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

Ammonia Toxicity Sampling and Analysis Plan

Final

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Title and Approval Sheet

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This Sampling and Analysis Plan is a companion document to the Quality Assurance Project Plan for 2008-2010 POD study "Effects of Toxic Contaminants on Invertebrates and Fish in the Sacramento-San Joaquin Delta" (Stillway 2008). All of the policies and procedures specified with regards to sampling in the POD 2008-2010 Quality Assurance Plan will be followed for this project.

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Introduction

Effluents from municipal wastewater treatment plants can be significant sources of ammonia and complex chemical mixtures (Kidd et al., 2007; Huang and Sedlak, 2001 and references therein). Potential sources of deleterious effects to fish in the Sacramento-San Joaquin Delta are of particular interest due to long-term declining trends and a precipitous drop in several pelagic fish species populations over the past several years. This trend, known as the pelagic organism decline (POD), has been the focus of an increasing number of investigations over the past several years with no single cause identified (Sommer et al., 2007). Delta smelt (*Hypomesus transpacificus*) is one of the species of concern in the POD that has been federally listed as threatened since 1993. Additional information about sensitivity of freshwater fishes to surface water constituents can be added to the current POD conceptual model and mathematical population models being developed under the guidance of the interagency ecological program (IEP) to investigate "what stressors, under what conditions, currently affect pelagic fish populations" in the San Francisco Estuary (IEP 2008a). The 2008 IEP POD work plan is also investigating more than 50 individual study elements on a variety of potential stressors (e.g., predation, water project operations, food limitation, temperature and salinity fluctuations). This pilot study will investigate the potential toxicity of ammonia and other chemicals in treated wastewater to delta smelt, and is a collaborative effort between the Central Valley Regional Water Quality Control Board (CVRWQCB), the UC Davis Aquatic Toxicity Laboratory (UCD-ATL), and the Sacramento Regional Wastewater Treatment Plant (SRWTP). This pilot study is also associated with a larger study being conducted by UCD-ATL for the Department of Water Resources (DWR) (Stillway 2008a,b).

Delta smelt toxicity testing methods are under development by the UCD-ATL, and water samples from the Sacramento-San Joaquin Delta in 2006 and 2007 yielded contradictory results. Results of 2006 testing suggested that delta smelt may be highly sensitive to ammonia (Werner et al. 2006; **Figure 1**). However, this relationship is questionable, due to the low strength of this correlation (r^2=0.27) and the heavy weight of one sample. These 2006 bioassays did not reveal any toxicity due to contaminants, but 4-day delta smelt survival showed a significant correlation with electrical conductivity. The fish age at testing (9 – 90 days) and the maximum ammonia measured during the tests (not ambient concentrations) were also significant factors. An estimated effect concentration of >0.02 mg/L unionized ammonia was reported. The unionized ammonia (NH_3) fraction is the form most toxic to fish and its equilibrium with ammonium (NH_4^+) is

temperature and pH dependent.

Figure 1. 2006 Delta smelt larvae survival (4-day) as a function of maximum unionized ammonia (calculated as mg/L NH_3 at 16-18ºC) in static renewal exposures. Symbols indicate different sample dates and fish ages tested; unpublished data, UCD-ATL.

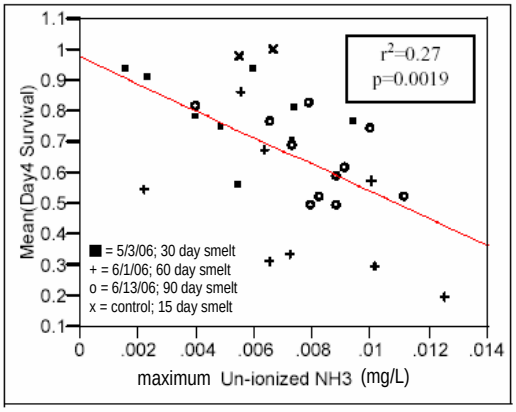
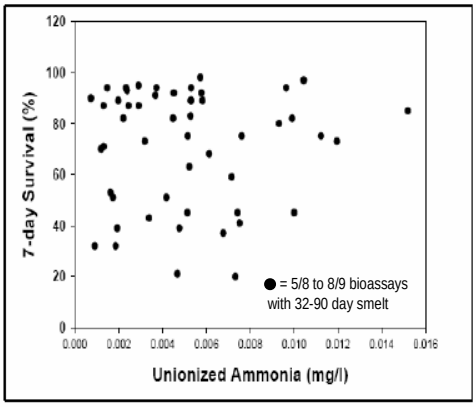


Figure 2. 2007 Delta smelt larvae survival (7-day) as a function of maximum unionized ammonia concentrations (calculated as mg/L NH_3 at 16-18ºC) in flow-through exposures with samples from various Delta locations and dates (Werner at al. 2007).

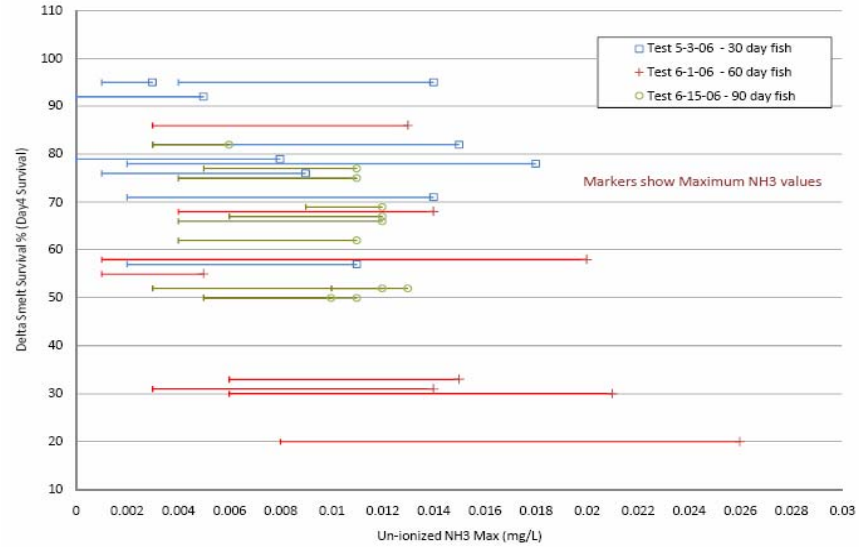


In contrast to the 2006 results, 2007 bioassays did not show any relationship between 7-day delta smelt survival and unionized ammonia (Werner et al. 2007; **Figure 2**). This may be due to refined test methods using flow through exposure tanks as opposed to the static renewal exposures used in 2006. The ranges of unionized ammonia concentrations determined from samples over the test duration are shown in **Figure 3**. This flow-through exposure is likely more representative of ambient conditions in the receiving water. Of the 42 surface water grab samples collected in 2007 (seven locations sampled on six occasions), only two from the lower Sacramento resulted in significantly reduced survival of delta smelt in laboratory toxicity tests.

