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Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species

FINAL REPORT

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Table of Contents

		Page
1.	**EXECUTIVE SUMMARY**	4
2.	**BACKGROUND**	5
3.	**MATERIALS AND METHODS**	6
	3.1 Test Animals	6
	3.2 SRWTP Effluent Test	7
	3.2.1 Test Design	7
	3.2.2 Sample Preparation	8
	3.2.3 Water Quality Parameters	8
	3.3 Tests with Delta Smelt	9
	3.3.1 SRWTP Effluent Exposures	9
	3.3.2 Reference Toxicant Tests	11
	3.4 Tests with Larval Rainbow Trout	14
	3.4.1 SRWTP Effluent Exposures	14
	3.4.2 Reference Toxicant Tests	14
4.	**RESULTS**	15
	4.1 Tests with Delta Smelt	15
	4.1.1 SRWTP Effluent Exposures	15
	4.1.2 Reference Toxicant Tests	20
	4.2 Tests with Larval Rainbow Trout	20
	4.2.1 SRWTP Effluent Exposures	20
5.	**QUALITY ASSURANCE/QUALITY CONTROL**	25
	5.1 Reference Toxicant Tests with Delta Smelt	25
	5.2 Reference Toxicant Tests with Rainbow Trout	25
	5.3 Receiving Temperature	25
	5.4 Test Temperature	26
6.	**DISCUSSION AND CONCLUSIONS**	26
7.	**UNCERTAINTIES AND RECOMMENDATIONS**	27
8.	**REFERENCES**	29
DATA APPENDIX		31
RESPONSE TO COMMENTS		55

1. Executive Summary

This study was performed as a collaborative effort between the Central Valley Regional Water Quality Control Board (CVRWQCB) and the UC Davis Aquatic Toxicology Laboratory (UCD-ATL), with assistance from the Sacramento Regional Wastewater Treatment Plant (SRWTP), to assess the potential toxicity of treated wastewater effluent from the Sacramento Regional Wastewater Treatment Plant to larval delta smelt (*Hypomesus transpacificus)* and rainbow trout *(Onchorhynchus mykiss)*. Concurrent tests with rainbow trout were performed at AquaScience Inc., Davis, CA.

Following previous studies performed in 2008 and 2009 (Werner et al., 2009 a, b), three exposure experiments were conducted during April-June, 2010 to measure effect concentration of SRWTP effluent. Test concentrations were 4.5, 9, 18 and 28 % effluent. The dilution water used for testing was ambient water collected from the Sacramento River at Garcia Bend upstream of the SRWTP. Garcia Bend water was collected daily, one day prior to being used for testing throughout the 7-d exposure periods. 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 (UC Davis Fish Culture and Conservation Laboratory, Byron, CA), water from the Sacramento River at Garcia Bend , and delta smelt culturing facility water adjusted with de-ionized water to the conductivity of Sacramento River water (low-EC control). Experiments were conducted concurrently with larval delta smelt and larval rainbow trout. Reference toxicant tests were performed for both species to account for differences in organism sensitivity. Test protocols specified that delta smelt survival in hatchery water controls be at least 60 percent for the test results to be considered acceptable. For rainbow trout, the test acceptability criterion was 90 % survival in laboratory control water.

All tests met test acceptability criteria. There were no significant effects of effluent on survival of larval delta smelt or rainbow trout. Mean total ammonia/um and un-ionized ammonia concentrations in the highest test concentrations of effluent (28 %) were 6.12 - 7.82 mg/L and 0.076 - 0.144 mg/L, respectively. In the test initiated May 20, 2010, survival in some treatments containing Sacramento River water was lower than in the respective low conductivity control suggesting that toxicants were present in the river.

2. Background

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

The main source of ammonia/um in the lower Sacramento River is the Sacramento Regional Wastewater Treatment Plant (SRWTP). The term ammonia/um refers to two chemical species which are in equilibrium in water (NH_3, un-ionized and NH_4^+, ionized) according to $NH_3 + H^+ \Longleftrightarrow NH_4^+$. Total ammonia plus ammonium can be measured, but toxicity is primarily attributable to the un-ionized form, which can be calculated based on pH, conductivity and temperature of the water. In general, more un-ionized ammonia and greater toxicity exist at higher pH, because its relative proportion increases with increasing pH according to the following equations (US EPA, 1985):

$$1 / (1 + 10^{\,pKa-pH}) = \% NH_3$$

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

Throughout this report, we refer to the sum of ammonia and ammonium as ammonia/um, and to the un-ionized form as ammonia.

The Sacramento River drains into delta smelt spawning and larval nursery areas, thus toxicants present in river water could potentially affect early life stages of delta smelt found downstream. Werner et al. (2010) found maximum ambient ammonia/um and ammonia concentration of 0.59 mg/L (at Hood) and 0.025 mg/L (Cache Slough near the confluence with Lindsey Slough), respectively. During 2006-2010, ammonia/um and ammonia concentrations monitored in the Delta were consistently highest in the Sacramento River at Grand Island (POD site 711) and Hood, and in Cache Slough near the confluence with Lindsey Slough.

The pH-dependent US EPA acute water quality criteria (criterion maximum concentration, CMC) for ammonia/um when salmonids are present ranges and the pH- and temperature-dependent chronic water quality criteria (30-day average) for water bodies where early life stages of fish are present are provided in Table 1 (USEPA 1999). Corresponding ammonia concentrations were calculated for pH and temperature extremes measured in the Sacramento River (Werner et al. 2008) and a water conductivity of 150 µS/cm, which is commonly measured in the lower Sacramento River.

Table 1. US EPA Acute and Chronic Criteria for total ammonia/um for salmonids and fish early life stages present (USEPA, 1999) and corresponding calculated un-ionized ammonia concentrations at pH and temperature extremes measured in the Sacramento River at Hood.

	T (°C)	pH	Acute Criterion (mg/L)	30-d Chronic Criterion (mg/L)
Ammonia/um	24	8.3	4.37	0.827
"	6.1	6.6	46.84	6.57
Ammonia	24	8.3	0.396	0.075
"	6.1	6.6	0.024	0.0034

In 2008 and 2009, we showed that ammonia/um at levels reported for the Sacramento River below SRWTP (0.6 – 1 mg/L) were not acutely toxic to larval (47-51 DPH) and juvenile (149 DPH) delta smelt. However, toxicity of SRWTP effluent was higher than would be expected based on ammonia/um concentration alone, and it was concluded that additional unknown contaminants increased effluent toxicity to larval delta smelt. Effluent effect concentrations determined in 2009 were 25.7% (7-d LC50), 18.3% (LOEC) and 9% (NOEC). This corresponded to total ammonia/um effect concentrations of 5.4 mg/L (7-d LC50), 3.92 mg/L (LOEC) and 1.96 mg/L (NOEC). In order to determine the variability of SRWTP effluent toxicity three additional toxicity tests with larval delta smelt were conducted in 2010. The suitability of rainbow trout as a surrogate test species was also investigated.

The study presented here addressed the following questions:

1. What is the range of no (NOEC) and low (LOEC) effect ranges of SRWTP effluent mixed into Sacramento River water from Garcia Bend for delta smelt?
2. Can larval rainbow trout be used as a surrogate species, if needed, for future toxicity identification evaluations in place of delta smelt?

3. Materials and Methods

3.1 Test Animals

Larval delta smelt *(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. Containers were placed in coolers packed lightly with ice to maintain a temperature of 16 ± 2ºC during transport. At test initiation, fish were 47-48 days old their fork length was 17.5 ± 1.8 (mean ± SD; n=10; April 2010), 13.6± 0.23 (May 2010), and 12.7 ± 1.4 (June 2010) mm, with corresponding wet weights of 16 ± 7, 7 ± 3, and 5 ± 3 mg. Water from FCCL was used

for performance control, low conductivity control treatments and reference toxicant tests (filtered). This water was pumped directly from the intake channel of the H.O. Banks Pumping Facility near Byron, CA, then passed through a series of sedimentation beds containing natural vegetation to allow any suspended solids in the water to precipitate. It was then exposed to an ozonation system to eliminate potentially harmful microbes. Ozonated FCCL water was transported to UCD-ATL, and appropriate control waters were prepared for the test one day before fish were collected.

Larval rainbow trout (*O. mykiss*) were obtained from Thomas Fish Company (Anderson, CA). Upon receipt at the lab, fish were acclimated in a 10-gal glass aquarium to moderately hard (US EPA, 2002) laboratory control water for 24 h prior to reference toxicant tests, and 48 h prior to effluent tests. Temperature was maintained at 12 ± 2 ºC, and fish were fed with Silver Cup(TM) trout chow #1 crumble three times a day.

