH}_3$$

where:  $\text{pKa} = 0.0902 + [2729.9/(\text{°C}+273.2)]$

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/ium shows no correlation with temperature (US EPA, 1999). This is probably due to an increase in the permeability of biological membranes such as gills by a factor of 2-3 for each 10°C increase in water temperature (Eddy, 2005). In this report, we refer to the sum of ammonia and ammonium as ammonia/ium, and to the unionized 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 unionized ammonia) 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, while pH at Grand Island was 6.6-8.3. Water temperature in the river was 6.1-25°C (Werner et al. 2008). Treated effluent discharged into the river by SRWTP contains ammonia/ium at an average concentration of  $24 \pm 3.4$  mg/L (2006-2007), and maximum ambient concentrations in the Sacramento River downstream of the point of discharge are approximately 1 mg/L ammonia/ium. For 2007/08, SRWTP reports mean daily ammonia concentrations of  $0.0085 \pm 0.005$  mg/L (SRWTP, unpublished data). For comparison, the pH- and

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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/ium at pH 8.3 and T=24°C (0.079 mg/L ammonia), to 6.57 mg/L at pH 6.6 and T=0-14°C (0.0066 mg/L ammonia at 14 °C) (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/ium 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. Is is a collaborative effort between the Central Valley Regional Water Quality Control Board (CVRWQCB), the UC Davis Aquatic Toxicity Laboratory (UCD-ATL), and SRWTP.

The study addressed the following hypotheses:

1. Larval delta smelt survival is negatively impacted by ambient ammonia/ium 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/ium 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/ium. Concentrations selected were based on environmental relevance and ammonia/ium effect concentrations determined in a related study, where the 96-h LC50 for 50-d old delta smelt was 12 mg/L ammonia/ium (0.147 mg/L ammonia). The no observed effect concentration (NOEC) was 5 mg/L ammonia/ium (0.066 mg/L ammonia) (UCD-ATL, unpublished data).

The sources of ammonia/ium were 1) the SRWTP effluent, and 2) a concentrated stock solution of ammonium chloride (4,000 ppm NH<sub>4</sub>Cl). Experiment I consisted of five concentrations of ammonia/ium from NH<sub>4</sub>Cl (0.25-4 mg/L) and 4 concentrations (0.25-2 mg/L) of ammonia/ium from SRWTP effluent (Table 1). Experiment II consisted of four NH<sub>4</sub>Cl treatments (1.0-8.0 mg/L ammonia/ium) and five SRWTP effluent treatments (0.5-8.0 mg/L ammonia/ium). 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 (*Hypomesus transpacificus*) and larval fathead minnow (*Pimephales promelas*). *P. promelas* was used in Experiment I only.

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

<b>Experiment I June 5-12, 2008</b>	<b>Experiment II July 17-24, 2008</b>
Sac River at Garcia Bend (SRGB)	Sac River at Garcia Bend (SRGB)
SRGB w/ 0.25 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	SRGB w/ 1.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl
SRGB w/ 0.50 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	SRGB w/ 2.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl
SRGB w/ 1.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	SRGB w/ 4.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl
SRGB w/ 2.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	SRGB w/ 8.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl
SRGB w/ 4.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	
SRGB w/ 0.25 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	SRGB w/ 0.50 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP
SRGB w/ 0.50 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	SRGB w/ 1.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP
SRGB w/ 1.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	SRGB w/ 2.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP
SRGB w/ 2.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	SRGB w/ 4.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP
	SRGB w/ 8.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 of water from mid-channel in the Sacramento River at Garcia Bend (SRGB) in 5-gallon 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 gallons 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/ium exposure concentrations (Table 1) for the larval delta smelt and a parallel larval fathead minnow test. Each day of the experiment, a stock solution of ammonium chloride (15.352g/L NH<sub>4</sub>Cl) was used to prepare exposure solutions. Dilutions of SRWTP effluent were also prepared daily. After each solution was thoroughly stirred, total ammonia/ium 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<sub>3</sub>), alkalinity (mg/L as CaCO<sub>3</sub>), specific conductance (SC), dissolved oxygen (DO), and total ammonia/ium. Ammonia/ium was measured within 30 min. of sample receipt. Data are shown in Tables 2 and 3.