The Sacramento River drains into delta smelt spawning/larval nursery areas and potential toxicants in river water could affect delta smelt found downstream. Ambient unionized ammonia concentrations were greatest at the Grand Island site (<0.012 mg/L), near the Sacramento River confluence with the shipping channel (**Figure 4**). However, ammonia concentrations in the Sacramento River samples at Hood were lower (<0.004 mg/L unionized ammonia) than at Grand Island, likely due to the lower pH of the water at Hood (Werner et al., 2008). SRWTP discharges treated effluent containing ammonia (2006-2007 average 24 ± 3.4 mg/L) into the Sacramento River and maximum ambient concentrations downstream of this discharge are approximately 1 mg/L total ammonia. Daily ambient Sacramento River ammonia measurements over the past two years (0.0085 +- 0.005 mg/L) also indicate that unionized ammonia concentration are below the proposed concentrations that could be lethal to sensitive fish (0.012 mg/L) when the unionized fraction was calculated based on daily concurrent pH and temperature river data. Total ammonia (NH_3 + NH_4^+) concentrations downstream of the SRWTP discharge were also well below the chronic national

recommended water quality criteria (USEPA 2005). Regardless of these data, SRWTP was selected for this study because it is a large point-source discharger and provides a source of ammonia containing effluent.

Figure 3. 2006 Delta smelt larvae survival (4-day) as a function of measured unionized ammonia (calculated as mg/L NH_3 at 16-18°C) from sampling through testing (Werner et al. 2006).



Based on the above discussion, the available information may not be adequate, to evaluate the impact of ammonia on delta smelt. The CVRWQCB and State Water Quality Control Board (SWRCB) have identified in recent resolutions that the impact of ammonia associated with the POD will be evaluated.

> "Recent studies have raised the possibility that ammonia concentrations in the Bay-Delta may be inhibiting primary production or contributing to fish toxicity. The Water Boards are organizing screening studies to investigate these effects further and will evaluate the need for additional toxicity monitoring of NPDES permitted discharges to Delta waterways…" (SWRCB Resolution 2007-0079 and CVRWQCB Resolution R5-2007-0161)

This is a pilot study intended to identify the potential for adverse effects of WWTP effluent, in particular ammonia, on delta smelt larvae. Follow-up investigations will be needed to make conclusive statements on the effect of ammonia in treated wastewater on delta smelt, and to determine the potential for adverse effects to delta smelt sensitive life stages in the appropriate habitat. Delta smelt do not typically reside near the SRWTP outfall in Freeport[1] and ammonia fate

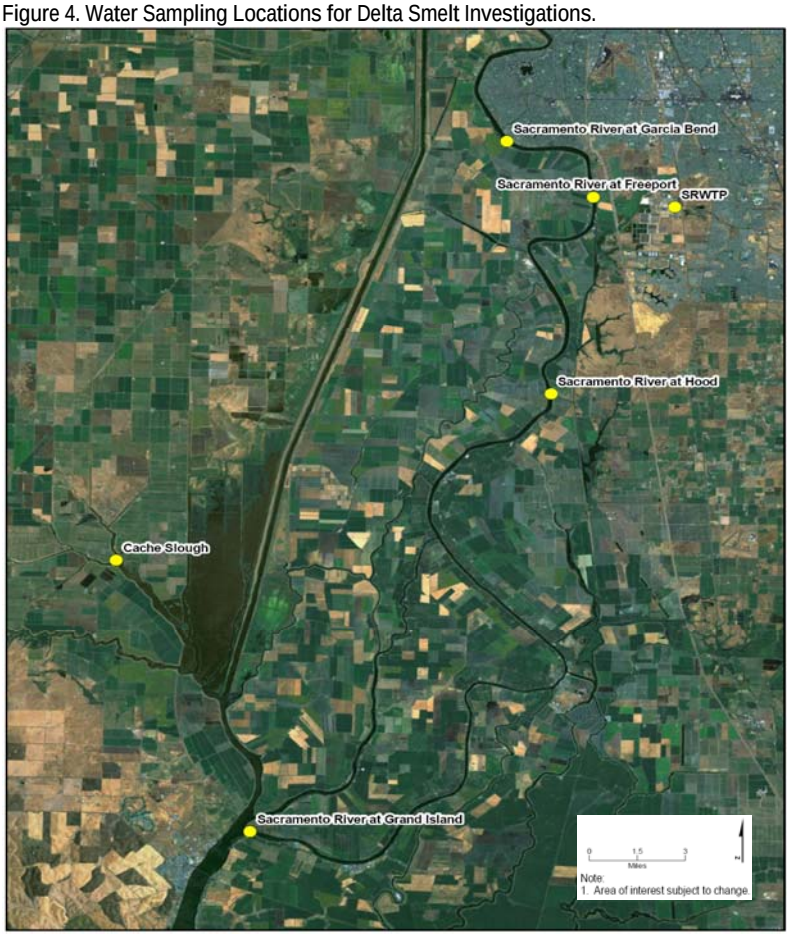
[1] Delta smelt have been regularly/seasonally caught in the Sacramento River and adjacent sloughs during US F&W trawls, including within the City of Sacramento (Pers. Comm. from Randy Baxter, California DF&G, June 13, 2008). The timing and extent of these catches will be clarified and discussed in the report generated from this pilot study.

and transport (i.e., the nitrogen cycle) can cause significant reductions to discharged ammonia between the Freeport discharge and the main delta smelt spawning and rearing habitat found approximately 20-30 miles downstream (e.g., Sacramento River at Isleton, Miner Slough, Cache Slough, and Steamboat Slough. This study also complements a POD investigation into SRWTP effluent-ammonia effects on diatom productivity with Dr. D. Dugdale (SFSU) and a pyrethroid source evaluation study with Dr. D. Weston (UC Berkeley).

Hypotheses

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

Figure 4. Water Sampling Locations for Delta Smelt Investigations.



Experimental Design

Definitive Testing

A clear relationship between ammonia toxicity and delta smelt survival without interferences from ambient samples is necessary for interpreting the data in this pilot study. Definitive toxicity tests to determine the sensitivity of 40-45 day old delta smelt to ammonia have been carried out by UCD-ATL as part of ongoing UCD-ATL/DWR investigations. Relative sensitivity analysis between delta smelt and standard EPA test species are also being conducted. The resulting 96-hour LC_{50} concentrations may support project team decisions and will be provided to the project team as soon as they are available.

Effluent/Ammonia Testing

The sensitivity of 40-45 day old delta smelt to ammonia and SRWTP effluent in Sacramento River water will be evaluated in paired 7-day acute bioassay experiments. This test will partially address the two hypotheses; although, follow-up investigations will likely be needed to clarify several outstanding questions and are discussed below. Testing details are also described below and will follow protocols developed for delta smelt toxicity testing (Appendix A) and in the QAPP (Stillway 2008). The experiment will be repeated if budget considerations permit.