3.2 SRWTP Effluent Tests

3.2.1 Test Design

Exposure experiments (7 d) were initiated with larval delta smelt and larval rainbow trout on April 22, May 20 and June 17, 2010. They consisted of a series of increasing effluent concentrations (4.5, 9, 18, 28 %) and controls (Table 2). Concentrations selected were based on effluent effect concentrations determined in 2009. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for SRWTP effluent determined in 2009 were 9 and 18.3 %, respectively, corresponding to 1.96 and 3.92 mg/L total ammonia/um (Werner et al. 2009 b). The LC50s were 25.7 % effluent and 5.4 mg/L total ammonia/um. The dilution water used for all tests was ambient water collected from the Sacramento River at Garcia Bend, approximately 2 miles upstream from the SRWTP. Garcia Bend water was collected daily, one day prior to being used for testing throughout the 7-d test. SRWTP effluent in the form of 24-h composite samples was also collected daily. Delta smelt hatchery water served as the control treatment and performance control for tests with this species. Laboratory control water (DIEPAMH) served as control treatment and performance control for concurrent rainbow trout tests. Additional reference treatments were: 1. Water from Sacramento River at Garcia Bend; 2. Low EC/turbidity control consisting of hatchery water diluted with de-ionized water to match EC and turbidity of Sacramento River at Garcia Bend (delta smelt tests only), and 3. Low EC control consisting of laboratory control water (DIEPAMH) diluted with de-ionized water to match EC of Sacramento River at Garcia Bend (rainbow trout test only). A mixture of antibiotics directed at gram-negative and gram-positive bacteria was added in all tests with delta smelt.

Table 2. Treatment list for larval delta smelt and rainbow trout tests.

Treatment*	Delta Smelt (Flow-Through)	Rainbow Trout (Static Renewal)
Sacramento River at Garcia Bend (SRGB)	X	X
SRGB w. 4.5% SRWTP	X	X
SRGB w. 9% SRWTP	X	X
SRGB w. 18% SRWTP	X	X
SRGB w. 28% SRWTP	X	X
Low Conductivity/ Low Turbidity Control	Delta smelt hatchery water adjusted to match SRGB conductivity and turbidity	Deionized water adjusted to US EPA moderately hard specifications (DIEPAMH) at SRGB conductivity
Performance Control	Hatchery water at delta smelt rearing conductivity and 11 NTU	DIEPAMH
Reference Toxicant	Copper Chloride ($CuCl_2$)	Sodium Chloride (NaCl)

*Antibiotics were added to all delta smelt tests.

3.2.2 Sample Preparation

On seven consecutive days, CVRWQCB staff collected 55-60 gal (approx. 220 L) of water from mid-channel in the Sacramento River at Garcia Bend (SRGB) in 5-gal clear plastic cubitainers. Samples were collected using a battery-operated bilge pump with a 20 ft hose mounted on a buoy. The pump and hose were flushed with river water for a minimum of three minutes each day prior to collecting the samples. Cubitainers were rinsed with river water three times prior to filling. On the same day, 5-6 gal of SRWTP effluent (24-h composite sample) were provided by SRWTP in 1-gal amber plastic cubitainers. Samples were transported on ice to UCD-ATL. Within one hour of sample delivery to UCD-ATL, the SRWTP effluent from different cubitainers was composited in a large low density polyethylene (LDPE) or high density polyethylene (HDPE) container. Ambient SRGB water was composited in a 55 gal HDPE container. Subsamples were used to prepare ammonia/um exposure concentrations (Table 2) for the larval delta smelt and the parallel larval rainbow trout test. Dilutions of SRWTP effluent were prepared daily. After each solution was thoroughly stirred, total ammonia/um was measured.

3.2.3 Water Quality Parameters

Sample Receipt: The following water quality parameters were measured upon sample receipt: turbidity, pH, temperature, total hardness (mg/L as $CaCO_3$), alkalinity (mg/L as $CaCO_3$), specific conductance (SC), dissolved oxygen (DO), and ammonia/um. Ammonia/um was measured within 30 min of sample receipt. Resulting data are shown in Tables 3 a-c.

Effluent Test: Each day, total ammonia/um, hardness, alkalinity, pH, DO, electrical conductivity (EC), SC, turbidity and temperature were measured in fresh test solutions prior to animal exposure. During the test, ammonia/um, turbidity, pH, DO, EC and temperature were measured in exposure aquaria twice daily at 9:00 AM and 4:00 PM. For measurements during exposure, a subsample was obtained by pooling approximately 50 ml from each of the replicate tanks per treatment.

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

3.3 Tests with Delta Smelt

3.3.1 Effluent Exposures

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

After arrival of larval delta smelt at UCD-ATL, fish used in effluent and low conductivity control treatments were acclimated for two days to the specific conductance of the Sacramento River water collected at Garcia Bend. The transport buckets containing the fish were placed into a temperature-regulated water bath maintained at 16ºC. One-liter beakers were used to carefully collect fish from the buckets, and fish were gently poured into a glass pan containing water at a depth of approximately 2 cm. Fish were then gently scooped up using 100 mL beakers and released into 2.5-gal exposure tanks at random, by submerging the beaker and allowing fish to swim freely into the tanks. Ten to twelve fish were placed into each of the test tanks (4 replicates per treatment) containing 7 L of hatchery water for a 48-h EC acclimation period (Werner et al., 2008).

Fish in all tanks except the performance controls were acclimated with hatchery water diluted with distilled water to match the conductivity of SRGB, while the fish in the performance control treatment were acclimated to the exposure chambers at a conductivity matching the fish's rearing conditions. Nanno 3600™, a concentrated *Nannochloropsis* algae solution (68 billion cells per ml; Reed Mariculture, Inc. Campbell, CA) was added to increase the turbidity of the acclimation water to minimize stress. Antibiotics (Maracyn and Maracyn-2, Virbac AH Inc., Fort Worth TX) were added at the manufacturer's recommended dose throughout the acclimating and testing period. Final concentrations were 5.3 mg/L Maracyn (erythromycin) and 0.26 mg/L Maracyn-2 (minocycline). A more detailed description of the acclimation procedure is provided by Werner et al. (2008).

At test initiation, and after 4 d of exposure, water was drawn down from 7 L to approximately 2 L to allow for an accurate count of living fish. If more than 10 fish were alive in a replicate, the extra fish were counted, but were not removed from the tank in order to minimize handling stress. During the exposure period, water was renewed daily by means of a drip system at a rate of 1 mL/min. Turbidity of hatchery control water was adjusted daily to 11 NTU using Nanno 3600™ to match rearing conditions. Turbidity and EC of Low EC Control water was adjusted to match Garcia Bend conditions. Dead fish were counted and removed daily, as well as any excess food and detritus. The feeding behavior of fish was monitored throughout the duration of the test.

3.3.2 Reference Toxicant Tests

In accordance with the protocols agreed to by SRWTP, and described in the "2008 Ammonia Toxicity Sampling and Analysis Plan: The Effects of Wastewater Treatment Effluent Associated Contaminants on Delta Smelt", each test included a static renewal reference toxicant (RT) test with copper as the toxicant to monitor the sensitivity of delta smelt larvae. Fish from each batch of delta smelt larvae used for the effluent tests were exposed to a range of copper concentrations for 96 h.

Fish were acclimated for 24 h to test conditions in containers used for transportation from FCCL to minimize handling stress. Hatchery water was adjusted with Instant Ocean to an SC of 900 µS/cm and a pH of 7.9. These conditions as well as the acclimation period were chosen based on the conditions of previous copper LC50 studies to ensure comparability, and designed to mimic average conditions in the Delta.

Static renewal tests were performed with hatchery water filtered using a 1 micron filter and adjusted to an SC of 900 µS/cm and a pH of 7.9. Copper was dissolved in water and spiked into treatment solutions prior to test initiation and again on day 2, when 80% water was renewed. Tests were conducted in a water bath maintained at 16 °C, under low light conditions, using 1-gal black buckets with lids each containing 3.5 L of sample water. Lids were allowed to rest on top 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.

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

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

Table 3 a. 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 April 22, 2010.

Water	Test Day	Date	Temp (°C)	EC (µS/cm)	SC (µS/cm)	DO (mg/L)	pH	Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)	Total Cl2 (mg/L)	Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Sac. River at Garcia Bend	0	4/21/2010	10.2	124	169	10.4	7.67	0.32	0.003	0.12	31.30	68	68
Sac. River at Garcia Bend	1	4/22/2010	10.4	136	162	9.6	8.03	0.28	0.005	0.09	10.19	68	68
Sac. River at Garcia Bend	2	4/23/2010	13.1	127	166	9.3	7.85	0.02	0.000	0.19	24.73	68	68
Sac. River at Garcia Bend	3	4/24/2010	12.6	118	155	9.5	8.03	0.06	0.001	0.18	26.10	68	66
Sac. River at Garcia Bend	4	4/25/2010	17.6	123	144	8.9	7.82	0.00	0.000	0.16	33.30	60	60
Sac. River at Garcia Bend	5	4/26/2010	13.8	113	143	9.0	7.73	0.07	0.001	0.18	26.50	56	64
Sac. River at Garcia Bend	6	4/27/2010	14.0	117	148	8.4	7.9	0.02	0.000	0.08	13.30	68	62
SRWTP	0	4/21/2010	4.0	560	812	13.4	7.64	28	0.127	0.14	5.84	116	148
SRWTP	1	4/22/2010	8.2	573	845	9.6	6.94	30	0.038	0.15	6.19	120	156
SRWTP	2	4/23/2010	7.8	584	872	10.3	7.00	26	0.037	0.09	5.48	128	168
SRWTP	3	4/24/2010	16.6	639	755	9.2	7.02	28	0.081	0.09	4.65	124	160
SRWTP	4	4/25/2010	9.6	531	759	9.6	6.97	24	0.037	0.14	4.40	124	152
SRWTP	5	4/26/2010	13.2	579	747	8.5	6.97	23	0.046	0.06	4.39	128	160
SRWTP	6	4/27/2010	6.5	513	789	8.8	6.98	30	0.037	0.11	5.33	132	164

Table 3 b. 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 May 20, 2010.

