Testimony before State Water Resources Control Board
Delta Flow Criteria Informational Proceeding

Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web

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My name is Diana Engle and I am providing this testimony regarding hypothesized direct and indirect effects of ammonia on the pelagic ecosystem of the upper San Francisco Estuary. I am an aquatic ecologist with over 20 years of experience evaluating the ecology and biogeochemistry of lakes, streams, large rivers and floodplains, estuaries, and wetlands. My education includes a doctorate in ecology from the University of California at Santa Barbara. I have authored extensive assessments of water quality in coastal and inland environments of California, and have published peer-reviewed articles on topics including floodplain nutrient dynamics and carbon cycling, watershed mass balances and stream export, and the ecology of aquatic macrophytes, floodplain algae, and riverine and lacustrine zooplankton. More detailed biographical information may be found in the Statement of Qualifications which accompanies this written testimony.

I am actively involved in forums addressing the POD and ammonia-related issues in the Delta. I have been a member of the IEP POD Contaminants Work Team since early 2008, and was an invited panel member at both the March 2009 CalFed *Ammonia Workshop* and the Central Valley Regional Board's August 2009 *Ammonia Summit*. I was an invited speaker at the October 2009 IEP Workshop: *Bay-Delta Monitoring Questions & Tools for the 21st Century*, and presented a comprehensive analysis of ambient ammonia data from the San Francisco Estuary at the *9th Biennial State of the San Francisco Estuary Conference* in September 2009.

1. Summary and Purpose of Testimony

Hypothesized effects of ammonia in the ecosystem of the upper San Francisco Estuary fall into two main categories:

- Direct effects on fish or invertebrates owing to acute or chronic toxicity
- Indirect effects of ammonia on the pelagic food web, via alterations of phytoplankton biomass or quality

The purpose of this testimony is to highlight key studies and findings which address whether ammonia is a direct or indirect determinant of biomass or species composition of pelagic organisms in the upper San Francisco Estuary (SFE)[1]. Section 2 provides an overview of research from the SFE regarding key issues related to the hypotheses above. Sections 3-6 provide more detailed discussion of evidence for selected issues. Attachments 1-4 contain supplemental material, and are referenced in the text.

There is now considerable agreement that ambient ammonia levels throughout the estuary are not acutely toxic to fish or their invertebrate prey, including Delta smelt and key calanoid copepod species. Hypotheses related to other direct or indirect effects of ammonia are being addressed by ongoing research. However, to date, information emerging from these research activities does not support an argument that ammonia is significantly contributing to the pelagic organism decline (POD) or to undesirable changes in the estuarine food web. *Consequently, there is no compelling need for information about ambient ammonia concentrations to influence a determination of the volume and timing of Delta exports and other Delta flow criteria.*

2. Overview of Scientific Evidence that Should be Considered by the SWRCB

Direct Toxicity. Ample evidence indicates that ambient ammonia concentrations throughout the upper SFE are not high enough to cause acute toxicity to Delta smelt or to the wide range of aquatic organisms explicitly protected by current USEPA ammonia criteria. In addition, preliminary tests in 2009 using calanoid copepods from the Delta (which are prey items for Delta smelt) indicated that ambient acute toxicity is highly unlikely for these organisms at prevailing ammonia and pH levels. This characterization of ambient conditions applies not only to "POD" years (e.g., 2002 onward), but also to the entire 35-year period for which long-term monitoring data are available. The characterization also applies to the reach of the Sacramento River below the discharge of the Sacramento Regional Wastewater Treatment Plant (SRWTP) (e.g., River Mile 44 and points downstream).

Three principal lines of evidence are currently available which indicate a lack of acute ammonia toxicity in the SFE:

1. ***Screening of ambient concentrations using USEPA ammonia criteria.*** A comprehensive compilation of publicly available data from long-term water quality monitoring programs currently allows comparison of USEPA acute and chronic criteria with ambient ammonia concentrations in almost 12,000 grab samples taken throughout the freshwater and brackish estuary from 1974 to the present. Ammonia concentrations have *never* exceeded the USEPA acute criterion; the chronic criterion was exceeded *only twice* in the available record (in 1976, 1991). Margins of safety are large: on average in the freshwater Delta, the acute and chronic criteria exceed ambient concentrations by factors of 300 and 80, respectively. This analysis shows that ambient concentrations of ammonia throughout the estuary, including in the Sacramento River below the SRWTP, have always met USEPA ammonia criteria by comfortable margins of safety.

   ⇒ More detailed information about the dataset referred to above, procedures used to screen the data using USEPA criteria, and results for data through January 2010, are presented in Section 3 below.

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[1] The upper San Francisco Estuary is used herein to refer to the legal Delta, Suisun Bay, and eastern San Pablo Bay.

2. ***Acute toxicity testing using Delta smelt***. The Central Valley Regional Water Quality Control Board (Regional Board) has funded several rounds of acute toxicity tests using Delta smelt, conducted by the UC Davis Aquatic Toxicology Laboratory (UCD-ATL; Werner et al. 2009a, b). Tests have been conducted using larval Delta smelt (47- and 51-day old) and juveniles (149-day old), and using both 96-hr and 7-day exposure periods. Both ammonium chloride and Sacramento Regional Wastewater Treatment Plant (SRWTP) effluent have been used as sources of ammonia in exposure tests. Depending on the test, endpoints (e.g., LC50, LC10, LOEC, NOEC) have been expressed in one or more of the following terms:

   - total ammonia (the analytical measurement)
   - un-ionized ammonia (the calculated fraction of total ammonia which is toxic, which is pH and temperature dependent)
   - percent SRWTP effluent (which can be compared to the dilution factors which occur in the Sacramento River below the SRWTP discharge)

   Testing indicates that Delta smelt have similar acute sensitivity to ammonia as rainbow trout. This is significant because the USEPA acute criterion for ammonia which applies to water bodies with salmonids was specifically derived to protect rainbow trout. The testing has also revealed that ambient concentrations which occur in the freshwater and brackish estuary are well below acute effects thresholds for Delta smelt.

   ⇒ Published effects thresholds for Delta smelt are compared to ambient ammonia data in Section 4 below.

3. ***Preliminary acute toxicity tests with Delta copepods.*** During summer 2009, acute exposure tests were conducted using two calanoid copepods which are important prey items for Delta smelt (*Eurytemora affinis* and *Pseudodiaptomus forbesi*). Preliminary acute thresholds were presented at the Regional Board Ammonia Summit (Teh et al. 2009a) for three test pHs: 7.2, 7.6, 8.1. Comparison of these effects thresholds with ambient pH and ammonia concentrations from the estuary currently indicate that acute toxicity is highly unlikely for these copepods.

   ⇒ Published effects thresholds for *Eurytemora affinis* (Teh et al. 2009b) are compared to ambient ammonia data in Section 4 below.