At test initiation, total ammonia/ium, 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/ium, 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/ium 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/ium 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 measurements obtained using the HACH AmVer™ Low Range Ammonia Test 'N Tube™ Reagent Set did not differ significantly from analytical chemistry measurements (R<sup>2</sup>=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/ium concentrations changed under experimental conditions. Over the 24-h period, the pH increased by an average of 0.37, and ammonia/ium 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.

Sample	Date	Test Day	Turbidity (NTU)	Ammonium (mg/L)	pH	Temp (°C)	Hardness (mg/L as CaCO <sub>3</sub> )	Alkalinity (mg/L as CaCO <sub>3</sub> )	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.

Sample	Date	Test Day	Turbidity (NTU)	Ammonia/ium (mg/L)	pH	Temp (°C)	Hardness (mg/L CaCO <sub>3</sub> )	Alkalinity (mg/L CaCO <sub>3</sub> )	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  $16 \pm 2^\circ\text{C}$  during transport. The control water used in both the ammonia/ium 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 is 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 is 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/ium Exposures

After arrival at UCD-ATL delta smelt used in ammonia/ium 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  $16^\circ\text{C}$ . 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. A more detailed description of the acclimation procedure is provided by Werner et al. (2007). 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 control and low conductivity control treatments. 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. 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/ium 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  $\mu\text{S}/\text{cm}$  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  $\mu\text{S}/\text{cm}$  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/ium, 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/ium, 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-gallon 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  $\mu\text{g}/\text{L}$   $\text{Cu}^{2+}$ , nominal) and a control. Concentrations were selected based on the previously determined 96-h LC<sub>50</sub> for larval delta smelt (85.2  $\mu\text{g}/\text{L}$   $\text{Cu}^{2+}$ ) 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

Concurrent tests with larval fathead minnow (*Pimephales promelas*) were conducted to compare delta smelt test results to a species commonly used in 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  $25 \pm 2^\circ \text{C}$ . De-ionized water amended with dry salts to EPA moderately hard standards 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/ium 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  $25 \pm 2^\circ \text{C}$  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 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 Exposures

Experiment I - June 5, 2008: 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  $\mu\text{S}/\text{cm}$ ) 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.

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  $\mu$ S/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 concentration 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  $\mu$ S/cm between the highest ammonium-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; SRWT= 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 (%) <sup>1</sup>	
	mean	se
Sacramento River at Garcia Bend (SRGB)	66.3	8.8
SRGB + 0.25 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	62.5	8.0
SRGB + 0.50 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	64.1	11.4
SRGB + 1.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	64.2	8.3
SRGB + 2.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	72.3	5.2
SRGB + 4.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	61.2	7.1
SRGB + 0.25 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	81.4	3.7
SRGB + 0.50 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	45.8	4.2
SRGB + 1.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	62.6	4.3
SRGB + 2.00 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	64.9	10.1
Low EC Control	81.3	7.1
Hatchery Water Control	91.7	3.4

<sup>1</sup> 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 (°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	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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/ium (mg/L)				Ammonia (mg/L) <sup>1</sup>				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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	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

<sup>1</sup> Unionized ammonia concentrations were calculated based on total ammonia/ium, pH and water temperature measured at test initiation.

Table 5-1. Percent survival of 43-d old delta smelt larvae after a 7-d test initiated 7/17/08; SRWT= 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 (%) <sup>1,2</sup>	
	mean	se
Sacramento River at Garcia Bend (SRGB)	65.0	8.7
SRGB + 1.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	47.5	6.3
SRGB + 2.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	60.0	7.1
SRGB + 4.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	75.0	2.9
SRGB + 8.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	40.0	12.9
SRGB + 0.5 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	55.0	5.0
SRGB + 1.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	50.0	4.1
SRGB + 2.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	47.5	4.8
SRGB + 4.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	60.0	5.8
SRGB + 8.0 mg/L NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from SRWTP	42.5	13.1
Low EC Control <sup>2,3</sup>	52.5	8.5
Hatchery Water Control	80.0	4.1

<sup>1</sup> The Low EC Control consisted of hatchery water diluted with distilled water to match SRGB conductivity.

<sup>2</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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/ium (mg/L)				Ammonia (mg/L) <sup>1</sup>				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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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

<sup>1</sup> Unionized ammonia concentrations were calculated based on total ammonia/ium, 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 ug/L Cu<sup>2+</sup>; 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<sup>1</sup>.