Water	Test Day	Date	Temp (°C)	EC (µS/cm)	SC (µS/cm)	DO (mg/L)	pH	Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)	Total Cl2 (mg/L)	Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Sac. River at Garcia Bend	0	5/19/2010	19.7	120	134	9.1	7.93	0.00	0.000	0.08	5.95	52	60
Sac. River at Garcia Bend	1	5/20/2010	11.7	99	132	10.5	7.86	0.02	0.000	0.10	4.62	56	54
Sac. River at Garcia Bend	2	5/21/2010	14.4	114	144	10.0	7.85	0.01	0.000	0.08	8.42	56	56
Sac. River at Garcia Bend	3	5/22/2010	11.8	97	130	9.4	7.58	0.05	0.000	0.08	7.25	52	56
Sac. River at Garcia Bend	4	5/23/2010	15.3	106	130	9.0	8.14	0.03	0.001	0.08	7.86	52	54
Sac. River at Garcia Bend	5	5/24/2010	15.3	104	127	9.0	8.35	0.02	0.001	0.03	6.57	52	52
Sac. River at Garcia Bend	6	5/25/2010	15.7	101	123	8.9	8.23	0.04	0.002	0.08	5.81	48	54
SRWTP	0	5/20/2010	6.7	574	876	11.3	6.94	23	0.026	0.13	5.55	132	152
SRWTP	1	5/21/2010	13.7	700	897	10.2	7.23	25	0.094	0.10	6.33	356	174
SRWTP	2	5/22/2010	10.9	645	882	9.3	6.90	26	0.037	0.93	5.62	132	168
SRWTP	3	5/23/2010	12.8	635	831	9.2	7.07	24	0.058	0.08	6.14	116	142
SRWTP	4	5/24/2010	13.8	632	803	8.9	7.05	25	0.063	0.05	5.42	112	138
SRWTP	5	5/25/2010	13.8	629	796	9.0	7.10	26	0.073	0.09	5.59	112	140
SRWTP	6	5/26/2010	10.3	599	838	8.5	6.90	25	0.034	0.10	6.38	112	142

Table 3 c. 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 June 17, 2010.

Water	Test Day	Date	Temp (°C)	EC (µS/cm)	SC (µS/cm)	DO (mg/L)	pH	Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)	Total Cl2 (mg/L)	Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Sac. River at Garcia Bend	0	6/16/2010	9.6	100	133	8.9	7.64	0.03	0.000	0.06	4.43	42	46
Sac. River at Garcia Bend	1	6/17/2010	13.4	91	115	8.8	7.57	0.00	0.000	0.03	4.77	44	50
Sac. River at Garcia Bend	2	6/18/2010	16.0	95	115	8.6	7.53	0.02	0.000	0.05	7.49	40	46
Sac. River at Garcia Bend	3	6/19/2010	12.3	99	131	8.9	7.56	0.02	0.000	0.09	8.12	44	48
Sac. River at Garcia Bend	4	6/20/2010	9.4	92	130	8.7	7.76	0.00	0.000	0.07	6.73	48	48
Sac. River at Garcia Bend	5	6/21/2010	15.2	105	127	8.3	7.59	0.01	0.000	0.04	6.06	44	52
Sac. River at Garcia Bend	6	6/22/2010	15.8	115	138	8.6	7.76	0.02	0.000	0.07	5.86	48	52
SRWTP	0	6/17/2010	11.6	606	810	9.1	6.04	23	0.005	0.11	4.55	112	140
SRWTP	1	6/18/2010	8.4	573	840	8.8	6.79	24	0.022	0.06	5.46	136	142
SRWTP	2	6/19/2010	9.4	588	834	9.0	6.80	22	0.022	0.07	5.02	112	132
SRWTP	3	6/20/2010	7.4	546	826	8.3	6.88	21	0.022	0.11	4.61	108	126
SRWTP	4	6/21/2010	6.0	512	788	8.9	6.89	17	0.016	0.09	3.67	112	122
SRWTP	5	6/22/2010	4.8	465	728	9.7	7.10	20	0.028	0.07	4.15	112	122
SRWTP	6	6/23/2010	12.6	565	738	9.6	6.87	21	0.032	0.11	4.32	108	136

3.4 Tests with Larval Rainbow Trout

3.4.1 Effluent Exposures

Tests with larval 15-30 day old rainbow trout were conducted concurrently with delta smelt exposures. Test protocols followed procedures described in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (US EPA, 2002), except that the test duration was 7 d instead of the standard 4 d. De-ionized water amended with dry salts to US EPA moderately hard standards (DIEPAMH) was the laboratory control water used in these tests. For the 7-d test, the test acceptability criterion was 90% control survival.

Test treatments included four concentrations of SRWTP effluent as described above and in Table 1, a standard laboratory control, and a Low-EC control. Tests initiated with 15-30 day-old *O. mykiss* used four replicate 5-L low-density polyethylene plastic buckets with lids loosely attached, each containing 4 L of test solution and ten fish. Eighty percent of the test solution was renewed daily, at which time debris and dead animals were removed. Fish were fed one pinch of Silver Cup™ Trout Chow #1 crumble (provided by the *O. mykiss* supplier) per replicate daily, two hours prior to water renewal. Test chambers were incubated in a temperature-controlled water bath maintained at 12 ± 2 ºC under fluorescent and ambient light with a 16h light: 8h dark photoperiod. Mortality was recorded daily and at test termination. Water quality measurements (DO, pH, total ammonia and temperature) were measured daily using pooled subsamples from replicate beakers.

3.4.2 Reference Toxicant Tests

Reference toxicant tests with *O. mykiss* were conducted with each effluent test, however, they were initiated one day prior in order to match the timing of the delta smelt reference toxicant tests. Methods followed the 96-h acute toxicity test protocols established by US EPA (2002). Tests consisted of a standard laboratory control, and four increasing concentrations of sodium chloride. Eighty percent of the test solution was renewed after 48 h, at which time dead fish and debris was removed. Fish were fed three times daily with Silver Cup™ Trout Chow #1 crumble. Test chambers were incubated in a temperature-controlled water bath maintained at 12 ± 2 ºC under fluorescent and ambient light with a 16h light: 8h 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.

4. Results

4.1 Tests with Delta Smelt

4.1.1 SRWTP Effluent Exposures

All delta smelt tests met test acceptability criteria with survival in performance controls being ≥84.2. There were no significant effects of effluent on survival of larval delta smelt (Tables 4a-c and 5a-c). Mean total ammonia/um and un-ionized ammonia concentrations in the highest test concentration of effluent (28 %) were 6.12-7.82 mg/L and 0.076 - 0.144 mg/L, respectively. In the test initiated May 20, 2010, survival in treatments containing Sacramento River (SRGB) water was lower than in the respective low conductivity control (Table 4 b), suggesting that Sacramento River water alone was somewhat detrimental to delta smelt survival. Variability in these treatments was high, but the difference was statistically significant in the 9 and 28 % effluent treatments.

Table 4 a. Percent survival of 48-d old delta smelt larvae during a 7-d test initiated April 22, 2010; SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean.

Treatment	Mean Measured Total Ammonia/um (mg/L)	Mean Un-ionized Ammonia (mg/L)	96-h Survival (%)		7-day Survival (%)	
			Mean	SE	Mean	SE
SRGB	0.11	0.002	95.8	4.2	95.8	4.2
4.5% SRWTP	1.10	0.022	95.5	2.6	93.2	4.4
9 % SRWTP	2.13	0.041	97.7	2.3	95.6	2.5
18 % SRWTP	4.22	0.075	100.0	0.0	97.7	2.3
28 % SRWTP	6.58	0.106	97.5	2.5	95.4	2.7
Low EC Control	0.19	0.003	95.6	2.5	93.6	2.2
Hatchery Water Control	0.24	0.006	100.0	0.0	95.8	2.4

Table 4 b. Percent survival of 47-d old delta smelt larvae during a 7-d test initiated May 20, 2010; SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean.

Treatment	Mean Measured Total Ammonia/um (mg/L)	Mean Un-ionized Ammonia (mg/L)	96-h Survival (%)		7-day Survival (%)	
			Mean	SE	Mean	SE
SRGB	0.08	0.003	79.9	7.8	75.4	10.2
4.5% SRWTP	1.31	0.039	85.4	6.3	77.6	8.3
9 % SRWTP	2.54	0.064	82.8*	3.9	73.7*	4.0
18 % SRWTP	5.04	0.100	81.4	6.3	74.4	10.5
28 % SRWTP	7.82	0.144	84.0*	2.4	70.3*	1.5
Low EC Control	0.18	0.008	97.5	2.5	90.5	5.5
Hatchery Water Control	0.20	0.004	86.3	4.6	84.2	2.6

*significantly different from Low EC Control.