Recent use of acute-to-chronic ratios (ACRs) to infer chronic toxicity in the Delta. Evidence that acute ammonia toxicity is not a key concern in the Delta has spurred interest in estimating chronic toxicity thresholds for selected Delta species which are not included in the USEPA database. Chronic test procedures are not available for Delta smelt. Chronic exposure tests for the calanoid copepod *Pseudodiaptomus forbesi* (life cycle tests), funded by the Regional Board, were planned between December 2009-April 2010; preliminary results were not available at the time of this writing. In the meantime, ACRs are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive Delta species. Recently, this approach has been applied to Delta smelt in a manner which is not consistent with USEPA derivation of ACRs and which supports assumptions about chronic toxicity that may not be warranted.

⇒ A brief discussion of concerns regarding how this approach has been applied to Delta smelt is provided in Section 5 below, and supported by more detailed information in *Attachment 3*.

Contaminant Mixtures. Information is currently lacking about whether ambient concentrations of other contaminants in the Delta affect sensitivity of organisms to ammonia. Test results reported in Werner et al. (2009b) (in which effects thresholds for delta smelt were higher in exposure tests using ammonium chloride than in those using SWRTP effluent) have entered the larger discussion of potential effects of contaminant mixtures. However, the concentrations of SRWTP effluent (as percentages of total flow in the river) that produced effects in these particular tests are well out of the range produced by the SRWTP discharge. The 7-day effects thresholds in Werner et al. (2009b) for 47-d old delta smelt, expressed as percent effluent, were as follows: LC50 (25.7%), LC10 (10.6%), NOEC (9%). In contrast, the percentages of effluent that occur in the Sacramento River below the SRWTP discharge are less than 3% the vast majority of the time[2]. <u>The environmental relevance of exposure concentrations has received less attention than deserved in investigations of lethal or sublethal effects of ammonia and other contaminants in the Delta.</u>

Studies Related to Indirect Effects of Ammonia on the Food Web

Ammonium Inhibition. Published work from field surveys and microcosm experiments has shown that, under certain conditions, ambient ammonium concentrations above ~4µM delay uptake of nitrate and development of diatom blooms in Suisun, San Pablo, and Suisun Bays (Dugdale et al. 2007, Wilkerson et al. 2006). This phenomenon, termed "ammonium inhibition" by the principal investigators, has been added to the list of factors that may be affecting the base of the pelagic food web in the SFE, and is currently being investigated in the freshwater Delta.

During 2008-2009, research addressing the relationship between ammonium, nitrate, and phytoplankton growth rates focused on the Sacramento River. Multiple transect studies were conducted betwen fall 2008 and spring 2009 by Regional Board staff and by researchers from San Francisco State University. To date, the results of this work are not yet publicly available as reports or in peer-reviewed literature. However, some of the results were presented at the Regional Board's Ammonia Summit (Foe et al. 2009, Parker et al. 2009a) and at the 2009 State of the San Francisco Estuary Conference (Parker et al. 2009b).

<u>Several key elements of the ammonium inhibition hypothesis were not confirmed by the Sacramento River studies.</u> Longitudinal patterns in biomass (of several taxa) and primary production rates were *not* explained by ambient ammonium concentrations or differential uptake of ammonium and nitrate. In incubations of river water, phytoplankton grew as well in water enriched with ammonium as they did in water enriched with nitrate. Significant increases in primary production rates occurred in the river between Rio Vista and Suisun Bay, despite the fact that inorganic nitrogen uptake in that reach was dominated by ammonium. This new information led principal investigators to conclude:

> "It is unclear from these data what drives declines in primary production of chl-a [in the Sacramento River]." (Parker et al. 2009b)

⇒ More information about ammonium inhibition studies and results is provided in Section 6.

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[2] Based on 7-day running averages for Sacramento River flow between 1998-2009, the 99.5th percentile percent effluent is 2.8%.

Microcystis. Toxic blooms of the colonial form of *Microcystis aeruginosa* have occurred in the north SFE during summer months (June-November) since 1999, and are the only recorded toxic phytoplankton blooms in the northern SFE to date. There is speculation, primarily based on information from highly eutrophic estuaries or laboratory work outside the Delta, that ammonia levels in the Delta might be contributing to the occurrence or toxin-production of *Microcystis*. However, field studies of *Microcystis* from the Delta do not confirm a relationship between ambient ammonia levels and the abundance or toxicity of *Microcystis*. Instead, physical factors such as water temperature, flow, and turbidity appear to best explain *Microcystis* abundance and toxicity in the SFE. Lack of a positive association between *Microcystis* and ammonia concentrations has been found in three separate studies in the estuary.

1. Lehman et al. (2008). Canonical analysis was performed on bi-weekly data for 17 environmental factors, *Microcystis aeruginosa* cell abundance, and microcystin cell content, from a sampling program in the freshwater and brackish estuary in 2004. East side stream-flow, Contra Costa Canal pumping, and water temperature were the primary factors explaining the abundance and microcystin content of *Microcystis* in the brackish and freshwater reaches of the Delta. Ammonia and nitrate concentrations were weakly *negatively* correlated with *Microcystis* abundance, meaning that higher ammonia and nitrate concentrations were associated with fewer *Microcystis*.

2. Lehman et al. (2010). Bi-weekly sampling throughout the estuary in late summer 2005 revealed no association between *Microcystis* abundance and ammonium-N or N:P ratios:

> "Although ammonium-N concentration was elevated at some stations in the western and central delta and the Sacramento River at stations at CS [Cache Slough] and CV [Collinsville], neither it nor the total nitrogen (nitrate-N and nitrite-N plus ammonium- N) to soluble phosphorus molar ratio (NP) was significantly correlated with Microcystis abundance across all regions or within the western and central delta separately. Plankton group carbon or plankton species abundance at 1 m was not significantly correlated with any of the water quality conditions measured, including the NP ratio." (Lehman et al. 2010, p. 237).

3. Cecile Mioni (CALFED post-doctoral study in progress). At the Regional Board Ammonia Summit, Cecile Mioni presented partial results from post-doctoral sampling work in the Delta in the summer of 2009 (Mioni & Paytan, 2009) which led to remarks in her presentation that *Microcystis* abundance appeared to be positively correlated with ammonium. However, subsequent analysis of more complete results from Dr. Mioni's research, including samples from October 2008, and June, July, August 2009, revealed a lack of correspondence between *Microcystis* cell abundance and ammonium concentrations. The lack of correspondence between *Microcystis* cell abundance and ammonium was particularly evident for sites where *Microcystis* was producing toxin. Based on the more recent analysis, Dr. Mioni now concludes that water temperature and secchi depth are more strongly correlated with *Microcystis* abundance than ammonium concentrations (the results of the study are currently in preparation for publication):

> "As you will see, the NH4 vs Microcystis abundance relationship does not appear to be very strong when we add the August 2009. I am seeing a stronger correlation with the water temperature and the secchi depth." (pers. comm. from C. Mioni to D. Engle, Dec. 16, 2009)

***Overall Quality of the Phytoplankton Assemblage*.** An observed shift in phytoplankton community composition from dominance by diatoms to increasing dominance by other, mostly smaller, taxa including miscellaneous (green) phytoflagellates, and the recent occurrence of blooms of *Microcystis*, underly a hypothesis that the quality of the phytoplankton assemblage as food for zooplankton is decreasing in the estuary. In turn, there is speculation that ammonium concentrations - or shifting N:P ratios - may be contributing to the observed shifts in species composition.