The first dilution series will consist of increasing ammonium-chloride concentrations (4.0, 2.0, 1.0, 0.5, 0.25 mg ammonia-N/l) amended into Sacramento River water collected upstream of the SRWTP[2]. This dilution series was selected to provide the greatest confidence at the expected maximum concentration in the river of approximately 1.0 mg/l total ammonium and is consistent with EPA's guidance for WET testing (USEPA 1991). Controls will consist of Sacramento River water, delta smelt hatchery water (control), and hatchery water adjusted to the conductivity (EC) of the Sacramento River water (low EC control). The second series will consist of four concentrations (2.0, 1.0, 0.5, 0.25 mg-ammonia-N/l) of SRWTP effluent amended into the same upstream river water as was used in the ammonia dilution series (**Table 1**). SRWTP effluent total ammonia concentrations typically range from 18-30 mg/L and comprise about 1-2 percent of the Sacramento River flows. Depending on the ambient effluent concentration for ammonia, the proposed effluent dilutions could range from 0.8 to 13 percent. The environmental relevance of tested dilutions will be considered in the data interpretation.

Concurrent tests with the fathead minnow (*Pimephales promelas*) will be performed to evaluate the relative sensitivity of delta smelt to this standard bioassay organism. Fathead minnow bioassays will follow the same sample/concentration matrix as the smelt, but will be done in standard beaker tests with a static renewal exposure according to standard procedures (USEPA 2002).

[2] Maximum ambient ammonia concentrations observed in the Sacramento River downstream of the SRWTP are approximately. 1 mg-N/l.

Table 1. Experimental Matrix

Amended into Sacramento River Water (mg NH3-N/l)	SRWTP effluent diluted in Sacramento River Water (mg NH3-N/l)	Estimated Unionized Ammonia (mg/L) at pH 7.6 and 61°F
4.0	-	0.0464
2.0	2.0	0.0232
1.0	1.0	0.0116
0.5	0.5	0.0058
0.25	0.25	0.0029
Sacramento River Water Control		
Delta Smelt Hatchery Water Control		
Low EC Control		
Ammonium-chloride (Reference Toxicity Concentration at LC50)		
Laboratory Water Control (Reference Toxicant Control)		

4 replicates for each treatment
Mg-N/L - total ammonia measurements

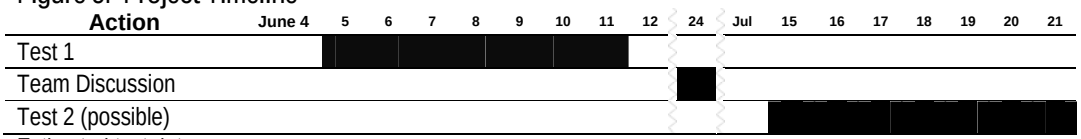
The ammonia-N concentrations in the SRWTP dilution series will be matched with those of the ammonia experiment as described above. The pH of ammonia-chloride treatments and diluted effluent treatments will be matched to the measured Sacramento River ambient pH (annual average of 7.4 ± 0.3). Both tests will be performed at 16^{o}C, a temperature that approximates Sacramento River temperatures during times when larval delta smelt are present in the bay-delta downstream of the Sacramento River (March – June)[3]. The entire experiment will consist of 12 treatments of four replicates each. Test will be performed for 7 days in a gravity-driven flow-through system (**Appendix A**). Temperature, ammonia concentration, conductivity, dissolved oxygen, turbidity, hardness, and pH will be monitored twice daily throughout the experiment. Each dilution series will be analyzed individually to determine whether there is a statistically significant inverse relationship between smelt survival and the independent variable. If so, then the LC_{50} concentration and associated 95% confidence limits will be calculated according to whole effluent toxicity test methods (USEPA 2002).

Schedule

Sampling is proposed to take place in early June with testing to begin within 36-hours of sample collection (**Figure 5**). Timing will greatly depend on the availability of test organisms from the Delta Smelt Hatchery at Tracy, CA. SRWTP will be notified at least 10-days prior to planned testing to coordinate effluent sampling. A second test may be conducted before the end of June. Results of the first test will be discussed by the project team as soon as they are available to help inform the second test.

[3] Note that delta smelt do not reside near the SRWTP outfall in Freeport.

Figure 5. Project Timeline



Estimated test dates

Sampling

Fifty gallons of Sacramento River water from a location several miles above SRWTP (Sacramento River at Garcia Bend). Samples will be collected daily by the Regional Board field team with the aid of a boat from the middle of the river (**Table 2**). River samples will also be collected at ebb tide to ensure samples are not tidally influenced. Four[4] (4) gallons of SRWTP effluent (flow-weighted 24-h composite sample) will be collected daily during the experimental period by SRWTP in low density polyethylene (LDPE) containers provided by UCD-ATL. Samples will be transported from SRWTP to the lab by the UCD-ATL field team. All samples will be stored at 4°C until used in the experiment. Field temperature, conductivity, dissolved oxygen, turbidity, hardness, and pH will be recorded. Temperature, ammonia concentration, conductivity, dissolved oxygen, hardness, turbidity, and pH of the effluent will be measured as soon as possible upon sample receipt and before preparing exposure solutions.

Samples will be archived for potential chemical analysis to confirm or determine causes of toxicity, if present.

Table 2. Sampling Roles and Responsibilities

Sample ID	Sac R. Control	Treated Effluent
Location	Garcia Bend	SRWTP
Collector	Regional Board	SRWTP
Volume (Gal)	50	4
Container	LDPE	amber LDPE
Storage	4°C; dark	4°C; dark
Holding Time	36 hrs	36 hrs
Sampling Frequency	Daily	Daily
Field Measurements	Temperature, conductivity, dissolved oxygen, turbidity, and pH.	Temperature, conductivity, dissolved oxygen, turbidity, and pH.
Lab Measurements (upon receipt and twice daily during tests)	Temperature, ammonia, conductivity, dissolved oxygen, turbidity, hardness, and pH	Temperature, ammonia, conductivity, dissolved oxygen, turbidity, hardness, and pH. Regional Board to measure & report TRC

Reference Toxicant Tests

To ensure comparable sensitivity between different batches of fish, acute 96-h survival reference toxicant tests using copper chloride (0.313, 0.625, 1.25 and

[4] A minimum of three gallons SRWTP composite effluent is needed, four will be provided to UCD-ATL if available.

2.5 mg/L) in laboratory control water (DIEPMH at 900 microS/cm, pH 7.9) will be performed simultaneously with each experiment (or started within a day of the ammonia test initiation). Due to the requirements of testing with a special status species, these reference tests will consist of four treatments and one control with three replicates each containing 5 fish per replicate.