Table 4 c. Percent survival of 48-d old delta smelt larvae during a 7-d test initiated June 17, 2010; SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean.

Treatment	Mean Measured Total Ammonia/um (mg/L)	Mean Un-ionized Ammonia (mg/L)	96-h Survival (%)		7-day Survival (%)	
			Mean	SE	Mean	SE
SRGB	0.08	0.001	100.0	0.0	91.3	3.7
4.5% SRWTP	1.06	0.015	100.0	0.0	84.8	4.5
9 % SRWTP	2.06	0.028	97.9	2.1	87.5	5.4
18 % SRWTP	3.91	0.047	96.2	3.8	92.3	7.7
28 % SRWTP	6.12	0.076	98.1	1.9	93.5	2.2
Low EC Control	0.16	0.002	97.9	2.1	95.8	4.2
Hatchery Water Control	0.19	0.003	97.9	2.1	95.8	2.4

Table 5 a. Water quality parameters measured during the 7-day test with 48-d old delta smelt initiated April 22, 2010. SRWTP = Sacramento Regional Wastewater Treatment Plant

Treatment	Temp (°C)				EC (uS/cm)				DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend	15.0	16.8	16.0	0.5	117.9	299.7	186	59	7.9	10.3	9.2	0.6	7.71	8.10	7.89	0.08
4.5 % SRWTP	14.9	17.1	16.0	0.5	141.6	314.6	208	56	8.2	10.1	9.3	0.5	7.75	8.03	7.86	0.07
9 % SRWTP	15.0	16.9	15.9	0.5	165.1	334.6	230	53	8.3	10.3	9.3	0.5	7.67	8.03	7.85	0.09
18 % SRWTP	15.0	16.9	15.9	0.5	208.2	342.4	265	41	8.4	10.3	9.4	0.5	7.54	8.01	7.80	0.14
28 % SRWTP	14.9	16.8	15.9	0.5	255.9	382.8	314	37	8.2	10.3	9.3	0.5	7.41	8.01	7.76	0.17
Low EC Control	15.1	16.8	16.0	0.5	127.1	273.7	188	45	8.0	9.7	8.9	0.4	7.48	8.23	7.79	0.26
Hatchery Water Control	15.1	16.6	16.0	0.4	1119	1393	1167	58	8.0	10.0	8.9	0.5	7.77	8.28	7.99	0.16

Treatment	Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)				Turbidity (NTU)				Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R at Garcia Bend	0.00	0.32	0.11	0.08	0.000	0.007	0.002	0.002	6.16	33.3	12.63	7.71	65	65
4.5 % SRWTP	0.17	1.59	1.10	0.33	0.004	0.039	0.022	0.008	6.78	35.7	12.99	8.07	67	66
9 % SRWTP	1.32	3.06	2.13	0.57	0.022	0.076	0.041	0.014	3.60	33.9	12.03	8.02	71	73
18 % SRWTP	2.28	6.44	4.22	1.14	0.036	0.135	0.075	0.029	6.19	30.1	11.62	6.71	75	82
28 % SRWTP	4.00	10.04	6.58	1.67	0.045	0.230	0.106	0.046	6.00	25.3	10.82	5.87	82	95
Low EC Control	0.03	0.60	0.19	0.14	0.000	0.009	0.003	0.002	4.40	10.7	7.82	1.31	38	29
Hatchery Water Control	0.10	0.59	0.24	0.13	0.002	0.012	0.006	0.003	5.76	10.7	7.97	1.23	209	92

Table 5 b. Water quality parameters measured during the 7-day test with 47-d old delta smelt initiated May 20, 2010. SRWTP = Sacramento Regional Wastewater Treatment Plant

Treatment	Temp (°C)				EC (uS/cm)				DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend	14.9	16.7	15.8	0.4	103.8	239.3	141	34	8.4	10.8	9.4	0.6	7.39	8.38	8.10	0.27
4.5 % SRWTP	15.0	16.7	15.8	0.4	129.2	274.5	168	36	8.5	10.7	9.5	0.6	7.36	8.25	8.02	0.23
9 % SRWTP	14.9	16.6	15.8	0.4	156.4	288.1	194	34	8.5	10.8	9.6	0.7	7.47	8.19	7.96	0.17
18 % SRWTP	14.9	16.7	15.8	0.4	208.3	344.4	243	33	8.4	11.3	9.5	0.7	7.48	8.04	7.86	0.14
28 % SRWTP	15.0	16.6	15.8	0.4	249.6	322.6	289	20	8.3	10.7	9.5	0.7	7.39	8.14	7.83	0.19
Low EC Control	15.1	16.7	15.9	0.4	103.0	382.7	155	59	8.0	10.2	9.0	0.6	7.66	8.57	8.18	0.24
Hatchery Water Control	15.0	16.7	15.8	0.4	1078	1371	1118	60	8.1	10.1	9.0	0.6	7.40	8.26	7.90	0.20

Treatment	Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)				Turbidity (NTU)				Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R at Garcia Bend	0.00	0.18	0.08	0.05	0.000	0.011	0.003	0.003	2.37	12.0	5.49	3.32	53	54
4.5 % SRWTP	1.02	1.46	1.31	0.12	0.009	0.065	0.039	0.015	2.23	11.3	5.32	2.85	55	58
9 % SRWTP	1.80	2.88	2.54	0.27	0.022	0.102	0.064	0.021	2.54	10.2	5.41	2.59	61	63
18 % SRWTP	3.96	5.64	5.04	0.49	0.043	0.150	0.100	0.029	2.96	9.1	5.44	2.29	65	72
28 % SRWTP	6.76	8.76	7.82	0.59	0.054	0.253	0.144	0.052	3.12	8.9	5.20	2.03	71	79
Low EC Control	0.01	0.71	0.18	0.20	0.000	0.030	0.008	0.009	1.82	12.4	7.57	2.41	33	27
Hatchery Water Control	0.05	0.42	0.20	0.12	0.000	0.010	0.004	0.003	4.47	12.1	7.89	2.63	161	60

Table 5 c. Water quality parameters measured during the 7-day test with 48-d old delta smelt initiated June 17, 2010. SRWTP = Sacramento Regional Wastewater Treatment Plant

Treatment	Temp (°C)				EC (uS/cm)				DO (mg/L)				pH			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac River at Garcia Bend	14.5	16.5	15.9	0.4	86.4	157.5	129	18	8.8	10.3	9.4	0.5	7.51	7.93	7.71	0.12
4.5 % SRWTP	14.5	16.5	15.9	0.4	110.9	172.9	151	15	8.7	10.5	9.5	0.5	7.57	7.92	7.71	0.10
9 % SRWTP	14.5	16.4	15.9	0.4	140.3	194.1	174	15	8.8	10.5	9.5	0.5	7.49	7.93	7.69	0.11
18 % SRWTP	14.5	16.4	15.9	0.4	193.0	233.7	219	13	8.7	10.6	9.5	0.5	7.39	7.88	7.64	0.14
28 % SRWTP	14.5	16.5	15.9	0.4	238.8	294.3	271	15	8.9	10.5	9.5	0.5	7.25	7.91	7.63	0.22
Low EC Control	14.5	16.7	15.9	0.5	93.0	168.3	135	22	8.1	9.5	9.0	0.3	7.30	8.00	7.58	0.15
Hatchery Water Control	14.5	16.5	15.9	0.4	523.0	578.0	555	16	8.2	9.3	8.8	0.3	7.59	7.96	7.75	0.10

Treatment	Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)				Turbidity (NTU)				Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R at Garcia Bend	0.00	0.17	0.08	0.05	0.000	0.004	0.001	0.001	1.86	12.0	5.13	3.07	47	48
4.5 % SRWTP	0.73	1.40	1.06	0.18	0.009	0.024	0.015	0.004	1.65	10.2	5.04	2.61	49	60
9 % SRWTP	1.42	2.84	2.06	0.34	0.017	0.046	0.028	0.008	1.76	9.2	4.93	2.36	51	55
18 % SRWTP	2.32	5.36	3.91	0.77	0.021	0.070	0.047	0.013	1.78	9.2	4.83	2.21	56	61
28 % SRWTP	3.76	9.12	6.12	1.26	0.023	0.152	0.076	0.033	2.27	8.1	4.88	1.89	65	62
Low EC Control	0.00	0.59	0.16	0.17	0.000	0.006	0.002	0.002	1.71	9.4	6.67	1.83	27	37
Hatchery Water Control	0.03	0.42	0.19	0.13	0.000	0.006	0.003	0.002	5.72	12.2	9.21	2.19	98	58

4.1.3 Reference Toxicant Tests

All reference toxicity tests with copper met test acceptability criteria. Effect concentrations for copper (Table 6, Appendix Tables A1-A3) were within the same range as in previous years, when 96-h LC50s were 85-150 µg/L Cu^{2+} (Werner et al. 2009 b). Like in 2009, larvae obtained earlier in the year were less sensitive than those obtained later.