Non-nutrient factors affecting diatom abundance in the SFE are rarely discussed. Lehman (1996, 2000) attributed a multi-decadal in the proportional biomass of diatoms in the Delta and Suisun Bay to climatic influences on river flow. Clam grazing selectively removes larger particles (Werner & Hollibaugh 1993); clams may consume a larger fraction of diatoms that nanoplanktonic taxa such as flagellates. Kimmerer (2005) used long-term dissolved silica dynamics, corrected for mixing in the low salinity zone, as an indicator of diatom productivity in the northern SFE. He showed that there was a step decrease in annual silica uptake after 1986, which he attributed to efficient removal of diatoms by *Corbula amurensis* after its introduction in 1986. Diatoms settle more rapidly than other taxa. The deep, pool-like bathymetry of the Stockton Deepwater Ship Channel is hypothesized by some investigators to function as a trap for diatoms in transport in the San Joaquin River; unless current speeds are very high, diatoms cannot remain in suspension for the length of the ship channel (P. Lehman, DWR, Feb. 2009, personal communication). The extent to which shifts in diatom abundance in the SFE is explained by benthic grazing, or interannual variation in freshwater flows remains unanswered in the Delta.

Regarding phytoplankton quality, Regional Board staff concluded in a summary of the August 2009 Ammonia Summit:

> "Finally, due to the lack of data on phytoplankton community composition, there is no consensus yet demonstrating that elevated ammonia levels in the Delta have caused a shift in the algal community from diatoms to less nutritious forms." (Foe 2009)

Also lost from the food web discussions are several studies from the SFE which indicate that non-diatom organisms occupy an important position at the base of the pelagic food web, and that detritus-based pathways for energy transfer may contribute more to the pelagic food web in the Delta than has been acknowledged. This information is important because it argues for a more holistic framework for understanding productivity than the "diatom→copepod→fish" paradigm that drives much of the POD discussion. Such information led the Interagency Ecological Program to make the following acknowledgement in its 2007 Synthesis of Results:

> "...it is possible that the hypothesis that the San Fransisco Estuary is driven by phytoplankton production rather than through detrital pathways may have been accepted too strictly." (Baxter et al. 2008)

Examples of pertinent findings are:

- Gifford et al. (2007): Several zooplankton species in the SFE can shift between consumption of phytoplankton and consumption of heterotrophic microbes. In feeding experiments using natural plankton assemblages from the SFE, a cladoceran (*Daphnia*), a calanoid copepod *Acartia*, and two cyclopoid copepods (*Oithona davisae* and *Limnoithona tetraspina*), all grazed heterotrophic ciliates at higher rates than diatoms.
- Bouley & Kimmerer (2006): Significant grazing on heterotrophic ciliates was observed for both the filter-feeding calanoid copepods *Pseudodiaptomus forbesi* (a common Delta smelt prey item) and *Eurytemora affinis.*

- Hall & Mueller-Solger (2005): *E. affinis* and *P. forbesi* were more successfully cultured in the lab when fed the motile cryptophyte alga *Cryptomonas* than when fed the diatom *Skeletonema* or the green alga *Scenedesmus* suggesting these calanoid copepods might prefer motile prey.
- Rollwagen-Bollens & Penry (2003): The diet of *Acartia* spp. (an important calanoid copepod genus in the estuary) in San Pablo Bay was dominated by heterotrophic prey (especially protozoans such as ciliates and non-pigmented flagellates).

3. Comparisons of Ambient Ammonia Data with USEPA Criteria

Available Data. In water, ammonia primarily occurs as two forms: ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). The sum of un-ionized ammonia and ammonium is commonly referred to as *total ammonia*. The un-ionized form (dissolved ammonia gas) is toxic to aquatic animals at concentrations which vary widely among taxa. Ammonium and un-ionized ammonia occur in an equilibrium ($NH_4^+ \leftrightarrow NH_3 + H^+$) which is affected by pH, temperature, and salinity. When measurements of total ammonia are accompanied by measurements of water temperature, pH, and either electrical conductivity (EC) or salinity, ambient concentrations of un-ionized ammonia can be calculated. Calculation of USEPA ammonia criteria also requires data for water temperature, pH, and (for saltwater samples) salinity or EC.

Publicly available, co-occurring measurements of total ammonia, pH, temperature, and EC from the last 35 years (1974-2010) are available from 80 long-term monitoring stations in the upper SFE. This dataset allows calculation of un-ionized ammonia concentrations (and USEPA criterion concentrations) for almost 12,000 ambient water samples obtained as monthly or bi-weekly grab samples. A breakdown of these stations by sampling entity is provided in Table 1. The location of these stations, and sample counts per station for the entire record, are illustrated in Figure 1. A detailed inventory of stations and sample counts is provided as *Attachment 1*.

Procedure. The dataset described above was screened for exceedances of applicable current USEPA-recommended acute and chronic ammonia criteria for freshwater (USEPA 1999) and saltwater (USEPA 1989). These criteria are designed to protect the most sensitive fish and aquatic invertebrate species for which acceptable test results are available. Criteria are revised periodically when new data become available and are vetted by the EPA[3]. In the USEPA databases which supported the 1999 freshwater and 1989 saltwater criteria development, the most sensitive freshwater species is rainbow trout and the most sensitive saltwater species is winter flounder. Owing to higher acute sensitivity of salmonids, compared to other fish taxa, and higher chronic sensitivity of early life stages of fish, compared to older fish, USEPA (1999) recommends different versions of the freshwater acute and chronic criterion depending on whether these sensitive taxa or life stages are present in a waterbody. For the screening exercise described herein, the more conservative "salmonids present" acute criterion and the "early life stages of fish present" chronic criterion were used. Formulas for calculating the criteria, and other formulas used in the screening procedure, are provided in *Attachment 2*.

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[3] In December 2009, USEPA released a draft update of freshwater ammonia criteria (USEPA 2009) for public comment.