Quality Assurance Project Plan (QAPP)

Sampling protocols and test methods have been described in more detail by Stillway 2008 a,b) for the studies currently being conducted with DWR. This study will defer to the QAPP developed for the overall study (Stillway 2008a), although deviations from this protocol or failures to comply with the QAPP will be documented in writing and discussed with the project team, rather than the contract manager (CM) as noted in the QAPP.

Delta smelt toxicity testing methods are still under development and basic method validation tests (e.g., control survival ≥ 80%) are a challenge. Test methods and results will meet validation criteria described in the QAPP which include a minimum 60% control survival. This does not meet the USEPA (2002) standard bioassay criteria. Therefore, bioassay results will be evaluated with care and reported with qualification. Hatchery water control survival less than 60 percent will invalidate test results and require re-testing. Other deviations from EPA recommendations include:

1. Sample collection is recommended in amber glass containers. LDPE will be used instead, due to large sample volume requirements.
2. Testing is recommended within 36 hours of sample collection. The QAPP indicates that UC Davis will begin testing within 72 hours to accommodate extensive sample collection times. This is not the case for Sacramento River /SRWTP sample tests and testing will commence within 36 hours of sample collection.
3. It is recommended that control water hardness match the hardness of test samples; however, sample conductivity[5] will be matched and manipulating both water hardness and conductivity is problematic. The relatively small volumes of SRWTP effluent in Sacramento River water are not expected to cause significant changes to water hardness. Hardness will be monitored in all treatments but not adjusted.

Study Team

The study team and contact information is provided in **Table 3**. The project manager will notify SRWTP two weeks prior to the proposed testing day to coordinate sampling. The entire project team will also be notified, and consulted if time permits, of results in a timely manner and immediately if there are deviations from the proposed protocol or in the QAPP. Good communication is an integral part of a strong study.

[5] Conductivity in Sacramento River water is typically 160 uS/cm.

Table 3. Project Team

Name	Affiliation	Responsibility	Contact Info
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Data Analysis and Interpretation

There are four possible outcomes of the hypotheses testing that are described below and diagramed in **Table 4**. All four outcomes exclude the possibility that Sacramento River water samples have inherent toxicity. If survival is less than 60% in any of the control treatments then the test is invalid and will be reported as such.

Outcome 1 - "Yes-Yes" (upper left quadrant) indicates that there are statistically significant positive correlations between delta smelt mortality and unionized ammonia in spiked river water and in diluted effluent. Unionized ammonia is the likely toxicant if the calculated LC_{50} concentrations of both dilution series are not significantly different. This outcome alone does not mean that SRWTP effluent is responsible for ambient sample ammonia toxicity; only that ammonia should be investigated further.

Outcome 2 – There is a statistically significant positive correlation between delta smelt mortality and unionized ammonia in spiked river water, but no such relationship in diluted effluent. Something or some characteristic in the effluent may be antagonizing ammonia toxicity or reducing the toxicity of ammonia.

Outcome 3 - There is a statistically significant positive correlation between delta smelt mortality and unionized ammonia in diluted effluent, but no such relationship in spiked river water. This suggests that ammonia levels are insufficient to cause mortality to delta smelt, but some other chemical or effluent characteristic may act synergistically with ammonia or cause toxicity.

Outcome 4 - Ammonium spiked into Sacramento River water and effluent dilution treatments do not cause toxicity. This "no-no" response (lower right quadrant) would indicate that ammonia concentrations and SRWTP effluent in the lower Sacramento River were not impacting Delta smelt during the period of the study and under these test conditions and at the time of sampling.

Table 4. Interpretation of Possible Delta Smelt Ammonia Toxicity Study Outcomes.

		Concentration-Response from SRWTP Effluent Diluted in Sacramento River Water	
		Yes	No
Concentration-Response in Ammonia Amendment into Sacramento River Water	Yes	Outcome 1 - NH_3 possible cause of toxicity in ambient samples if similar effect concentrations between treatments. Conduct follow-up investigations to identify sources/ fate and transport, and evaluate environmental relevance of tested concentrations.	Outcome 2 - Something in effluent may be inhibiting NH_3 toxicity. Conduct follow-up investigations to identify sources/ fate and transport, and evaluate environmental relevance of tested concentrations.
	No	Outcome 3 - Something in effluent other than NH_3 may cause toxicity to delta smelt. Conduct follow-up investigations to determine the environmental relevance of these results.	Outcome 4 - Neither NH_3 levels in the Sacramento River nor other contaminants in SRWTP effluent are acutely toxic to delta smelt at these concentrations and study conditions

Note: If control survival in hatchery water or Sacramento River water is less than 60% then the test is invalid and this outcome matrix is not appropriate.

Reporting

Results from this project will provide information on the acute toxicity of ammonia and SRWTP effluent diluted in Sacramento River water to delta smelt. Comparisons of results from definitive ammonia toxicity bioassays and spiked river water samples may help answer urgent questions regarding the relative sensitivity of delta smelt to contaminants of concern and focus decision-making for future toxicity testing in the Delta. Two documents will result from this pilot study:

1) The lab will provide a draft lab report of the data with appropriate qualifiers and conclusions that will include an analysis of the results in light of the possible outcomes listed below. The draft lab report will be reviewed by the project team.
2) A technical staff report including the lab report, and with staff technical evaluation, will be developed by the CVRWQCB. A draft of this technical report will be reviewed by the project team. This technical report will also be reviewed by the IEP technical committee prior to finalizing.

All valid test outcomes will be interpreted in light of several factors to put these results in perspective and consider environmental relevance:

1) Ammonia effect concentrations compared to ambient concentrations,
2) SRWTP effluent is discharged approximately 20-30 miles upstream of the main delta smelt spawning and rearing areas, over which distance fate

and transport (i.e., the nitrogen cycle) will affect concentrations of chemicals in the treated wastewater.
3) Other sources of ammonia or environmental conditions that give the Grand Island samples greater concentrations than upstream samples.
4) The environmental relevance of tested dilutions (e.g., percent effluent/flow/timing).
5) The relevance of study timing relative to when and where critical life stages of delta smelt are present.
6) Any test with non-standard organisms should be evaluated with care and reported with qualification.
7) Results of acceptability criteria (e.g., control survival and reference toxicity results) will be reported.

Potential Future Follow-up Investigations

If valid effluent-ammonia toxicity relationships are determined then follow-up investigations can be conducted to determine the likelihood of adverse effects to delta smelt in their native habitat (e.g., Cache Slough):

1) Characterize the spatial and temporal ammonia concentrations in the Sacramento River and Delta, extending from upstream of the SRWTP to smelt spawning/larval nursery areas. Sources and concentrations of ammonia determined from characterizing the spatial and temporal trends can be used to develop a fate and transport model for ammonia. This model will help identify the sources and relative contributions of ammonia to potential POD issues.
2) Compare the ammonia concentration-response relationship to the observed ambient unionized ammonia concentrations in the river. This comparison will need to consider the spatial-temporal variability in ammonia and the uncertainty/variability in the ammonia concentration-response relationship.
3) Evaluate the effects of ammonia on Delta smelt at earlier life stages and in areas/conditions where they are more likely to occur.
4) Evaluate potential deleterious effects of effluent-associated chemicals other than ammonia on delta smelt, determine the environmental relevance of these any potential effects from effluent-associated chemicals, and determine the relative contribution of any effluent-associated effect on the POD.