Table 6. Copper effect concentrations derived from reference toxicant tests with larval delta smelt used in testing during 2010.

RT Test	Fish Age (days old)	Control Survival (%)	NOEC (ppb)	LOEC (ppb)	LC50 (ppb)
April 21, 2010	47	100	106	213	141.3 (NA)
May 19, 2010	46	87	53	106	99.9 (28.6-190.4)*
June 16, 2010	47	100	53	106	83.2 (61.5-158.6)*

*95% Confidence Interval

4.2 Tests with Rainbow Trout

4.2.1 SRWTP Effluent Exposures

All rainbow trout tests met test acceptability criteria, with survival in performance controls being 100 %. No significant reduction in 7-d survival was detected (Tables 7 a-c, 8 a-c).

Table 7 a. Percent survival of 15-30-d old rainbow trout exposed for 7 d to SRWTP effluent diluted with Sacramento River water. The experiment was initiated April 22, 2010. SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean.; DIEPAMH = laboratory control water.

Treatment	96-h Survival (%)		7-d Survival (%)	
	Mean	SE	Mean	SE
Sacramento River at Garcia Bend (SRGB)	100.0	0.0	100.0	0.0
4.5% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
9% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
18% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
28% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
Low EC Control	100.0	0.0	100.0	0.0
DIEPAMH	100.0	0.0	100.0	0.0

Table 7 b. Percent survival of 15-30-d old rainbow trout exposed for 7 d to SRWTP effluent diluted with Sacramento River water. The experiment was initiated May 20, 2010. SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean.; DIEPAMH = laboratory control water.

Treatment	96-h Survival (%)		7-d Survival (%)	
	Mean	SE	Mean	SE
Sacramento River at Garcia Bend (SRGB)	100.0	0.0	100.0	0.0
4.5% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
9% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
18% SRWTP diluted with SRGB	97.5	2.5	97.5	2.5
28% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
Low EC Control	100.0	0.0	100.0	0.0
DIEPAMH	100.0	0.0	100.0	0.0

Table 7 c. Percent survival of 15-30-d old rainbow trout exposed for 7 d to SRWTP effluent diluted with Sacramento River water. The experiment was initiated June 17, 2010. SRWTP = Sacramento Regional Wastewater Treatment Plant; SRGB = Sacramento River at Garcia Bend; SE=standard error of the mean.; DIEPAMH = laboratory control water.

Treatment	96-h Survival (%)		7-d Survival (%)	
	Mean	SE	Mean	SE
Sacramento River at Garcia Bend (SRGB)	100.0	0.0	100.0	0.0
4.5% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
9% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
18% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
28% SRWTP diluted with SRGB	100.0	0.0	100.0	0.0
Low EC Control	100.0	0.0	100.0	0.0
DIEPAMH	100.0	0.0	100.0	0.0

Table 8 a. Water quality data for the 7-day effluent test with rainbow trout initiated April 22, 2010.

Treatment	Temp (°C)				EC (µS/cm)				DO (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac R Garcia Bend	11.7	13.4	12.7	0.5	113	159	128	17	6.2	11.0	9.0	1.7
4.5% SRWTP diluted with SRGB	11.6	13.8	12.7	0.6	132	179	150	18	6.1	10.9	8.9	1.7
9% SRWTP diluted with SRGB	11.8	13.6	12.8	0.5	152	208	172	21	6.0	11.1	8.8	1.9
18% SRWTP diluted with SRGB	11.7	13.4	12.7	0.5	191	250	214	24	6.4	11.0	8.8	1.8
28% SRWTP diluted with SRGB	11.6	13.3	12.6	0.5	236	306	263	28	6.0	11.2	8.8	1.9
Low EC Control	11.7	13.7	12.7	0.5	110	173	130	25	6.3	11.3	8.8	1.6
DIEPAMH	12.1	13.6	12.9	0.4	196	303	239	33	6.3	10.6	8.4	1.5

Treatment	pH				Ammonia Nitrogen (mg/L)				Un-ionized Ammonia (mg/L)				Mean Hardness (mg/L as $CaCO_3$)	Mean Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R Garcia Bend	7.31	8.31	7.71	0.28	0.01	1.03	0.35	0.34	0.000	0.007	0.003	0.002	65	65
4.5% SRWTP diluted with SRGB	7.24	8.13	7.67	0.27	0.81	2.36	1.43	0.43	0.009	0.024	0.015	0.005	67	66
9% SRWTP diluted with SRGB	7.26	8.23	7.65	0.27	1.62	3.48	2.46	0.52	0.011	0.057	0.027	0.014	71	73
18% SRWTP diluted with SRGB	7.29	8.20	7.62	0.23	3.68	6.00	4.67	0.89	0.025	0.128	0.046	0.027	75	82
28% SRWTP diluted with SRGB	7.34	7.95	7.56	0.16	5.80	8.60	7.05	1.17	0.041	0.109	0.057	0.017	82	95
Low EC Control	7.07	8.60	7.66	0.45	0.00	0.96	0.33	0.32	0.000	0.009	0.002	0.002	38	29
DIEPAMH	7.27	8.24	7.76	0.36	0.03	1.07	0.37	0.36	0.001	0.006	0.003	0.002	209	92

Table 8 b. Water quality data for the 7-day effluent test with rainbow trout initiated May 20, 2010.

Treatment	Temp (°C)				EC (µS/cm)				DO (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac R Garcia Bend	12.3	13.9	12.9	0.5	98	102	99	2	7.2	10.6	8.8	1.3
4.5% SRWTP diluted with SRGB	12.3	13.3	12.8	0.3	122	126	124	1	7.1	10.6	8.8	1.3
9% SRWTP diluted with SRGB	12.3	13.3	12.8	0.3	146	153	150	3	7.1	10.9	8.8	1.4
18% SRWTP diluted with SRGB	12.2	13.2	12.7	0.3	194	205	199	4	7.1	10.6	8.8	1.4
28% SRWTP diluted with SRGB	12.2	13.2	12.7	0.4	246	261	253	6	7.1	10.5	8.8	1.4
Low EC Control	12.2	13.2	12.8	0.4	97	112	104	5	7.4	10.3	8.7	1.1
DIEPAMH	12.2	13.7	12.9	0.4	217	234	229	6	7.6	10.0	8.5	0.8

Treatment	pH				Ammonia Nitrogen (mg/L)				Un-ionized Ammonia (mg/L)				Mean Hardness (mg/L as $CaCO_3$)	Mean Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R Garcia Bend	7.27	8.42	8.00	0.30	0.00	0.77	0.31	0.32	0.000	0.003	0.001	0.001	53	54
4.5% SRWTP diluted with SRGB	7.36	8.27	7.91	0.23	1.26	1.99	1.58	0.29	0.007	0.051	0.033	0.014	55	58
9% SRWTP diluted with SRGB	7.32	8.11	7.83	0.22	2.50	3.42	2.91	0.31	0.012	0.069	0.054	0.020	61	63
18% SRWTP diluted with SRGB	7.54	8.12	7.79	0.21	5.04	6.08	5.50	0.31	0.040	0.140	0.102	0.035	65	72
28% SRWTP diluted with SRGB	7.47	8.09	7.71	0.22	7.64	9.28	8.35	0.43	0.068	0.198	0.132	0.050	71	79
Low EC Control	7.36	8.69	8.07	0.38	0.00	0.85	0.37	0.35	0.000	0.005	0.002	0.002	39	29
DIEPAMH	7.11	8.52	7.87	0.33	0.03	0.74	0.34	0.30	0.000	0.007	0.002	0.003	90	63

Table 8 c. Water quality data for the 7-day effluent test with rainbow trout initiated June 17, 2010.