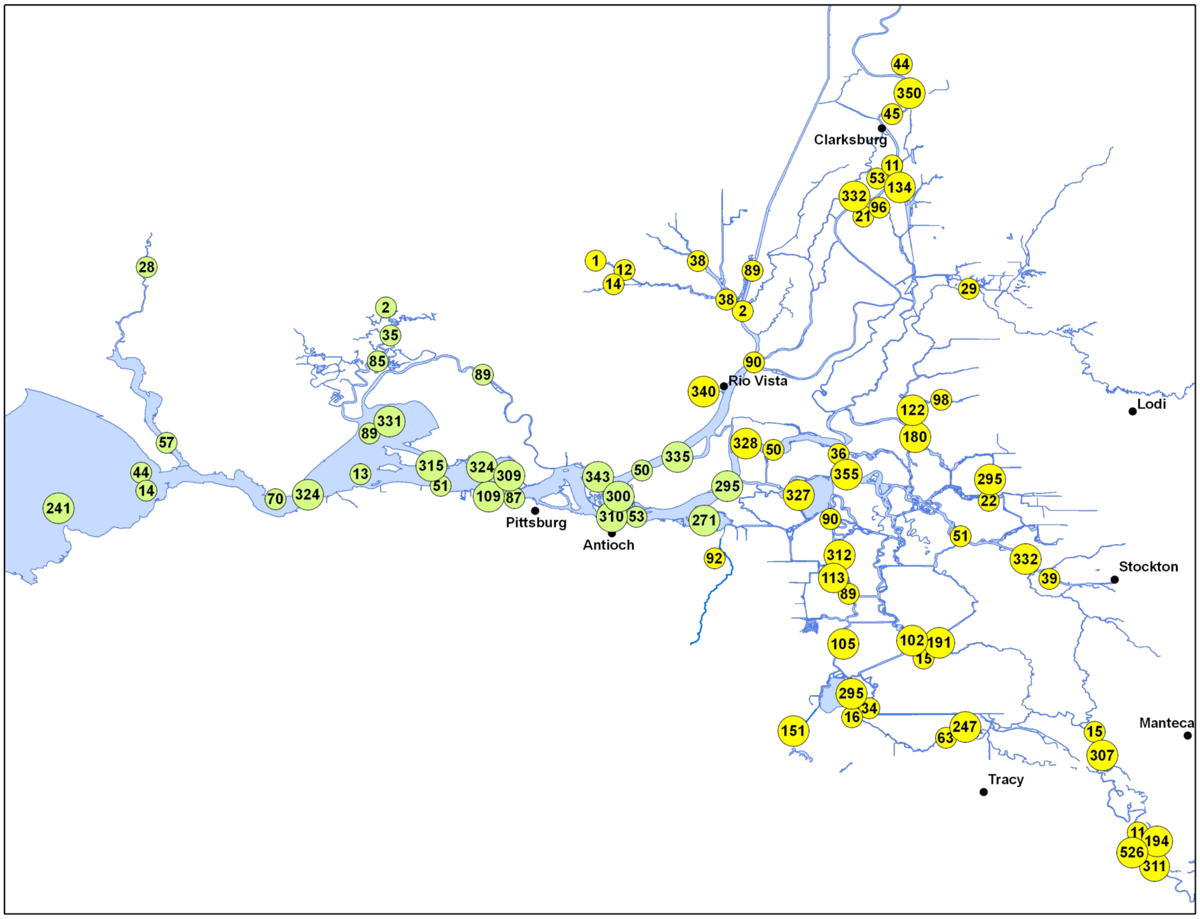

Figure 1. Long-term estuarine (green symbols) and freshwater (yellow symbols) monitoring stations in the Upper San Francisco Estuary providing co-occurring measurements of pH, water temperature, and total ammonia. Values inside symbols are numbers of monthly or bi-weekly grab samples taken during the period 1974-2010. Stations were classified as estuarine or freshwater based on procedures in the California Toxics Rule (see text). Monitoring programs are identified in Table 1.

Table 1. Availability of Co-Occurring Measurements of Total Ammonia, pH, Water Temperature, and Salinity/Electrical Conductivity in the Freshwater and Brackish Delta from Long-term Monitoring Programs in the Upper San Francisco Estuary(1).

Monitoring Program	Estuarine Reaches(2)				Freshwater Reaches(2)			
	1974-2010		"POD" era 2000-2010		1974-2010		"POD" era 2000-2010	
	Stations	Samples	Stations	Samples	Stations	Samples	Stations	Samples
California Department of Water Resources, Municipal Water Quality Investigations (DWR-MWQI)	1	109	1	85	14	917	7	656
Interagency Ecological Program Environmental Monitoring Program (IEP-EMP)	15	3840	5	49	22	4610	5	74
Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta, UC Davis Aquatic Toxicology Laboratory (UCD ATL POD Investigation)	12	625	12	625	11	663	11	663
US Geological Survey (USGS)	--	--	--	--	4	974	3	291
Sacramento Regional County Sanitation District Coordinated Monitoring Program (SRCSD-CMP)	--	--	--	--	2	89	2	89
Total	28	4574	18	759	53	7253	28	1773

(1) As used herein, the term upper San Francisco Estuary Includes the legal Delta, Suisun Bay, Suisun Marsh, and eastern San Pablo Bay. Stations are illustrated in Figure 1.

(2) Stations were classified as estuarine (brackish) or freshwater using long-term records of salinity and the procedure outlined in the California Toxics Rule (CTR; USEPA 2000). Stations were classified as follows: "freshwater" if salinity was ≤1 ppt in ≥ 95% of samples, "saltwater" if salinity was ≥ 10 ppt in ≥ 95% of samples, and "estuarine" if salinity was between 1-10 ppt in ≥ 95% of samples.

Salinity is variable at the "estuarine" stations in the dataset. The California Toxics Rule (CTR) provides guidance on when freshwater versus saltwater objectives are applicable in estuarine water bodies (USEPA 2000, Section G2). Following the procedure outlined in the CTR for priority toxicants, exceedances at estaurine stations were determined as follows:

1. Both the Freshwater and Saltwater criterion was calculated for each ambient sample using ambient pH, temperature and salinity.
2. For each sample, the stricter (lower) criterion concentration was compared to the ambient concentration of total ammonia.[4,5]

Results. The results of the screening indicate that ambient ammonia concentrations meet USEPA acute and chronic criteria by comfortable margins of safety throughout the upper SFE. <u>For data spanning the period 1974-2010 (a total of 11,827 samples), the screening resulted in zero exceedances of the acute criterion, and only two exceedances of the chronic criterion</u>[6]. Neither of the two exceedances of the chronic criterion occurred during POD years. Based on the numbers of samples available for freshwater stations only, State Listing Policy (SWRCB 2004)[7] would require 622 exceedances for the period 1974-2010, or 101 exceedances for the period 2000-2010, to justify a 303(d) listing for ammonia toxicity in the Sacramento-San Joaquin Delta.

Margins of safety can be estimated by dividing USEPA criterion values (expressed as total ammonia) for each sample by the corresponding ambient concentration of total ammonia. Margins of safety obtained using this ratio are summarized in Table 2. The analysis indicates that, on average, the acute criterion exceeds ambient ammonia concentrations in the upper SFE by factors between 200-300. On average, the chronic criterion exceeds ambient concentrations by factors ranging from 40-80. Ample separation between ambient ammonia concentrations in the Sacramento River near Hood and acute and chronic criteria (calculated per sample based on ambient pH and temperature) is illustrated by the time series in Figure 2.

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[4] At estuarine stations, the freshwater acute criterion was stricter than the saltwater acute criterion for ~90% of samples, but the saltwater chronic criterion was stricter than the freshwater chronic criterion for ~80% of samples.