Treatment	Measured Cu <sup>2+</sup> Concentration (ppb)		Survival (%) <sup>2</sup>	
	Total	Dissolved	Mean	SE
Filtered Hatchery Water (FHW)	3	2	67	17.6
FHW + 27 ppb Cu <sup>2+</sup>	28	28	93	6.7
FHW + 53 ppb Cu <sup>2+</sup>	54	48	73	13.3
FHW + 106 ppb Cu <sup>2+</sup>	115	95	53	24.0
FHW + 213 ppb Cu <sup>2+</sup>	210	178	7	6.7

<sup>1</sup> 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	Estimate	Copper Concentration (ppb)			
		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) <sup>2</sup>				Temp (°C)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW) <sup>1</sup>	-	-	779	-	16.8	17.3	17.1	0.4
FHW + 27 ppb Cu <sup>2+</sup>	-	-	778	-	16.4	17.1	16.8	0.5
FHW + 53 ppb Cu <sup>2+</sup>	-	-	784	-	16.4	16.9	16.7	0.4
FHW + 106 ppb Cu <sup>2+</sup>	-	-	779	-	16.5	16.7	16.6	0.1
FHW + 213 ppb Cu <sup>2+</sup>	-	-	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) <sup>1</sup>	9.4	10.2	9.8	0.3	7.92	8.03	7.98	0.06
FHW + 27 ppb Cu <sup>2+</sup>	9.4	9.8	9.7	0.2	7.99	8.05	8.01	0.03
FHW + 53 ppb Cu <sup>2+</sup>	9.8	10.1	9.9	0.1	8.02	8.06	8.03	0.02
FHW + 106 ppb Cu <sup>2+</sup>	9.7	10.0	9.9	0.1	7.96	8.09	8.04	0.07
FHW + 213 ppb Cu <sup>2+</sup>	9.1	9.9	9.7	0.4	7.98	8.11	8.02	0.08

<sup>1</sup> 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/ium: 0.000 mg/L, Ammonia: 0.000 mg/L).

<sup>2</sup> 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<sup>1</sup>.

Treatment	Measured Cu <sup>2+</sup>		Survival (%) <sup>2</sup>	
	Concentration (ppb)		Mean	SE
	Total	Dissolved		
Filtered Hatchery Water (FHW)	2	2	78	11.7
FHW + 27 ppb Cu <sup>2+</sup>	38	37	72	6.0
FHW + 53 ppb Cu <sup>2+</sup>	98	89	7	6.7
FHW + 106 ppb Cu <sup>2+</sup>	149	136	7	6.7
FHW + 213 ppb Cu <sup>2+</sup>	269	242	0	0.0

<sup>1</sup> 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 (°C)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW) <sup>1</sup>	730	751	741	15	16.5	17.8	16.9	0.6
FHW + 27 ppb Cu <sup>2+</sup>	740	764	752	17	16.3	17.7	16.8	0.6
FHW + 53 ppb Cu <sup>2+</sup>	744	750	747	4	16.4	17.8	16.8	0.7
FHW + 106 ppb Cu <sup>2+</sup>	743	758	751	11	16.4	17.9	16.8	0.7
FHW + 213 ppb Cu <sup>2+</sup>	-	-	756	-	16.4	16.4	16.4	-

Treatment	DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW) <sup>1</sup>	9.0	9.8	9.6	0.4	7.77	8.04	7.94	0.12
FHW + 27 ppb Cu <sup>2+</sup>	9.1	9.6	9.5	0.2	7.86	8.00	7.94	0.06
FHW + 53 ppb Cu <sup>2+</sup>	9.4	9.8	9.6	0.2	7.86	8.04	7.98	0.08
FHW + 106 ppb Cu <sup>2+</sup>	9.1	9.8	9.5	0.3	7.84	8.01	7.92	0.08
FHW + 213 ppb Cu <sup>2+</sup>	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/ium: 0.04 mg/L, Ammonia: 0.001 mg/L).

## 4.2 Tests with Larval Fathead Minnow

### 4.2.1 Ammonia/ium 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  $\text{NH}_4\text{Cl}$  and diluted SRWTP effluent. Test was initiated 6/05/08.