Treatment	Temp (°C)				EC (µS/cm)				DO (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Sac R Garcia Bend	11.9	13.8	12.8	0.6	85	112	100	10	7.9	10.5	9.3	1.1
4.5% SRWTP diluted with SRGB	11.8	13.4	12.7	0.5	110	143	122	11	8.0	10.5	9.3	1.1
9% SRWTP diluted with SRGB	11.9	13.7	12.8	0.6	133	163	145	11	7.7	10.6	9.3	1.1
18% SRWTP diluted with SRGB	11.8	13.6	12.7	0.6	181	206	190	9	7.8	10.6	9.3	1.2
28% SRWTP diluted with SRGB	11.8	13.8	12.9	0.6	176	246	229	24	7.6	10.6	9.2	1.2
Low EC Control	11.8	13.6	12.9	0.6	87	120	104	12	7.6	10.4	9.0	1.0
DIEPAMH	12.0	14.1	13.1	0.6	228	256	238	9	7.6	10.3	9.0	1.0

Treatment	pH				Ammonia Nitrogen (mg/L)				Un-ionized Ammonia (mg/L)				Mean Hardness (mg/L as $CaCO_3$)	Mean Alkalinity (mg/L as $CaCO_3$)
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
Sac R Garcia Bend	7.24	7.95	7.61	0.26	0.00	1.51	0.31	0.41	0.000	0.006	0.002	0.002	47	48
4.5% SRWTP diluted with SRGB	7.21	7.97	7.59	0.27	0.73	2.72	1.38	0.49	0.005	0.020	0.012	0.005	49	48
9% SRWTP diluted with SRGB	7.17	7.96	7.55	0.25	1.42	4.64	2.50	0.77	0.007	0.042	0.021	0.010	51	56
18% SRWTP diluted with SRGB	7.23	7.86	7.55	0.20	2.32	6.76	4.46	1.19	0.015	0.061	0.035	0.014	56	63
28% SRWTP diluted with SRGB	7.24	7.82	7.54	0.17	3.76	9.12	6.75	1.59	0.024	0.088	0.053	0.023	65	64
Low EC Control	6.97	8.05	7.40	0.32	0.00	0.74	0.29	0.29	0.000	0.003	0.001	0.001	34	25
DIEPAMH	7.21	8.12	7.68	0.33	0.00	0.75	0.29	0.29	0.000	0.004	0.002	0.001	85	61

5. Quality Assurance/Quality Control

All toxicity testing performed at UCD-ATL was supervised by the Project and Laboratory Managers to ensure data quality. The UCD-ATL Quality Assurance Officer has reviewed all work performed to date to assess its quality and credibility. The following is a summary of the QA/QC work completed during the 2010 project period.

5.1 Reference Toxicant Tests with Delta Smelt

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

For this project, 96 h reference toxicant tests were conducted using the same batch of delta smelt used to perform the ammonia/um exposure experiments. Tests with copper chloride were initiated 24 h prior to the initiation of ammonia exposures due to the shorter period of time required to acclimate the fish from rearing water conductivity (~1500 µS/cm) to RT test conductivity (900 µS/cm). Due to the sensitive nature of the delta smelt, fish are not held in the laboratory longer than necessary to minimize stress. All RT tests met test acceptability criteria, and effect concentrations fell within the same range as in 2009 (Werner et al. 2009 b). Results suggest that the organisms' response was consistent and data are reliable.

5.2 Reference Toxicant Tests with Rainbow Trout

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

5.3 Receiving Temperature

Water collected from the Sacramento River at Garcia Bend arrived at UCD-ATL with temperatures exceeding the USEPA criterion of 0-6^{o}C. Temperatures reflected environmental temperatures in the river, as the time between water collection and arrival at the laboratory was too short to cool the water to 6^{o}C. Upon arrival, water samples were stored in the dark at 0-4 ºC. It is unlikely that elevated receiving temperatures had a negative impact on sample integrity.

SRWTP effluent samples were received at UCD-ATL with temperatures exceeding the USEPA criterion of 0-6^{o}C on April 22, 2010, May 21-22, 2010, and June 17, 2010.

Receiving temperatures ranged from 6.6 to 9.6 ºC. Subsequently, additional ice was added to transport coolers, and subsequently receiving temperatures were within specified limits. Elevated sample receiving temperatures likely did not affect test results. While warm temperatures increase the chances of sample toxicant degradation, temperatures were relatively close to the US EPA criterion of 0-6 ºC, and samples were placed in cold storage in the dark immediately upon receipt.

5.4 Test Temperature

During the test initiated on May 20, 2010, the temperature-controlled water bath which held the delta smelt test malfunctioned on the evening of May 21, 2010, causing bath temperatures to drop to approximately 9 ºC by the morning of May 22, 2010. Water temperatures in test chambers dropped to approximately 9-10 ºC overnight, until this was remedied the following morning. This drop in test chamber temperature may have had minimal adverse effects on the fish utilized in the test; however treatment survival ranged from 70-91% and met all test acceptability criteria. Therefore the data from this test is considered reliable.

6. Discussion and Conclusions

Results from this project provide information on the acute toxicity of SRWTP effluent to larval delta smelt (47-48 d old) and rainbow trout (15-30 d old) during April-June 2010. Below we discuss our results in the context of the data and questions on which the experimental design for these tests was based, address uncertainties, and provide recommendations for future studies.

Question 1: What is the range of no (NOEC) and low (LOEC) effect ranges of SRWTP effluent mixed into Sacramento River water from Garcia Bend for delta smelt?

We did not observe the same level of toxicity as seen in our 2009 study. There was no significant reduction in larval delta smelt survival at effluent concentrations of ≤ 28 %. This concentration is above the concentration released by SWRTP to the Sacramento River.

The average test concentrations of ammonia/um in the 28 % effluent treatments were 6.12 - 7.82 mg/L, which was close to the 7-d LC50 for pure ammonia/um determined in 2009 (7.45 mg/L) and above the 7-d LC50 of ammonia/um in effluent (5.40 mg/L). Similarly, the highest un-ionized ammonia concentrations tested in this study (0.076 - 0.144 mg/L) were within the range of the 7-d LC50 determined (for effluent) in 2009, which was 0.090 mg/L. Acute 96-h LC50 values for larval delta smelt determined in previous years were 11.63 and 11.81 mg/L total ammonia/um, and 0.147 and 0.164 mg/L un-ionized ammonia at pH 7.9, T=16^{o}C, and EC=900 µS/cm.

Effect concentrations may vary in response to changes in effluent quality or unknown variations in delta smelt sensitivity, but the underlying reasons for the different results between tests conducted in 2009 and 2010 are not known. The fish used in toxicity tests were similar in age and size, and reference toxicant tests show that their sensitivity to copper was similar in both years, with 96-h LC50s of 80-150 µg/L Cu^{2+} in 2009 (Werner et al. 2009 b) and 83-141 µg/L Cu^{2+} in 2010. The size of fish used for effluent testing in 2010 was similar or smaller than those used in 2009. The average fork length of 47-48 day old delta smelt used in 2010 was 17.5 ± 1.8 mm (mean ± SD; n=10; April 2010), 13.6± 0.23 mm (May 2010), and 12.7 ± 1.4 mm (June 2010), with corresponding wet weights of 16 ± 7, 7 ± 3, and 5 ± 3 mg. The fork length of fish used in testing in 2009 was 17.8 ± 1.4 mm (mean ± SD; n=10; 2009; Joan Lindberg, UC Davis Fish Culture and Conservation Laboratory, Byron, CA, personal communication), similar to the fish used for testing in May 2010, however, weight information for fish used in 2009 is not available.

Based on test results obtained in this study, we conclude that average as well as maximum permissible SRWTP effluent concentrations (effluent: Sac River water 14:1) in the Sacramento River below SRWTP are not likely to affect 7-d survival of 47-d old delta smelt larvae.

Question 2: Can larval rainbow trout be used as a surrogate species, if needed, for future toxicity identification evaluations in place of delta smelt?

No acute toxicity was seen in following effluent exposures with larval rainbow trout or delta smelt. We are therefore unable to address this question.

Based on 96-h LC50 data, delta smelt larvae at the age of 47-51 DPH are about as sensitive to ammonia/um as rainbow trout (*Oncorhynchus mykiss*) with a species mean acute value of 11.23 mg/L ammonia/um at pH 8.0 (US EPA, 1999).

7. Uncertainties and Recommendations for Future Studies

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

(1) Acute and chronic effects on newly hatched larvae: Tests conducted in 2009 demonstrate that sensitivity of delta smelt to ammonia/um declines with increasing size and age, thus very early stages of delta smelt may be more vulnerable to this and potentially other effluent-associated contaminants. Such testing would require changes in hatchery rearing conditions to acclimate delta smelt to low conductivity water as found in the Lower Sacramento River/Cache Slough complex.

(2) Effects of multiple stressors. Many environmental factors can modify the toxicity of a single contaminant such as ammonia/um. Pre-exposure or simultaneous exposure to elevated temperature, disease, other contaminants 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.

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

Recommendations for Future Research

- Information should be generated on the susceptibility of early larval stages of delta smelt (<47 DPH) to ammonia/um and SRWTP effluent. Embryos should be reared under water quality conditions typical for the Lower Sacramento River, and exposed to a range of ammonia/um and effluent concentrations.
- Acute-to-chronic ratios should be established for delta smelt using sublethal endpoints such as swimming ability, histopathologic lesions and cellular responses.
- More information is needed on the toxicity of ammonia/um to fish when other stressors are present, in particular under conditions of food deprivation, elevated temperature, and in mixture with other contaminants, such as copper and current-use pesticides.
- Every attempt should be made to use ecologically significant, sublethal toxicity endpoints, such as growth, reproductive success, and swimming ability to evaluate the effects of ammonia/um on Delta fish species.
- Biomarkers can provide important information on biologically active toxicants present in the Delta and affecting species of concern, especially, if they can be linked to individual survival, growth or reproduction. Well characterized biomarkers should be integrated into monitoring efforts, especially where other sublethal endpoints (growth, behavior) are difficult to obtain.
- Where possible, *in situ* methods should be used to monitor ambient toxicity.