[5] Normally chronic criteria apply to 4-day averages of ambient concentrations (in saltwater), or 30-day averages of ambient concentrations (freshwater), not to monthly grabs. In absence of long-term monitoring data collected more frequently than monthly or bi-weekly, an underlying assumption of the screening exercise is that grab samples represent 4-day or 30-day average concentrations.

[6] The two exceedances occurred at IEP-EMP station C3 (Sacramento River at Greene's Landing) in October 1991, and at IEP-EMP station P8 (San Joaquin River at Stockton) in April 1976.

[7] The State Listing Policy (SWRCB 2004) contains procedures for determining how many exceedances of a particular Basin Plan objective must be observed before a water body can be placed on the 303(d) list as impaired by a given constituent or parameter. The procedure is based on the total number of measurements available from a water body, and the number of exceedances contained in the overall data set. The State Listing Policy procedure for toxicants involves using the binomial distribution to calculate the number of exceedances for which the probability of Type 1 and Type 2 error are minimized for an acceptable exceedance proportion of $\leq 3\%$ and an unacceptable exceedance proportion of 18%.

Table 2. Mean Margins of Safety Separating US EPA Criterion Concentrations from Ambient Ammonia Concentrations in the Upper San Francisco Estuary

	Mean Margin of Safety (Criterion/Ambient Concentration)			
	Using Acute Criterion		**Using Chronic Criterion**	
	1974-2010	**2000-2010**	**1974-2010**	**2000-2010**
Freshwater Stations	295	312	74	80
Estuarine Stations	243	185	51	40

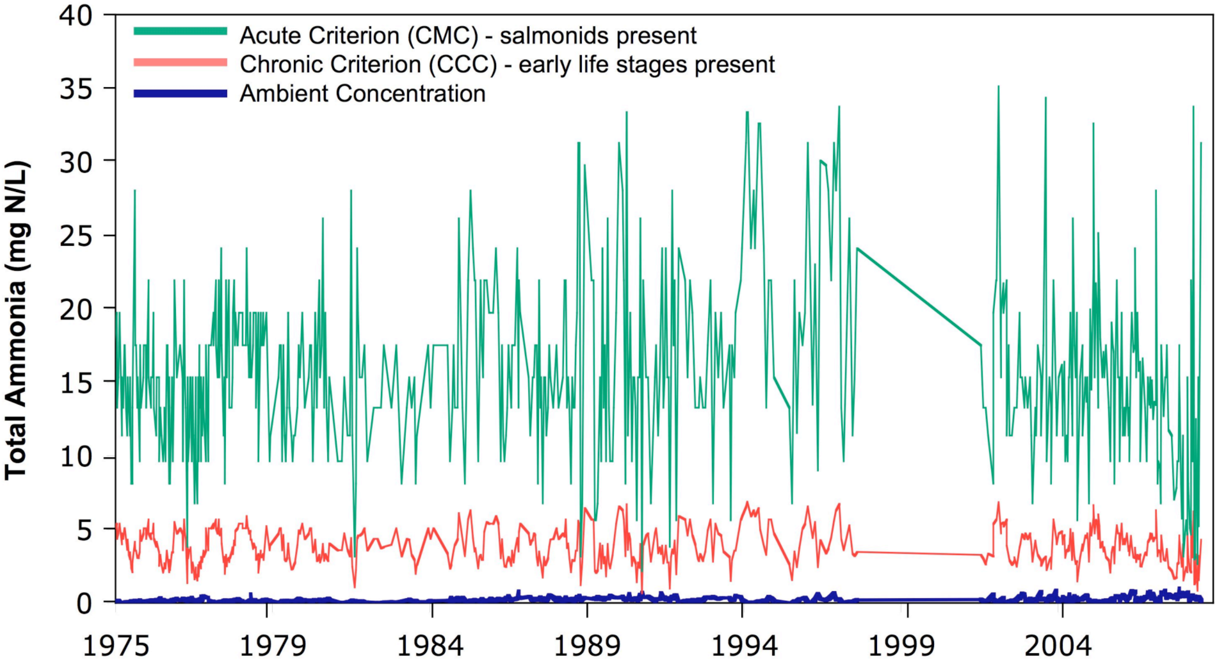

Figure 2. Comparison of ambient total ammonia concentrations in the Sacramento River (lower blue line) with the USEPA acute criterion (upper green line) and chronic criterion (middle red line). Data are from stations located at River Mile 44, Hood, and Greene's Landing.

4. Comparison of Ambient Ammonia Data with Effects Thresholds for Delta smelt and the Copepod *Eurytemora affinis*

Preliminary acute effects thresholds for ammonia, obtained in exposure tests using ammonium chloride, have been reported for larval and juvenile Delta smelt (Werner 2009), and one of its important prey items, the calanoid copepod *Eurytemora affinis* (Teh et al. 2009); thresholds expressed as un-ionized ammonia concentrations, are summarized in Table 3[8].

Table 3. Acute Effects Thresholds for Ammonia for Delta smelt and *Eurytemora affinis* from Exposure Tests using Ammonium Chloride.

Test Organism	Effects Threshold		References
	Threshold Type	Un-ionized Ammonia (mg N/L)	
larval Delta smelt (47-day old)	96-hr LC10	0.084, 0.105	Werner et al. (2009b) p. 15, 19
	96-hr LC50	0.164	
	7-day LC10	0.094	
	7-day LC50	0.113	
larval Delta smelt (51-day old)	96-hr LC10	0.096	Werner et al. (2009b) p. 17
	96-hr LC50	0.147	
juvenile Delta smelt (149-day old)	96-hr LC10	0.400	Werner et al. (2009b) p. 21
	96-hr LC50	0.557	
	7-day LC10	0.398	
	7-day LC50	0.515	
Calanoid copepod *Eurytemora affinis*	96-hr LC10 (pH 7.6)	0.08	Teh et al. (2009b)
	96-hr LC 50 (pH 7.6)	0.12	

In Figure 3, the ranked distributions of un-ionized ammonia concentrations for POD years for the freshwater and estuarine datasets, including the 99th percentile values, are compared to the lower effects thresholds for Delta smelt and *Eurytemora affinis* in Table 3. The comparison indicates that a significant margin of safety separates ambient ammonia concentrations in the upper SFE from acute effects thresholds so far reported for these two species.

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[8] Copepod sensitivity varied inversely with pH in tests by Teh et al. (2009b). For example, the 96-hr LC10 and LC50 obtained at a test pH of 7.2 were 0.011 and 0.068 mg N/L, respectively. Analogous values for a test pH of 8.1 were 0.46 and 0.78 mg N/L. Thresholds presented in Table 3 are for the test pH which best represents ambient conditions in the upper SFE. Using the dataset described in this document, median and mean pH for estaurine stations for 2000-2010 are 7.7 and 7.6, respectively. Median and mean pH for freshwater stations for 2000-2010 both equal 7.6. Between 1974-2010, un-ionized ammonia concentrations exceeded the *lowest* LC10 (0.011 mg N/L) in Teh et al. (2009b) in only six samples with less-than-median pH (pH ≤ 7.6). This indicates that comparison of ambient ammonia concentrations with the copepod test results obtained at the median pH is a reasonable approach.