Treatment	Survival (%) <sup>1</sup>	
	x	se
Sacramento River at Garcia Bend (SRGB)	100.0	0.0
SRGB + 0.25 mg/L $\text{NH}_3/\text{NH}_4^+$ from $\text{NH}_4\text{Cl}$	100.0	0.0
SRGB + 0.50 mg/L $\text{NH}_3/\text{NH}_4^+$ from $\text{NH}_4\text{Cl}$	100.0	0.0
SRGB + 1.00 mg/L $\text{NH}_3/\text{NH}_4^+$ from $\text{NH}_4\text{Cl}$	97.5	2.5
SRGB + 2.00 mg/L $\text{NH}_3/\text{NH}_4^+$ from $\text{NH}_4\text{Cl}$	100.0	0.0
SRGB + 4.00 mg/L $\text{NH}_3/\text{NH}_4^+$ from $\text{NH}_4\text{Cl}$	100.0	0.0
SRGB + 0.25 mg/L $\text{NH}_3/\text{NH}_4^+$ from SRWTP	100.0	0.0
SRGB + 0.50 mg/L $\text{NH}_3/\text{NH}_4^+$ from SRWTP	95.0	5.0
SRGB + 1.00 mg/L $\text{NH}_3/\text{NH}_4^+$ from SRWTP	100.0	0.0
SRGB + 2.00 mg/L $\text{NH}_3/\text{NH}_4^+$ 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 (°C)				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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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)				Unionized 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> from NH <sub>4</sub> Cl	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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 <sub>3</sub> /NH <sub>4</sub> <sup>+</sup> 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 tests were conducted with delta smelt twice during the study period, using copper (II) chloride ( $\text{CuCl}_2$ ) 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 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  $\mu\text{S}/\text{cm}$ ) to RT test conductivity (900  $\mu\text{S}/\text{cm}$ ). 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  $\text{CuCl}_2$  (27, 56, 106, and 213 ppb) with three replicates per treatment and five fish per replicate. Concentrations were based on the copper  $\text{LC}_{50}$  for delta smelt larvae determined in May 2008 at UCD-ATL. Test results yielded a  $\text{CuCl}_2$   $\text{LC}_{50}$  between 76-95 ppb (95% CI), with a NOEC of 37.5 ppb, and a LOEC of 75 ppb. This  $\text{LC}_{50}$  test was conducted using 49 d old delta smelt larvae. Reference toxicant tests conducted for this project were initiated following protocols identical to the  $\text{LC}_{50}$  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  $\text{LC}_{50}$  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 all test acceptability criteria. Test results yielded an  $\text{LC}_{50}$  of 39.84 ppb, with a NOEC of 27 ppb, and a LOEC of 53 ppb. As these RT tests met all test acceptability criteria, the delta smelt data for June and July, 2008, are considered reliable.

Although there are only three data points ( $\text{LC}_{50}$  and two reference toxicant tests), there is some indication that younger fish are more sensitive to  $\text{CuCl}_2$ . However, it is unknown whether age has any effect on smelt sensitivity to ammonia, and this warrants further investigation.

## 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<sub>50</sub> or EC<sub>25</sub> 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/ium 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/ium 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/ium, 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 approximately 1 mg/L ammonia/ium and  $0.0085 \pm 0.005$  mg/L ammonia (mean daily concentrations during 2007/08; SRWTP, unpublished data). During the experimental period, Sacramento River water upstream of SRWTP (Garcia Bend) had ammonia/ium concentrations of  $<0.17$  mg/L and ammonia concentrations of



<0.007 mg/L. Based on test results obtained in this and related studies, we conclude that average ammonia/ium concentrations reported for the Sacramento River immediately below SRWTP are about 3.6 times lower than the highest no observed effect concentration (NOEC) tested in this study, and are not likely to affect 7-d survival of 55-d old delta smelt larvae.

Results obtained to date are consistent with ammonia/ium 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 and LOEC for ammonia/ium were 5.0 mg/L and 9.0 mg/L, respectively, with an LC50 of 12.0 mg/L (pH 7.9, T=16°C). The 96-h NOEC and LOEC for ammonia were 0.066 and 0.105 mg/L, respectively, with an LC50 of 0.147 mg/L. Delta smelt larvae at 50 dph are >5-fold more sensitive to ammonia/ium than larval fathead minnow (UCD-ATL, unpublished data), 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/ium (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/ium and ammonia concentrations in the Sacramento River below SRWTP are therefore within a safety factor of approximately 10 based on acute effect concentrations reported for 50-d old delta smelt larvae and other sensitive fish species. It should also be noted that Thurston and Russo (1983) demonstrated that large rainbow trout were measurably more sensitive than other life stages.