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Appendix

Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species

Reference Toxicant Summary Tables
and Water Quality Data

A. Delta Smelt Reference Toxicant Tests with Copper

Table A1 a. Results of a delta smelt 96-hour toxicity test initiated on 4/21/10 examining the toxicity of copper.

Treatment	24-hour Survival (%)		48-hour Survival (%)		72-hour Survival (%)		96-hour Survival (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Filtered Hatchery Water (FHW)	100	0	100	0	100	0	100	0
FHW + 53 ppb Copper	93	7	80	12	80	12	67	24
FHW + 106 ppb Copper	87	7	87	7	87	7	80	12
FHW + 213 ppb Copper	60	23	60	23	60	23	60	23
FHW + 300 ppb Copper	57	12	42	10	35	13	35	13

Table A1 b. Water chemistry during a delta smelt 96-hour toxicity test initiated on 4/21/10 examining the toxicity of copper.

Treatment	Temp (°C)				EC (uS/cm)				DO (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)	15.9	16.9	16.4	0.4	732	791	754	32	9.1	9.6	9.2	0.3
FHW + 53 ppb Copper	15.9	16.9	16.3	0.5	733	774	751	21	8.9	9.7	9.3	0.3
FHW + 106 ppb Copper	15.9	16.9	16.4	0.5	736	807	763	38	9.0	9.6	9.2	0.3
FHW + 213 ppb Copper	15.9	16.9	16.4	0.5	740	795	761	30	9.1	9.6	9.3	0.2
FHW + 300 ppb Copper	15.9	17.1	16.4	0.6	738	793	759	30	9.1	9.7	9.3	0.3

Treatment	pH				Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)	7.89	8.31	8.19	0.20	0.02	0.09	0.06	0.05	0.001	0.004	0.003	0.002
FHW + 53 ppb Copper	7.90	8.26	8.17	0.18	0.02	0.05	0.04	0.02	0.001	0.002	0.002	0.001
FHW + 106 ppb Copper	7.88	8.29	8.16	0.19	0.04	0.09	0.07	0.04	0.002	0.005	0.003	0.002
FHW + 213 ppb Copper	7.90	8.31	8.17	0.18	0.00	0.07	0.04	0.05	0.000	0.004	0.002	0.003
FHW + 300 ppb Copper	7.92	8.27	8.15	0.16	0.02	0.16	0.09	0.10	0.001	0.008	0.004	0.005

Table A2 a. Results of a delta smelt 96-hour toxicity test initiated on 5/19/10 examining the toxicity of copper.

Treatment	24-hour Survival (%)		48-hour Survival (%)		72-hour Survival (%)		96-hour Survival (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Filtered Hatchery Water (FHW)	100	0	100	0	87	7	87	7
FHW + 53 ppb Copper	93	7	93	7	80	12	73	18
FHW + 106 ppb Copper	87	13	80	12	67	24	40	20
FHW + 213 ppb Copper	33	7	20	12	13	7	13	7
FHW + 300 ppb Copper	30	15	8	8	8	8	8	8

Table A2 b. Water chemistry during a delta smelt 96-hour toxicity test initiated on 5/19/10 examining the toxicity of copper.

Treatment	Temp (°C)				EC (uS/cm)				DO (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)	15.2	16.6	15.9	0.6	757	774	763	8	8.3	9.4	9.0	0.5
FHW + 53 ppb Copper	15.3	16.3	15.9	0.4	757	761	759	2	8.2	9.3	8.9	0.5
FHW + 106 ppb Copper	15.5	16.2	15.9	0.3	747	767	755	9	8.6	9.2	9.0	0.3
FHW + 213 ppb Copper	15.6	16.1	15.9	0.2	747	779	761	13	8.6	9.3	9.1	0.3
FHW + 300 ppb Copper	15.7	16.3	16.0	0.3	755	775	766	10	9.0	9.5	9.2	0.2

Treatment	pH				Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)	8.02	8.13	8.08	0.05	0.00	0.11	0.05	0.06	0.000	0.004	0.002	0.002
FHW + 53 ppb Copper	7.98	8.10	8.06	0.05	0.00	0.07	0.04	0.04	0.000	0.002	0.001	0.001
FHW + 106 ppb Copper	7.94	8.09	8.05	0.07	0.00	0.07	0.03	0.04	0.000	0.002	0.001	0.001
FHW + 213 ppb Copper	7.94	8.17	8.06	0.09	0.00	0.08	0.04	0.04	0.000	0.003	0.001	0.001
FHW + 300 ppb Copper	7.96	8.14	8.08	0.09	0.00	0.07	0.04	0.04	0.000	0.002	0.001	0.001

Table A3 a. Results of a delta smelt 96-hour toxicity test initiated on 6/16/2010 examining the toxicity of copper.

Treatment	24-hour Survival (%)		48-hour Survival (%)		72-hour Survival (%)		96-hour Survival (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Filtered Hatchery Water (FHW)	100	0	100	0	100	0	100	0
FHW + 53 ppb Copper	100	0	100	0	100	0	93	7
FHW + 106 ppb Copper	87	7	33	13	33	13	27	18
FHW + 213 ppb Copper	33	7	7	7	0	0	0	0
FHW + 300 ppb Copper	40	20	7	7	0	0	0	0

Table X. Water chemistry during a delta smelt 96-hour toxicity test initiated on 6/16/10 examining the toxicity of copper.

Treatment	Initial EC (uS/cm)	Temp (°C)				pH			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)	743	15.8	16.9	16.4	0.5	7.83	7.90	7.86	0.03
FHW + 53 ppb Copper	717	15.7	16.4	16.1	0.3	7.78	7.92	7.85	0.06
FHW + 106 ppb Copper	737	16.0	16.7	16.3	0.3	7.82	7.90	7.85	0.04
FHW + 213 ppb Copper	869	15.9	16.3	16.1	0.2	7.79	7.82	7.81	0.02
FHW + 300 ppb Copper	741	15.9	16.3	16.1	0.2	7.76	7.91	7.82	0.08

Treatment	DO (mg/L)				Ammonia Nitrogen (mg/L)				Unionized Ammonia (mg/L)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Filtered Hatchery Water (FHW)	8.7	9.7	9.1	0.4	0.00	0.20	0.11	0.09	0.000	0.200	0.002	0.002
FHW + 53 ppb Copper	8.8	9.1	9.0	0.1	0.07	0.12	0.10	0.03	0.001	0.120	0.002	0.000
FHW + 106 ppb Copper	8.9	9.3	9.1	0.2	0.07	0.15	0.12	0.04	0.001	0.150	0.003	0.001
FHW + 213 ppb Copper	8.8	9.2	9.1	0.2	0.07	0.08	0.08	0.01	0.001	0.080	0.001	0.000
FHW + 300 ppb Copper	8.8	9.2	9.0	0.2	0.05	0.10	0.08	0.04	0.001	0.100	0.001	0.001

B. Water Quality Data

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 5 a. Effect of 96-h exposure to copper on percent survival of 47-d old delta smelt larvae. This test was initiated on April 21, 2010.

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

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

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

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

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

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

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

FHW + 106 ppb Cu^{2+}	40	23.1
FHW + 213 ppb Cu^{2+}	13	6.7

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

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

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

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

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

Treatment	96-hr Survival (%)	
	mean	se
Filtered Hatchery Water (FHW) @ 900 uS/cm	93	6.7

FHW + 27 ppb Cu^{2+}	80	11.5
FHW + 53 ppb Cu^{2+}	67	6.7
FHW + 106 ppb Cu^{2+}	33	17.6
FHW + 213 ppb Cu^{2+}	20	20.0

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

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

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

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

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

Treatment	96-hr Survival (%)	
	mean	se

Filtered Hatchery Water (FHW) @ 900 uS/cm	100.0	0.0
75 ppb Cu^{2+}	93.3	6.7
150 ppb Cu^{2+}	13.3	13.3
300 ppb Cu^{2+}	6.7	6.7

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

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

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

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

Responses to Comments

Comments on the Draft Report submitted August 23, 2010, were received from representatives of the Sacramento Regional County Sanitation District (SRCSD). The document provided by SRCSD and responses to comments can be found below.

Comments 1-3: We use maximum concentrations and pH and T extremes to account for worst case scenarios, which must be considered in environmental regulation. However, SRCSD comments and associated data provided are included in the final report.

Comment 4. We agree that this paragraph is somewhat confusing and have added "…suggesting that Sacramento River water alone was somewhat detrimental to delta smelt survival." We hope that this, along with the data in the respective table, is now easy to understand.

Comments 5, 6. We are not familiar with the term "unbounded NOEC" and will therefore abstain from including the text suggested by the reviewers. However, we added "Effect concentrations may vary in response to changes in effluent quality or unknown variations in delta smelt sensitivity…"

Comment 7. See above.

Comment 8. We included "…especially, if they can be linked to individual survival, growth or reproduction."

Comment 9. Much to our regret, the *in situ* tests with larval delta smelt performed by Werner et al. were unsuccessful. This species is too delicate to expose in currently available *in situ* systems.

Comment 10. Seven-day LC50s were determined in 2009 and reported in Werner et al. 2009 b.