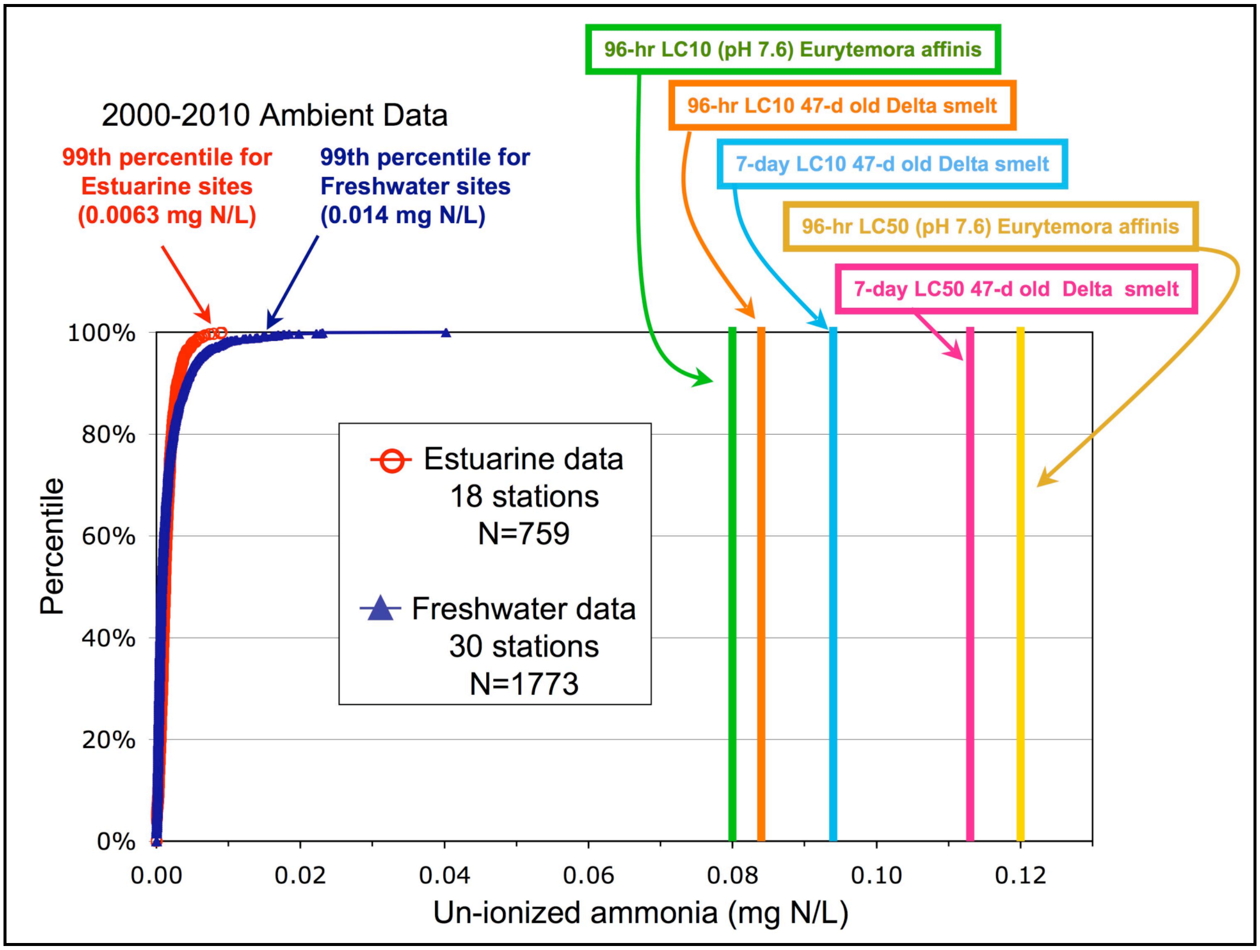

Figure 3. Ranked Distribution of Ambient Concentrations of Un-ionized Ammonia from Estuarine Stations (red circles) and Freshwater Stations (blue triangles) in the upper San Francisco Estuary for POD years 2000-2010. Datasets are described in Table 1. Included are acute effects thresholds for un-ionized ammonia from exposure tests using Delta smelt and *Eurytemora affinis*. Additional effects thresholds for these species that were too high to display in the graph are provided in Table 3.

5. Use of Acute-Chronic Ratios to Infer Chronic Toxicity in the Delta

As stated in the introduction, acute-to-chronic ratios (ACRs) are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive Delta species such as Delta smelt or calanoid copepods. For example, hypothetical ACRs for rainbow trout were used at the Regional Board Ammonia Summit (Werner 2009), and in recent reports to the Regional Board (Werner et al. 2009a,b), to support an argument that ambient levels of ammonia in some Delta locations may be causing chronic toxicity for Delta smelt. The logic behind the argument can be summarized as follows:

- Chronic toxicity test results are lacking for Delta smelt.
- Delta smelt appear to be as acutely sensitive to ammonia as rainbow trout (*Oncorhynchus mykiss*).
- Therefore, chronic toxicity values for Delta smelt are probably similar to those for rainbow trout.
- Hypothetical ACRs for rainbow trout are alleged to be in the range 14.6-23.5.
- One can divide the LC50 for Delta smelt (acute value) by the hypothetical ACRs for rainbow trout to estimate the concentration of ammonia that would cause chronic toxicity to Delta smelt
- Some ambient ammonia concentrations in the Delta are higher than the values that result from this exercise.

The hypothetical ACRs for rainbow trout listed above (14.6 and 23.5) are not based on evidence for chronic effects of ammonia effects on survival, reproduction, or growth of rainbow trout and were derived using test data that was excluded by USEPA in 1999 (and in the Draft 2009 update) for use in developing the chronic ammonia criterion. In fact, to date, the USEPA has determined that the available chronic test results for rainbow trout do not meet USEPA standards for use in calculating species mean chronic values (SMCVs), or for calculating a genus mean chronic value (GMCV) for its genus *Oncorhynchus*:

> "As noted in the 1999 AWQC document, five other studies have reported results of chronic tests conducted with ammonia and other salmonids including *Oncorhynchus mykiss* and *Oncorhynchus nerka*. There is a lack of consistency among the chronic values obtained from these tests, and several tests produced "greater than" and "less than" values (Table 5). Consequently, in keeping with the decision made in the 1999 AWQC document, a GMCV is not derived for *Oncorhynchus*." (2009 Draft Update of Freshwater Ammonia Criteria; USEPA 2009, p. 21)

Attachment 3 describes the derivation of the hypothetical ACRs for rainbow trout listed above, and explains why the derivation represents a significant departure from USEPA guidance concerning chronic test design and endpoints, methods for ACR derivation, and interpretation of chronic test data for the species. USEPA-vetted genus mean ACRs (GMACRs) for fish occupy the range 2.7-10.9 (USEPA 1999, 2009). In summary, assertions about chronic toxicity for Delta smelt - or other sensitive species - based on hypothetical ACRs for rainbow trout in the range 14.6-23.5 should be avoided. At a minimum, such assertions must be carefully qualified as not being based on evidence for population-level effects of ammonia on sensitive fish.