Fate and transport of SRWTP effluent likely affects concentrations and potential toxicity of ammonia/ium discharged into the Sacramento River. SRWTP discharges treated effluent containing ammonia/ium at an average concentration of  $24 \pm 3.4$  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 a pH of 8.3 about 30 miles downstream at Grand Island with water temperatures as high as 25°C during summer months (Werner et al. 2008). Maximum ambient concentrations downstream of the point of discharge are approximately 1 mg/L total ammonia/ium. While this concentration is below pH- and temperature-dependent US EPA chronic water quality criteria (30-d average) for water bodies where early life stages of fish are present (US EPA 1999) at Hood, it could be above the US EPA water quality criterion at Grand Island at pH 8.3 and T=24°C. It is therefore possible that downstream river conditions with regard to pH and temperature could lead to violations of the US EPA chronic water quality criteria. However, biological uptake, adsorption to aquatic sediments and nitrification may reduce ammonia concentrations in the aquatic environment. More detailed studies of environmental conditions are needed before the risk of effluent-associated ammonia/ium toxicity to delta smelt can be accurately assessed.

Exposure duration is another important factor influencing the toxicity of ammonia. Acute 7-d toxicity tests, as performed in this study, are unable to detect the potential chronic effects of ammonia/ium 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/ium 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/ium sensitivity similar to delta smelt, have a ratio between 14.6 and 23.5, respectively (US EPA, 1999; Passell et al., 2007). If a safety factor of 23.5 were applied to acute ammonia effect concentrations for delta smelt larvae (ammonia 96-h LC<sub>50</sub>: 0.15 mg/L) then the resulting threshold concentration would be 0.0064 mg/L ammonia. Reported unionized ammonia concentrations in the Sacramento River immediately below the SRWTP are 0.0085±0.005 and would exceed potentially chronic safe values for delta smelt. During January-June 2008, maximum ammonia concentrations measured down the river at Hood and Grand Island (POD site 711) were 0.019 mg/L and 0.021 mg/L, respectively (Werner I., UCD-ATL, unpublished data). The chronic values derived above are similar to those reported by other studies. Dodds and Welch (2000) suggest that chronic effects of ammonia on fish may occur at concentrations as low as 0.005 mg/L.

The effects of ammonia/ium on most important invertebrate species of the SSJ Delta are presently unknown, but 2006-07 data for the SSJ Delta showed that ammonia/ium was negatively correlated with 10-day growth of the amphipod species *Hyaella 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/ium at 25°C and pH 7.94 (equal to 0.085 mg/L ammonia).

In conclusion, our study showed that ammonia/ium at levels detected in the Sacramento River was not acutely toxic to 55-d old delta smelt. However, based on information provided by USEPA (1999) and other related studies, it is possible that concentrations measured in the Sacramento River below SRWTP may be chronically toxic 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. This test should be repeated.

## 7. Uncertainties and Recommendations for Future Studies

Significant uncertainties remain with respect to the deleterious effects of ammonia/ium 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/ium. 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 unionized 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/ium can cause loss of equilibrium, hyperexcitability, increased respiratory activity and oxygen uptake, and increased heart rate. Increased ammonia/ium 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/ium.
- 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/ium toxicity to POD species spawning in these areas.

- Source analysis: Information on sources of ammonia/ium (agricultural, residential, atmospheric) in the Delta, in particular in the vicinity of important fish habitat should be generated.
- Information on toxic effects of ammonia/ium at lower trophic levels needs to be integrated and possibly generated to assess potential effects of reduced food availability on fish species of concern.
- Sources and concentrations of ammonia determined from characterizing spatial and temporal trends should be used to develop a fate and transport model for ammonia/ium (see Passell et al., 2007).
- More information is needed on the toxicity of ammonia/ium 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/ium 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.