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APPENDIX A – Lab Methods

Detailed Bioassay Method - 7-Day Delta Smelt (*Hypomesus transpacificus*)

Test Organisms: Delta Smelt are hatched and raised in large tanks at the hatchery in Byron, CA. At this facility, the delta smelt are kept in water pumped directly from the Delta. *Nannochloropsis* algae are added to increase turbidity and *Artemia* are added for food. Younger animals are also fed rotifers. Conductivity of the hatchery water is generally higher than the ambient Delta water due to the high conductivity of the water *Artemia* are raised in.

Control Water Collection: Water collected from the delta smelt hatchery is used for all control and acclimation treatments. This water is pumped directly from the intake channel of the H.O Banks Pumping Facility near Byron, CA, then passed through a series of sedimentation beds containing natural vegetation to allow any suspended solids in the water to precipitate. This less turbid water is then exposed to an ozonation system to kill any potentially harmful microbes. One day before fish are collected for testing at the UC Davis Aquatic Toxicology Laboratory (UCD-ATL), about 340 gallons of ozonated water are transported to UCD-ATL, and appropriate control waters (see below) are prepared for the tests.

Fish Collection and Transport: Fish are maintained in large flow-through tanks at the Byron Hatchery. Using a drain valve, the water level is dropped to approximately one third of the initial volume of water to increase fish density and thus facilitate collection of the fish. One liter beakers are used to scoop up fish. These are then gently poured into a 11" x 15" metal pan containing ~ ½ inch of water. When the pan contains approximately 30- 40 fish they are gently poured into black plastic buckets containing hatchery water at a depth of 3-4 inches. Once the desired fish number is reached, the transport bucket is filled to the brim with hatchery water and bucket lids are sealed to prevent water leakage. Buckets are loaded into coolers packed very lightly with ice to keep the water temperature at 14-16º C. Small pieces of foam are placed around the buckets to reduce vibration during transport. Fish are then transported to the UCD-ATL in Davis. Ice in coolers is replenished periodically during transport to maintain a water temperature of 14-16º C. EC and SC are measured, and dissolved oxygen content was initially monitored during transport. It was determined that it is not necessary to aerate the water during transport.

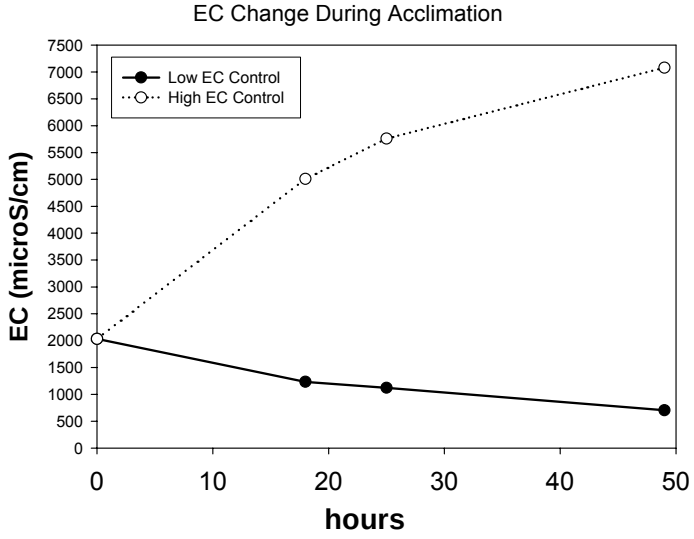
Testing Procedures: Upon receipt at UCD-ATL, the animals are put into a temperature-regulated water bath maintained at 16º C. One-liter beakers are used to collect fish from the buckets, and fish are gently poured into a metal pan containing ~ 1/2" of water. The fish are gently scooped up using 100 mL beakers and released into the replicate tanks at random, submerging the beaker and allowing fish to swim freely into the tanks. Numbers of fish loaded into each tank are recorded.

Tests will be set up with approx. 45-day old fish. Upon arrival at UCD-ATL, 12 fish are immediately placed into the test tanks with no secondary holding units, for EC acclimation. During acclimation and testing, fish are fed three times a day with 1mL of *Artemia* and 1mL of rotifers at each feeding. Just before test initiation, the salinity adjusted control water is drawn down from 7 liters to approximately two liters to allow for an accurate count of living fish.

Sacramento River water, hatchery water and EC-adjusted hatchery water will be used as acclimation and control water. EC is adjusted with distilled water (Low EC Control) to match the Sacramento River water samples. Water quality parameters (EC, pH, temperature, DO and ammonia concentration) will be measured twice daily, and dead fish are counted and removed daily. The feeding behavior of fish is monitored throughout the duration of the test. At test termination, surviving fish are counted.

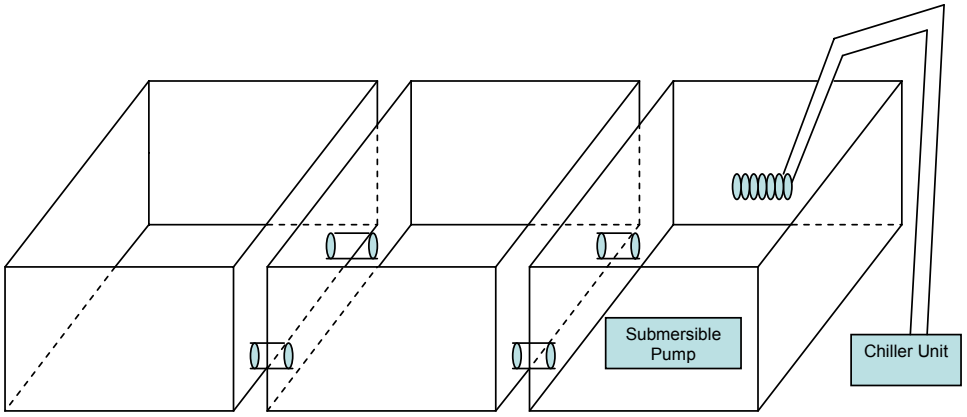
EC Acclimation

Upon receipt 10 fish are placed directly into each test container for electrical conductivity acclimation. The flow-through drip system (1.5 ml/min) is used to gradually add EC modified hatchery (control) water to adjust the EC to match the EC of the Sacramento River water for 48 h.