6. Ammonium Inhibition

Published observations from field surveys and microcosm experiments have indicated spring blooms of phytoplankton in Central, San Pablo, and Suisun Bays (which are dominated by large diatoms) may occur when *at least* two conditions are satisfied: (1) vertical salinity stratification improves light conditions, and (2) ambient concentrations of ammonium are below a threshold of about 4 μM (Wilkerson et al. 2006). Tracer additions during container incubations indicated that significant increases in phytoplankton biomass in water from these locations did not occur until ammonium dropped below about 1 μM, and phytoplankton uptake of inorganic N switched from ammonium to nitrate (Dugdale et al. 2007). Owing to these studies in the brackish estuary, "ammonium inhibition" of nitrate uptake, and associated delays in bloom development, have been added to the list of factors that may be affecting the base of the pelagic food web in the SFE, and are currently being investigated in the freshwater Delta.

During 2009, research addressing the relationship between ammonium, nitrate, and phytoplankton growth rates focused on the Sacramento River. Preliminary data from several synoptic surveys conducted in fall 2008 and spring 2009 by Regional Board staff, and by researchers from San Francisco State University, provide snap shots of longitudinal patterns in the Sacramento River in nutrient concentrations, phytoplankton abundance (based on pigment and particle concentrations), phytoplankton taxonomic composition (based on pigment type and size spectra of particles), primary production (based on carbon uptake rates), and rates of ammonium and nitrate uptake (based on incubations with isotopic tracers).

Some of the results of the 2008/2009 research were presented at the Regional Board Ammonia Summit (Foe 2009, Parker et al. 2009a) and in a poster presented at the September 2009 State of the San Francisco Estuary Conference (Parker et al. 2009b). <u>Several results from these studies (bulleted below) contradict elements of the ammonium inhibition hypothesis - as it applies to the freshwater Delta - and indicate that phytoplankton responses to ammonium in the Sacramento River are different than those reported from the Suisun, San Pablo, and Central Bays</u>.

- When removed from light limitation, phytoplankton accumulation was not slower in Sacramento water collected below the SRWTP discharge, compared to water collected above the discharge (see slides 8, 11 in Parker et al. 2009a).
- In the Sacramento River, maximum cell-specific uptake rates for ammonium were not lower than those for nitrate (see slides 9, 10, 11 in Parker et al. 2009a).
- Small-celled phytoplankton and green algae exhibited similar longitudinal trends as large diatoms between the Yolo/Sacramento County line and Suisun/San Pablo Bays (see figures in Parker et al. 2009b [provided in Attachment 4]).
- No step-change in phytoplankton biomass or carbon fixation rates was associated with either (1) the location of the SRWTP discharge, or (2) a shift from primarily nitrate uptake by phytoplankton to primarily ammonia uptake below the discharge. Carbon fixation rates decreased upstream of the SRWTP, despite the fact that nitrate dominated N uptake in that reach of the river (see figures in Parker et al. 2009b [provided in Attachment 4]).
- Significant increases in phytoplankton concentration and carbon fixation can occur between Rio Vista and Suisun Bay, even when inorganic nitrogen uptake is dominated by ammonium (see slide 8 in Foe 2009b, and figure in Parker et al. 2009b [provided in Attachment 4]).
- Factors unrelated to the SRWTP discharge are apparently responsible for declines in chlorophyll-a (and other indices of phytoplankton biomass) which were observed between the Yolo/Sacramento County line and the Rio Vista locale during Spring 2009 (see slide 8 in Foe 2009b, and figure in Parker et al. 2009b [provided in Attachment 4]).

<u>The possibility is raised by these studies that ammonia inhibition (of nitrate uptake) does not influence the timing or magnitude of phytoplankton blooms in the Sacramento River (and potentially elsewhere in the freshwater Delta)</u>. This possibility is supported by long term grab

sample data for chlorophyll-a and ammonia in the freshwater reaches of Sacramento River downstream of Hood (i.e., outside of the area directly or indirectly influenced by grazing by the invasive clam *Corbula amurensis*). In Figure 4, a scatter plot is presented showing available paired measurements (from monthly or bi-weekly grab samples) of chlorophyll-a and ammonium (or total ammonia) from USGS and IEP monitoring stations located between Hood and Three-Mile Slough for the period 1975-2008. Visual inspection of the scatter plot suggests that historically, high biomass of riverine phytoplankton has not been constrained to windows when ammonium concentrations were below 4 µM (equivalent to 0.56 mg N/L on the x-axis; see shaded portion of the graph). Interpretation of this type of data is limited by the frequency of collection -- ideally chlorophyll-a would be sampled more frequently to better coincide with algal blooms of short duration.

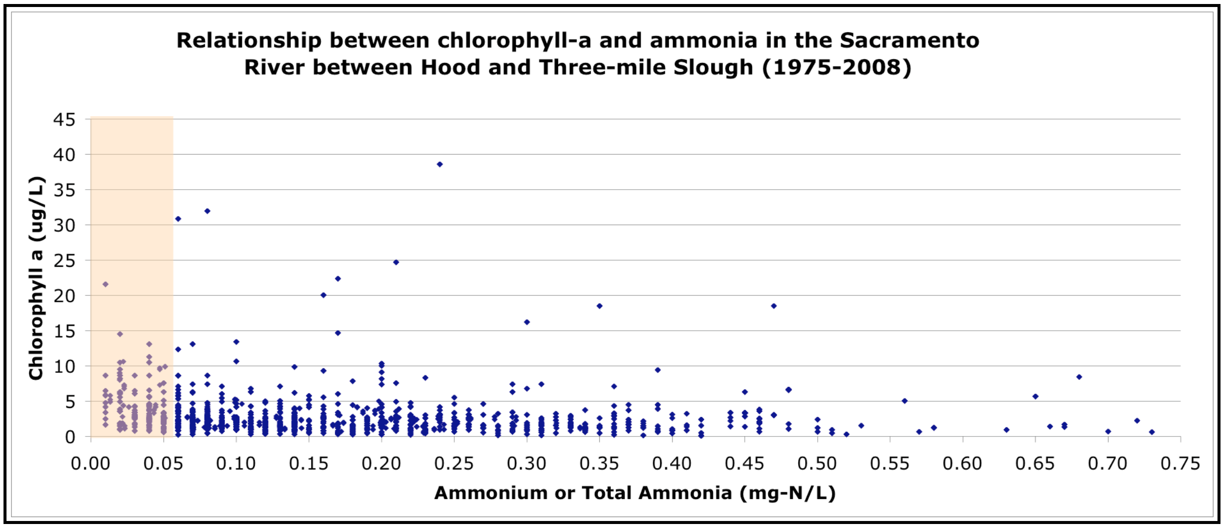

Figure 4. Relationship between chlorophyll-a (µg/L) and ammonium (mg-N/L) in the Sacramento River between Hood and Three-mile Slough between 1975-2008. Shaded area shows ammonia levels below R. Dugdale's hypothesized threshold for ammonium inhibition (4 µM, or 0.056 mg-N/L). Data are from surface water samples at USGS and IEP/DWR monitoring stations for which chlorophyll-a and either ammonium or total ammonia were measured. When ammonium data were not available, total ammonia values were used.