## 8. References

- Buhl, K. J. 2002. The relative toxicity of waterborne inorganic contaminants to the Rio Grande silvery minnow (*Hybognathus amarus*) and fathead minnow (*Pimephales promelas*) in a water quality simulating that in the Rio Grande, New Mexico. Final Report to the U.S. Fish and Wildlife Service, Study Number 2F33-9620003. U.S. Geological Survey, Columbia Environmental Research Center, Yankton, South Dakota, USA.
- Dodds, W. K., and E. B. Welch. 2000. Establishing nutrient criteria in streams. *Journal of the North American Benthological Society* 19:186–196.
- Feyrer F., Nobriga M.L., Sommer T.R., 2007. Multidecadal trends for three declining fish species: habitat patterns and mechanisms in the San Francisco Estuary, California, USA. *Can. J. Fish. Aquat. Sci.* 64, 723-734.
- Howard D. Passell,1,4 Clifford N. Dahm, And Edward J. Bedrick 2007. Ammonia modeling for assessing potential toxicity to fish species in the Rio Grande, 1989–

- 2002, *Ecological Applications*, 17(7), 2007, 2007 By The Ecological Society Of America
- Huang C.-H., Sedlak D.I., 2001. Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. *Environmental Toxicology and Chemistry*, Vol. 20, No. 1, pp. 133–139.
- Inter-Agency Ecological Program (IEP). 2008. Pelagic Organism Decline Progress Report: 2007 Synthesis of Results. Prepared by Randall Baxter (DFG), Rich Breuer (DWR), Larry Brown (USGS), Mike Chotkowski (USBR), Fred Feyrer (DWR), Marty Gingras (DFG), Bruce Herbold (USEPA), Anke Mueller-Solger (DWR), Matt Nobriga (CALFED), Ted Sommer (DWR), and Kelly Souza (DFG). January 15.
- Mitsch, W. J., J. W. Day, and J. Gilliam, Jr. 2001. Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River basin: strategies to counter a persistent ecological problem. *BioScience* 51:373–388.
- Prenter J., MacNeil C., Dick J.T.A., Riddell G. E., Dunn A. M. 2004. Lethal and sublethal toxicity of ammonia to native, invasive, and parasitised freshwater amphipods. *Water Research* 38 (2004) 2847–2850.
- Stillway, M. 2008a. Quality Assurance Project Plan. Pelagic Organism Decline (POD). Effects of Toxic Contaminants on Invertebrates and Fish in the Sacramento-San Joaquin Delta. April 2008 – March 2010. Final Version 1.0. Prepared for the Department of Water Resources. 44 pages plus appendix.
- Stillway, M. 2008b. Sampling and Analysis Plan, Pelagic Organism Decline (POD). Effects of Toxic Contaminants on Invertebrates and Fish in the Sacramento-San Joaquin Delta. January 2008 – December 2009. Final Version 1.0. Prepared for the Department of Water Resources. 23 pages.
- Thurston R.V., Russo R.C. 1983. Acute toxicity of ammonia to rainbow trout. *Trans. Amer. Fish Soc.* 112:696-704.
- USEPA. 1985. Ambient water quality criteria for ammonia. EPA-440/5-85/001. Office of Water Regulations and Standards. Criteria and Standards Division. Washington, DC.
- USEPA. 1991. Technical support document for water quality-based toxics control. EPA/505/2-90-001. US Environmental Protection Agency, Office of Water. Washington, D.C.
- USEPA. 1999. Update of ambient water quality criteria for ammonia. EPA-822-R-99-014, US Environmental Protection Agency, Office of Water, Washington, D.C.
- USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. October. EPA-821-R-02-012, US Environmental Protection Agency, Office of Water, Washington, D.C.
- Werner I., L. Deanovic, D. Markiewicz, M. Stillway, N. Offer, R. Connon, S. Brander, 2008. Final Report. Pelagic Organism Decline (POD): Acute and Chronic

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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/ium 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 <sup>2</sup>	Slope	95% Interval	Confidence
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/ium 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