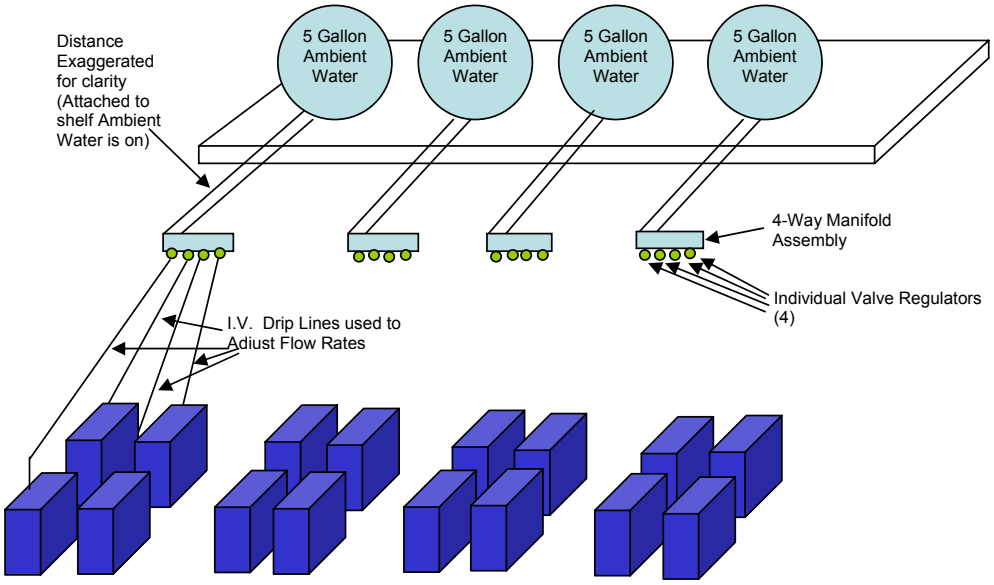


The Flow-Through Exposure System

Delta Smelt Exposure System: 3 sections with 16 x 2.5 gallon tanks per section Schematic Diagram



Tank and Manifold Assembly Schematic Diagram: Each of the following occupies 1 Section of the Three Section Bath



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APPENDIX B - Comments

Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt

Ammonia Toxicity Sampling and Analysis Plan

Response to Comments from the Pelagic Organism Decline – Contaminants Work Team

Background

The sampling and analysis plan for the study of the effects of wastewater treatment effluent-associated contaminants on Delta smelt was reviewed by members of the Interagency Ecological Program's Pelagic Organism Decline Contaminants Work Team. Members of the review team included:

- Frances Brewster, Santa Clara Valley Water District
- Debra Denton, US EPA
- Cameron Irvine, CH2M HILL
- Karen Larsen, Central Valley Regional Water Board
- Mitch Maidrand, Sacramento Regional County Sanitation District
- Thomas Maurer, US Fish & Wildlife Service
- Anke Mueller-Solger, CA Department of Water Resources
- Bob Seyfried, Sacramento Regional County Sanitation District
- Swee Teh, UC Davis
- Inge Werner, UC Davis Aquatic Toxicology Laboratory

On 21 May 2008 the review team met to review comments and determine how best to address them. Below is a summary of the comments received, the review team's responses, and the justification for decisions made.

Comments & Responses (comments are shown in bold text, responses are shown immediately below the comment in italicized text)

Comments submitted by Debra Denton, US EPA

1. **On page 3, the pilot study reword, "the effect of ammonia in treated wastewater on delta smelt." I believe that the study is to evaluate the effect of ammonia in treated wastewater. Keep in mind that ammonia maybe causing toxicity solely or in combination with other toxicants in the effluent or the ambient waters downstream of the discharge.**
   This was a typographical error and will be corrected in the document.

2. **On page 3, second objective under follow-up investigations "compare the ammonia concentration-response relationship of Delta smelt to the observed ammonia concentrations in the wastewater effluent. I'm not sure what is meant by the uncertainty/variability in the ammonia concentration-response relationship? Variability of within-test (measured via PMSD responses), the**

variability of the effluent in terms of varying concentrations of multiple chemicals over time?
Page 3, paragraph 4, sentence 1, no. 2: The last part of the sentence after the word "concentrations" will be deleted and replaced with "in the river". This section was also moved to the "Potential Future Follow-up Investigations" section.

3. **On page 4, under hypotheses #1, if the hypothesis is Delta smelt survival is negatively impacted (i.e. increased mortality) by ambient ammonia concentrations in the Sacramento River. Then the study would need to look at multiple test events not just one time sampling event.**
Hypothesis number 1 will be modified to indicate that the impacts to Delta smelt will be evaluated considering the current study conditions.

4. **On page 4, Under hypotheses #2, "positively correlated with ammonia" not sure what is intended by positively correlated. It doesn't necessarily have to be positively correlated to be a problem.**
The review team discussed the thinking behind this statement and agreed that no change to the document was necessary.

5. **On page 4, under experimental design - what is the LC 50 of ammonia to Delta smelt? I believe these toxicity tests are underway? In fact, I would suggest conducting this ammonia Delta smelt sensitivity (definitive tests) concurrently with the EPA standard fish species fathead minnow to determine the relative sensitivity of Delta smelt to fathead minnow.**
Inge Werner confirmed that this work had already been conducted. The document will be modified to reflect that.

6. **On page 5, it is not clear what is the "part of a tiered study approach"?**
The document will be modified to include a section titled "Potential Future Follow-up" to clarify the discussion of how results from the current study could be used to identify needed follow-up studies versus what will be done during the screening studies that will be conducted under this sampling and analysis plan.

7. **On page 7, under reference toxicant tests just provide the copper chloride concentrations to be tested instead of TUs. I am supportive of running these reference toxicant tests with the concurrent effluent receiving water testing.**
Concurrent reference toxicant tests will be done as stated in the revised workplan.

8. **On page 8, it is stated that the river and effluent samples and testing will commence within 36 hours of sample collection. However, under (see schedule), the time period is within 24 hours. Please be consistent.**
This was a typographical error. The correct holding time is 36 hours and the document will be modified to reflect that.

9. **On page 8, number 3, the samples will be matched to River water hardness. What value will you use the mean, 90th percentile, etc?**

Due to difficulties with matching hardness, the UCD ATL will match the electrical conductivity. The document will be modified to reflect that.

10. **On page 9, under outcome number four, with this one-time study there is no way that you can say that there is a no-no response. Therefore, this option should be removed or lots of caveats need to be expressed such as the fact that it was a one-time event. Another suggestion under these outcomes could be concurrent permittee testing of the permit required standard three freshwater species concurrently with this experiment. Then if toxicity is demonstrated with any the effluent three-species, a TIE could be conducted to assess whether ammonia and/or another toxicant is causing toxicity.**
The document will be modified to be clear that the no-no response refers only to results obtained under the testing conditions and time periods described in this sampling and analysis plan and that the implications for conditions in waters downstream of the locations being tested cannot be determined from the current study. The District also will check whether their permit-required whole effluent toxicity monitoring can be conducted concurrently with the Delta smelt exposures.