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September 21, 2010

Dr. Inge Werner
Aquatic Toxicology Program
School of Veterinary Medicine
Department of Anatomy, Physiology and Cell Biology
University of California
Davis CA 95616

Via email to iwerner@ucdavis.edu

Re: Comments on *Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species* - Draft Final Report. Submitted to the Central Valley Regional Water Quality Control Board on August 23rd, 2010

Dear Dr. Werner:

The Sacramento Regional County Sanitation District (SRCSD) is pleased to provide you with the attached comments on your draft final report *Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species* by Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. We appreciate the opportunity to comment on this important study as a member of the Pelagic Organism Decline (POD) Contaminant Work Team and as a partner with the Central Valley Regional Water Quality Control Board (CVRWQCB) and UC Davis Aquatic Toxicity Lab (ATL). We are pleased to continue providing our technical input and logistical support that have helped make these studies successful and have added value due to SRCSDs knowledge of water quality.

This draft report gives a clear presentation of the study findings and SRCSD agrees with the report conclusions. We are providing supplemental information and minor editorial suggestions in the attached specific comments that we believe will help clarify the environmental relevance of the data. Environmental relevance has always been a focus of these studies as defined in the original sampling and analysis plan "The Effects of Wastewater Treatment Effluent Associated Contaminants on Delta Smelt Ammonia Toxicity Sampling and Analysis Plan - Final. Werner, Irvine, and Foe 7/28/08." As an example of the focus on environmental relevance, the hypothesis from the original plan states:

> *"Hypothesis 1: Delta smelt survival is negatively impacted (i.e., increased mortality) by ambient ammonia concentrations in the Sacramento River with increasing concentrations causing increased mortality under the study conditions."*

SRCSD was under the impression that follow-up testing was to be performed with the same focus. However, when ambient conditions are discussed in the report they reflect only extreme conditions. Including averages or likely environmentally relevant conditions in the Delta would further support the original intent of the work effort. SRCSD suggests that the report describe the environmental relevance related to the effect levels from SRWTP effluent (i.e., that no adverse effects to smelt and rainbow trout were observed at over 20 times the average percent effluent and ammonia concentrations found in the Sacramento River at the time of this testing).

SRCSD is pleased to have been a partner in these investigations with the UC Davis ATL and CVRWQCB and is available to continue searching for solutions to the POD. If you would like further information, please contact me at dornl@sacsewer.com or 916-876-6030.

Sincerely,

Linda Dorn

Linda Dorn
Environmental Program Manager

Attachment: SRCSD specific comments on *Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species* Draft Final Report Submitted to the Central Valley Regional Water Quality Control Board on August 23rd, 2010, by Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz.

cc: Pamela Creedon, Central Valley Regional Water Quality Control Board
Chris Foe, Central Valley Regional Water Quality Control Board
Stephanie Fong, Central Valley Regional Water Quality Control Board
Stan Dean, District Engineer
Prabhakar Somavarapu, Director of Policy and Planning
Rueben Robles, Director of Operations
Mitch Maidrand, Principal Civil Engineer
Terrie Mitchell, Legislative and Regulatory Affairs Manager
Debbie Webster, Executive Officer, Central Valley Clean Water Agencies

ATTACHMENT

SRCSD specific comments on *Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species* Draft Final Report Submitted to the Central Valley Regional Water Quality Control Board on August 23rd, 2010, by Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz.

SECTION 2 – BACKGROUND

Comment 1: Page 5, fourth paragraph 1. It would be very helpful, and support the environmental relevance of tested concentrations, to describe the average ammonia/ium and ammonia concentrations in the Sacramento River that you have encountered in addition to the maximum concentrations that are discussed.

Comment 2: Page 6, Table 1. This table presents the Ambient Water Quality Criteria (USEPA 1999) for extreme pH and temperatures measured in the Sacramento River at Hood. It would be very helpful to provide averages in addition to these extreme values to support the environmentally relevance of the test conditions.

Comment 3: Page 6, first paragraph. It would be very helpful to describe the environmental relevance of these test conditions by presenting the actual dilution ratios of SRWTP effluent in the Sacramento River during the time of testing, and the average dilution ratio. The percent of SRWTP effluent typically present in the Sacramento River is averages approximately 2% over a year, while the instantaneous maximum permitted dilution ratio – which is seldom met and never sustained – is 7% (14:1). These dilution ratios averaged 1.2% of river flows during sampling days for 2010 smelt tests (Table 1).

Table 1. Effluent dilution ratios on sample collection days for 2010 Smelt toxicity studies.

Date	Average Dilation Ratio of SRWTP Effluent in the Sacramento River (60 MIN EFFLUENT DILUTION RATIO AVG) (permitted maximum is 14:1)	Mean Percent Effluent in the Sacramento River below the SRWTP Discharge (%)
4/21/2010	90.2 : 1	1.1%
4/22/2010	105.6 : 1	0.9%
4/23/2010	118.8 : 1	0.8%
4/24/2010	119.5 : 1	0.8%
4/25/2010	109.3 : 1	0.9%
4/26/2010	96.4 : 1	1.0%
4/27/2010	90.4 : 1	1.1%
5/19/2010	68.1 : 1	1.5%
5/20/2010	72.8 : 1	1.4%
5/21/2010	70.3 : 1	1.4%
5/22/2010	75.1 : 1	1.3%
5/23/2010	76.1 : 1	1.3%
5/24/2010	71 : 1	1.4%
5/25/2010	70.6 : 1	1.4%
6/16/2010	99.2 : 1	1.0%
6/17/2010	91.7 : 1	1.1%
6/18/2010	82.7 : 1	1.2%
6/19/2010	83.7 : 1	1.2%
6/20/2010	81.9 : 1	1.2%
6/21/2010	78.9 : 1	1.3%

Date	Average Dilation Ratio of SRWTP Effluent in the Sacramento River (60 MIN EFFLUENT DILUTION RATIO AVG) (permitted maximum	Mean Percent Effluent in the Sacramento River below the SRWTP
6/22/2010	80.3 : 1	1.2%
Average	*93.8 : 1*	*1.2%*

SECTION 4 – RESULTS
SECTION 4.1.1 SRWTP EFFLUENT EXPOSURES

Comment 4: **page 15, first paragraph.** Several comparisons are made in this paragraph between the smelt survival in various controls and effluent dilutions. Readers may be confused when they read in the second sentence that there were no significant effects, but then learn in the last sentence that there were significant effects. It would be helpful to clarify in the second sentence that there were no significant differences between smelt survival in effluent dilutions compared to Garcia Bend reference site samples.

SECTION 6 – DISCUSSION AND CONCLUSIONS

Comment 5: **page 26, fourth paragraph.** While not all of the information desired in the attempt to answer question 1 was obtained, these tests did identify unbounded NOECs for percent SRWTP effluent, ammonia/ammonium, and ammonia. Please indicate that the highest tested concentrations represent unbounded NOECs for 48-day old delta smelt survival are 28% effluent, 6.12 – 7.82 mg/L ammonia/ium, and 0.076 – 0.144 mg/L ammonia. These unbounded NOECs are critical to answering question 1 with all toxicity data where delta smelt are exposed to effluent and ammonia mixtures because these NOECs overlap other effect concentrations.

Comment 6: **page 26, fifth paragraph**. Since identifying "the range of no (NOEC) and low (LOEC) effect ranges of SRWTP effluent mixed into Sacramento River water" was a goal of this study, it would be extremely helpful to indicate the ranges of NOEC and LOEC concentrations determined for delta smelt in previous studies in addition to the range of LC50s that are currently discussed. Reporting the current unbounded NOECs in relation to previous test results (NOECs and LOECs) demonstrates that these values vary in response to changes in effluent quality or unknown variations in smelt sensitivity.

SECTION 7 – UNCERTAINTIES AND RECOMMENDATIONS FOR FUTURE STUDIES

Comment 7: **page 27, second paragraph.** The final conclusion could be further supported by describing the environmental relevance related to the effect levels from SRWTP effluent (i.e., that no adverse effects to smelt and rainbow trout were observed at over 20 times the average percent effluent and ammonia concentrations found in the Sacramento River at the time of this testing).

Comment 8: **page 28, fifth bullet**. It might be helpful to mention that biomarkers are excellent measures of exposures, but should be linked to individual survival, growth, and reproduction in order to be relevant in comparison to USEPA water quality criteria.

Comment 9: **page 28, sixth bullet**. This comment indicates that *in situ* methods should be used to monitor ambient toxicity. It would be helpful to refer the readers to Werner et al. (2010) which describes preliminary *in situ* toxicity testing with delta smelt in several locations in the Delta, including at Hood.

Comment 10: **page 28.** Please consider adding the recommendation to conduct 7-day LC50 data for larvae delta smelt exposed to ammonia/ium. This is a critical data gap to allow comparison with the 7-day bioassays. Only 96-hour (four-day) ammonia LC50s are compared with the 7-day bioassays used in the current study. To our knowledge, only one 7-day LC50 study has been conducted, and this was with juvenile smelt (149 dph) which were shown to be less sensitive to ammonia than larvae used in effluent-ammonia bioassays.