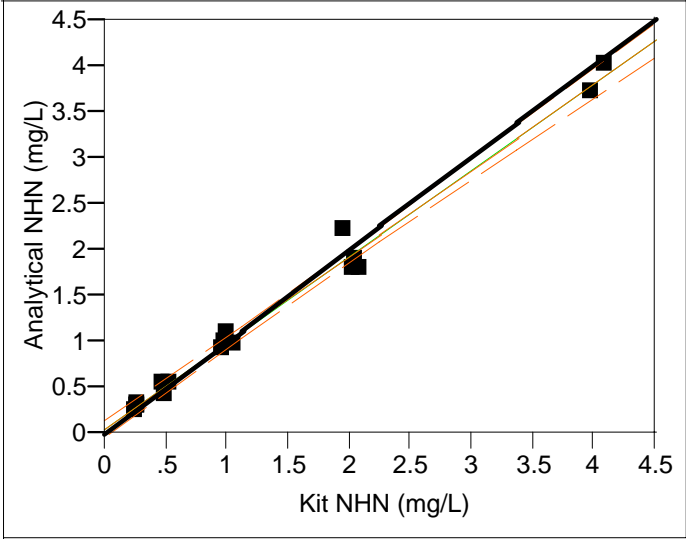


Figure A2. Regression of dataset #2

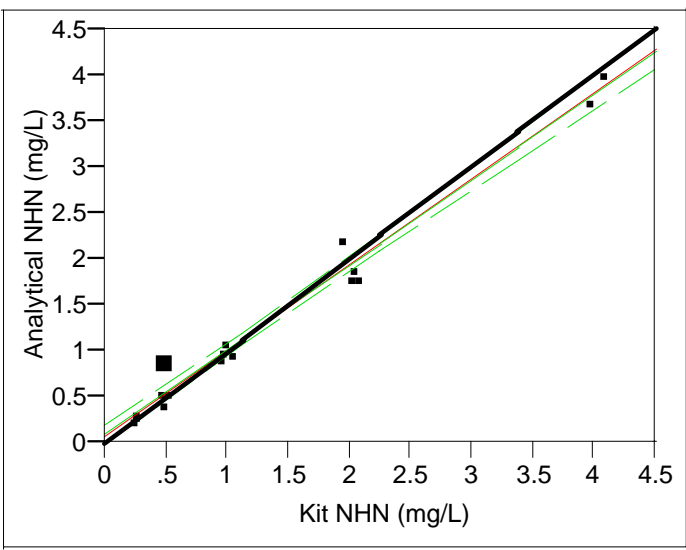
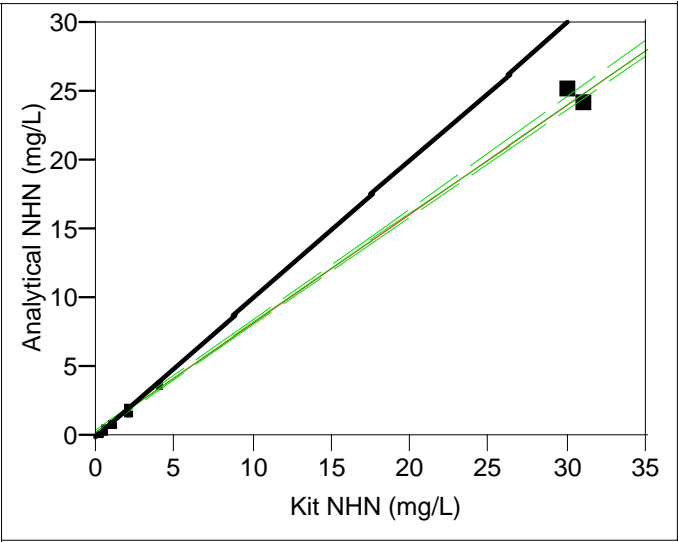


Figure A3. Regression of dataset #3



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## B. Results of the pH Drift Study

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

ID	Treatment	5/13/2008 17:00		5/14/2008 9:00		5/14/2008 17:30	
		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°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 <sub>3</sub> )	Ammonia /ium (mg/L)	Unionized 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 <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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 <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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 <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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 <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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 <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from Ammonia-Chloride.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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/ium from SRWTP Effluent.

Time (hrs)	Timepoint Name	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	Hardness (mg/L as CaCO <sub>3</sub> )	Ammonia Nitrogen (mg/L)	Unionized 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		100
6/5/2008	144	26	6.4			75.3	9	7.6		
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		95
6/9/2008	140.4		6.4			75.6	7	4.9		
6/10/2008	143.4	26	6.4			75.9	8	5.1		1.2
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		
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		
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		
7/22/2008	168.7	22	6.2	6.5	6.5	78.2	7	4.7	90	

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