11. **The team is very experienced in this work and will be very able to conduct the experiments.**
Comment noted.

Comments Received from Frances Brewster, Santa Clara Valley Water District

12. **Thank you for the opportunity to provide comments on the study proposal. My main comment is that I question the pH and temperature selected for the test. The study proposes to use the measured Sacramento River ambient pH and temperature; approximately 7.4 units and 16 degrees Celsius, respectively. If we want to know whether Sac Regional effluent is toxic to Delta smelt in the vicinity of the discharge, I suppose this is an appropriate test condition. However, ammonia from Sac Regional and other sources potentially reaches the Cache Slough complex (via Elk, Sutter and Steamboat Sloughs and tidal flows) where younger, more sensitive than 40-45 day old smelt are developing. While there may be significant loss and transformation during transit, this area is closer than the stated 30-40 miles downstream and probably at times is warmer and has higher pH than Sacramento River water. And, there may not be significant loss or transformation. If you want to know whether ammonia, from Sac Regional and other sources combined, is a problem for Delta smelt (hypothesis #1) it seems that the study should capture the range of measured ammonia concentrations at the range of measured pH and temperatures that Delta smelt might be exposed to (not the levels at the point of a single discharge).**
The review team recognizes the limitations associated with interpreting the results of this study, which is intended as a preliminary screening study the results of which will be used to determine necessary follow-up. That follow-up will need to include investigating the effects of ammonia on Delta smelt at earlier life stages and in areas they are more likely to occur such as the Cache Slough complex where pH and

temperatures differ from measurements in the Sacramento River near the wastewater treatment plant outfall. As such, the review team agreed to clarify that additional study would be necessary to evaluate these potential effects but that the screening would be conducted at the temperatures and pH as described in the sampling and analysis plan. The justification is that it is difficult to adjust pH, particularly considering the sensitivity of the Delta smelt in laboratory exposures. The review team also expressed reluctance to modify the testing temperature for similar reasons and to be consistent with previous Delta smelt testing.

13. **On page 9, outcome 4 only provides evidence that ammonia levels in the Sacramento River are not acutely toxic to 40-45 day old Delta smelt at the tested pH and temperature. It does not answer whether ammonia from Sacramento River sources contributes to acute or chronic effects in downstream areas. Tier II follow-up investigations could be conducted on different life stages, different exposure durations, and different environmentally relevant pH and temperatures**.
See response to comment number 10 above.

14. **It seems that we should first answer whether ammonia is potentially harmful to Delta smelt under a worst case, environmentally relevant scenario. In a VERY cursory scan of the BDAT data I saw ammonia levels up to 0.5 mg/L and pH approaching 9 units in Delta smelt habitat (not necessarily at the same location and time, and not necessarily the most current, or best data available). At 16 degrees, this would equate to unionized ammonia approaching 0.1 mg/L. If environmentally relevant concentrations are harmful, then look at relative contributions from different sources, including Sac Regional.**
See response to comment number 12 above.

Additional Comments During Review Team Discussion

15. **The review team questioned how this study fits into all of the other investigations related to the pelagic organism decline.**
Anke Mueller-Solger agreed to write this summary, which will be included in the document.

16. **The review team expressed concern about whether the sampling site upstream of the wastewater treatment plant discharge at Garcia Bend was tidally influenced, which could confound interpretation of the study results.**
The review team agreed that samples should be collected on the ebb tide. The District staff also indicated that the wastewater discharge is diverted (i.e., sent to storage ponds rather than discharged to the river) during low river flow periods. Due to this practice, the District does not expect effluent mixing as far upstream as Garcia Bend under any circumstances.

17. **It is not clear from the study plan that the testing duration is 7-days for the river and effluent study and that duration for the reference toxicant and laboratory ammonia LC50 studies is 96-hours.**

The document will be modified to make this clear.

18. There was a suggestion that the study include trout exposures to compare the sensitivity of the Delta smelt to a salmonid species.
The review team agreed that this would be good information but that the budget and laboratory capacity may not be able to accommodate this additional testing. Karen Larsen and Inge Werner agreed to check on the possibility of adding this element to the study plan.

Subsequent to the review team meeting, the following comments were received from Randy Baxter, CA Department of Fish & Game

19. The sampling and analysis plan was explained well, and provided an effective review of important results to date. In particular, I found the discussion of conflicting past results, potential confounding factors in current plan, rationale for current testing process well reasoned.
Comment noted

20. The Experimental Matrix as presented and interpretation table (#4) seem like the appropriate direction to take initially.
Comment noted. Caveats for interpreting the current study results will be added to the document (see comment number 10 above).

21. Recognizing and testing for contaminants correlated with ammonia seems wise.
Comment noted.

22. Temperature target of 16 is OK for the first tests.
The testing temperature will be consistent with previously conducted Delta smelt exposures (see comment number 12 above).

23. QAPP section is confusing. It appears to be written for previous tests using delta-wide water collection and adapted. The adaptations in one or more cases bring the QAPP back into EPA conformity and should be left at that.
Comment noted.

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APPENDIX C – Bench Sheets

Initial Water Chemistry for the Delta Smelt SRWTP/Ammonia Test (Page 1 of 2)
Today's Date:
Day of Test:

Treatment Description	Treatment ID	Initial Turbidity	Initial Ammonia	Initial pH	Initial Temp	Initial Hardness	Initial SC	Initial EC	Intitial DO
Sac River at Garcia Bend (SRGB)	1								
SRGB w/ 0.25 mg/L NH_3-N from NH_4Cl	2								
SRGB w/ 0.50 mg/L NH_3-N from NH_4Cl	3								
SRGB w/ 1.00 mg/L NH_3-N from NH_4Cl	4								
SRGB w/ 2.00 mg/L NH_3-N from NH_4Cl	5								
SRGB w/ 4.00 mg/L NH_3-N from NH_4Cl	6								
SRGB w/ 0.25 mg/L NH_3-N from SRWTP	7								
SRGB w/ 0.50 mg/L NH_3-N from SRWTP	8								
SRGB w/ 1.00 mg/L NH_3-N from SRWTP	9								
SRGB w/ 2.00 mg/L NH_3-N from SRWTP	10								
Low Conductivity Control[1]	11								
Hatchery Water Control[2]	12								

1. Diluted hatchery water, matches the specific conductance and turbidity of SRGB
2. Hatchery water salted up with Instant Ocean to match the specific conductance of rearing conditions. Turbidity is 11 NTU.

Final Water Chemistry for the Delta Smelt SRWTP/Ammonia Test (Page 2 of 2)
Today's Date:
Day of Test:

		~9:00 AM				~4:00 PM				
Treatment Description	**Treatment ID**	**Final Ammonia**	**Final pH**	**Final Temp**	**Final DO**	**Final Turbidity**	**Final Ammonia**	**Final pH**	**Final Temp**	**Final DO**
Sac River at Garcia Bend (SRGB)	1									
SRGB w/ 0.25 mg/L NH_3-N from NH_4Cl	2									
SRGB w/ 0.50 mg/L NH_3-N from NH_4Cl	3									
SRGB w/ 1.00 mg/L NH_3-N from NH_4Cl	4									
SRGB w/ 2.00 mg/L NH_3-N from NH_4Cl	5									
SRGB w/ 4.00 mg/L NH_3-N from NH_4Cl	6									
SRGB w/ 0.25 mg/L NH_3-N from SRWTP	7									
SRGB w/ 0.50 mg/L NH_3-N from SRWTP	8									
SRGB w/ 1.00 mg/L NH_3-N from SRWTP	9									
SRGB w/ 2.00 mg/L NH_3-N from SRWTP	10									
Low Conductivity Control[1]	11									
Hatchery Water Control[2]	12									

1. Diluted hatchery water, matches the specific conductance and turbidity of SRGB
2. Hatchery water salted up with Instant Ocean to match the specific conductance of rearing conditions. Turbidity is 11 NTU.

Addendum

Addendum to bioassay study plan titled "*Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*" dated 06/24/08. – written by Chris Foe (07/10/08); revisions by C. Irvine (07/11/08)

A second iteration of delta smelt ammonia acute (i.e., mortality) bioassay tests are scheduled for 17-23 July, 2008, at the UC Davis Aquatic Toxicity Laboratory. Planned treatments are summarized in Table 1. In accordance with the protocols agreed to with SRCSD, and documented in the Sampling and Analysis Plan, this test will include a copper chloride reference toxicant series (0.31, 0.63, 1.25, and 2.5 mg/l) in laboratory water to determine the relative sensitivity of delta smelt.

Effluent toxicity testing has occurred at ammonia concentrations up to 2 mg/l (~9.5 % effluent). A new objective is to quantify potential interactions between effluent and ammonia toxicity to delta smelt. Note that ambient receiving water total ammonia concentrations range from 0.5-1.0 mg/L and effluent concentrations average 2% of river flows. The planned test concentrations of ammonia greater than 2 mg/L and with effluent concentrations greater than 3% are not environmentally relevant. This is a screening level study designed with conservative methods to determine if further studies are necessary. Potential follow-up studies will be discussed by the study team once results are received.

Table 1. Treatments for the July 2008 delta smelt ammonia tests.

Sacramento River water amended with NH_3Cl (mg/l)	**Sacramento River water amended with effluent in terms of NH_3 (mg/l)**
hatchery control	
Low electrical conductivity adjusted hatchery control	
Upstream Sac River Control	
~~0.25 mg/l~~	~~0.25 mg/l~~
~~5.0 mg/l~~	0.5 mg/l l (~2.1% effluent)
1.0 mg/l	1.0 mg/l (~4.25% effluent)
2.0 mg/l	2.0 mg/l (~9.5% effluent)
4.0 mg/l	4.0 mg/l new (~19% effluent)
8.0 mg/l new	8.0 mg/l new (~38% effluent)

Notes:
Concentrations stricken out were tested in the first delta smelt ammonia/effluent bioassays but will be excluded from the second round testing.
Effluent concentrations based on volume requirements for the initial delta smelt effluent dilution bioassays (9.5 % for the 2.0 mg/L dilution).

The purpose of this testing is two fold.

- First, verify earlier acute no observed effect concentrations (NOECs) for ammonia to delta smelt in river water, and to possibly compare lowest observed effect concentrations (LOECs) for acute ammonia toxicity to delta smelt in river water with LOECs determined in laboratory water. This will be accomplished by repeating the higher concentrations in the earlier screening tests with SRWTP

effluent and ammonium chloride (NH_4Cl) spiked river water (described in the sampling and analysis plan).

- Second, test ammonia concentrations that bracket low effect levels (e.g., LC_{10} – LC_{20}) to determine whether other agents (e.g., chemicals) that could contribute to delta smelt toxicity are present in SRWTP effluent at these concentrations. A previous test determined NH_4Cl toxicity to smelt in laboratory water. This part of the test is designed to evaluate whether SRWTP effluent adds to, subtracts, or has no effect on acute ammonia toxicity.
    - o If toxicity in effluent dilutions is greater (more mortality) than the toxicity of NH_4Cl dilutions in river water, then it would indicate some other substance in the effluent could be contributing to toxicity.
    - o If toxicity in effluent dilutions is lower (less mortality) than the toxicity in NH_4Cl dilutions then some other substance in the effluent could be moderating ammonia toxicity. Note: This could be analogous to the changes in copper toxicity as water hardness increases or decreases. Toxicity is often dependent upon a complex combination of chemical and physical conditions in the water, but this study is not designed to determine the mechanisms of any interactions between effluent and ammonia. .
    - o If both treatments (effluent dilutions and NH_4Cl dilutions) are equally toxic, then effluent has no influence on ammonia toxicity.
    - o If there is no toxicity in either treatment, then there are no acute toxicity problems for smelt under these test conditions.

The following summarizes in greater detail how the new bioassay results will be interpreted:

1) Survival of smelt in both hatchery control and hatchery control diluted to the EC of the Sacramento River upstream of the SRWTP discharge at Garcia Bend will be reviewed. If 7-day survival in either treatment is less than 60 percent, then all test results will be considered invalid.

2) If the performance of both control treatments are acceptable, then smelt survival in both the NH_4Cl and effluent spiked treatments will be compared against upstream Sacramento River water control to determine the NOEC and LOEC. Analysis of variance at $p<0.05$ will be used to establish statistical differences. A previous test in laboratory water determined NOEC and LOEC concentrations for total ammonia (primarily ammonium or NH_4) at 5 and 9 mg/l, respectively. It is the unionized form, or ammonia (NH_3) that is toxic to fish; therefore, this fraction will be calculated based on pH and temperature for each test day (USEPA 1999). Statistical analyses to determine potential toxicity differences between treatments will repeat all calculations using this unionized ammonia concentration, and use this information when interpreting results. The previous test in laboratory water determined NOEC and LOEC concentrations for unionized ammonia at 0.066 and 0.105 mg/l, respectively.

3) If a LOEC is measured in either dilution series, then mortality in those paired ammonia concentrations will be compared between effluent and ammonia spiked treatments with a two tailed paired t-test. As stated above, it may be concluded that the SRWTP effluent

contains chemicals that have the potential to moderate ammonia toxicity in River water if the NH_4Cl treatment has significantly greater mortality than the paired effluent dilution. Alternatively, if mortality is greater in the effluent spiked river water than in the paired NH_4Cl spiked river water, then it may be assumed that the effluent contains one or more contaminants that could contribute to toxicity.

The results of this test will be interpreted based on unionized ammonia concentrations and discussed in terms of the environmental relevance.