7. References Cited

Note: References are provided as pdfs on the CD-ROM which accompanies written testimony submitted to the SWRCB by Sacramento Regional County Sanitation District.

Baxter, R., R. Breuer, L. Brown, M. Chotkowski, F. Feyrer, M. Gingras, B. Herbold, A. Müller-Solger, M. Nobriga, T. Sommer, and K. Souza. 2008. Pelagic organism decline progress report: 2007 Synthesis of results. Interagency Ecological Program for the San Francisco Estuary.

Bouley, P., and W. J. Kimmerer. 2006. Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. Mar. Ecol. Prog. Ser. 324: 219-228.

Dugdale, R. C., F. P. Wilkerson, V. E. Hogue, and A. Marchi. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. Estuar. Coast. Shelf Sci. 73: 17-29.

Foe, C. 2009. *August 2009 Ammonia Summit Summary*. Technical Memo to Jerry Bruns and Sue McConnell, Central Valley Regional Water Quality Control Board, 24 September 2009.

Foe, C., A. Ballard, & R. Dahlgren. 2009. *Preliminary Ammonia Results from an Ongoing Monitoring Program*. Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Gifford, J.M., G. Rollwagen-Bollens, and S.M. Bollens. 2007. Mesozooplankton omnivory in the upper San Francisco Estuary. Mar. Ecol. Prog. Ser., 348: 33-46.

Hall, C., and A. Mueller-Solger. 2005. Culturing delta copepods. IEP Newsletter., Vol. 18, No. 3, Summer 2005. (entire Issue is on CD, refer to pages 13-17)

Kimmerer, W.J. 2005. Long-term changes in apparent uptake of silica in the San Francisco Estuary. Limnol. Oceanogr 50: 793-798.

Lehman, P. W. 1996. Changes in chlorophyll-a concentration and phytoplankton community composition with water-year type in the upper San Francisco Estuary. (pp. 351-374) *In* Hollibaugh, J.T, (ed.) San Francisco Bay: the ecosystem. San Francisco (California): Pacific Division, American Association for the Advancement of Science. (book chapter, not included in CD)

Lehman, P.W. 2000. The influence of climate on phytoplankton community biomass in San Francisco Bay Estuary. Limnol. Oceanogr. 45: 580-590.

Lehman, P.W., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in the San Francisco Estuary. Hydrobiologia 600: 187-204.

Lehman, P.W., S.J. Teh, G.L. Boyer, M.L. Nobriga, E. Bass, and C. Hogle. 2010. Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia 637: 229-248.

Mioni, C.E., and A. Paytan. 2009. Does ammonia control harmful algae abundance & toxicity in the San Francisco Estuary, CA? Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Parker A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J.Davidson-Drexel, S. Blaser, and J. Fuller. 2009a. Effect of wastewater treatment plant effluent on algal productivity in the Sacramento River Part 1: Grow-out and wastewater effluent addition experiments. Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Parker A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J.Davidson-Drexel, J. Fuller, & S. Blaser. 2009b. *Transport and Fate of Ammmonium Supply from a Major Urban Wastewater Treatment Facility in the Sacramento River, CA*. 9th Biennial State of the San Francisco Estuary Conference, Oakland, CA, September 29-October 1, 2009.

Rollwagen-Bollens, G.C., and D.L. Penry. 2003. Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). Mar. Ecol. Prog. Ser. 257: 139-158.

State Water Resources Control Board (SWRCB). 2004. Water Quality Control Policy for Developing California's Clean Water Act Section 303(d) List. State Water Resources Control Board, Sacramento, CA. Adopted September 2004.

Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009a. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Teh, S. J, S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009b. *Final Report. Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis, of the San Francisco Estuary*. Appendix A *In*: Reece, C., D. Markiewicz, L. Deanovic, R. Connon, S. Beggel, M. Stillway, and I. Werner. 2009. *Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delt*a. UC Davis Aquatic Toxicology Laboratory, Progress Report, 29 September 2009.

USEPA. 1985a. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. United States Environmental Protection Agency.

USEPA. 1985b. *Ambient Water Quality Criteria for Ammonia - 1984*. EPA 440/5-85-001. United States Environmental Protection Agency, January 1985.

USEPA. 1989. *Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989*. EPA 440-5-88-004. United States Environmental Protection Agency, April 1989.

USEPA. 1999. *1999 Update of Ambient Water Quality Criteria for Ammonia*. EPA 822-R-99-014. United States Environmental Protection Agency, December 1999.

USEPA. 2000. California Toxics Rule [CTR], Water Quality Standards; Establishment of Numeric Criteria for Priority Toxic Pollutants for the State of California; Federal Register Rule 40CFR Part 131. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 2009. *Draft 2009 Update Aquatic Life Ambient Water Quality Criteria for Ammonia-Freshwater.* EPA 822-D-09-001. United States Environmental Protection Agency, December 2009.

Werner, I. 2009. *Effects of Ammonia/um and Wastewater Effluent Associated Contaminants on Delta Smelt.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Werner, I., and J.T. Hollibaugh. 1993. *Potamocorbula amurensis*: Comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Limnol. Oceanogr. 38: 949-964.

Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009a. *The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*. Draft Final Report to the Central Valley Regional Water Quality Control Board. January 28, 2009.

Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009b. *Acute toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminant on Delta Smelt - 2009*. Draft Report to the Central Valley Regional Water Quality Control Board. October 30, 2009.

Wilkerson, F.P., R.C. Dugdale, V.E. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. Estuaries Coasts 29: 401-416.

Attachment 1. Inventory of Co-Occurring Measurements of pH, water temperature, and Total Ammonia from the upper San Francisco Estuary, 1974-2010

Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
Freshwater Reaches								
USGS	11303500	San Joaquin R nr Vernalis CA	37.6760406	-121.2663293	526	163	12/14/1977	1/5/2010
USGS	11447650	Sacramento R @ Freeport CA	38.45601954	-121.5013437	350	126	9/14/1977	12/16/2009
USGS	11447810	Sacramento R a Greens Landing CA	38.345745	-121.5460657	96	0	10/3/1974	9/15/1980
USGS	381427121404901	Lower Yolo Bypass nr Rio Vista CA	38.2407475	-121.6813446	2	2	3/3/2000	3/22/2000
UCD-ATL	711	Sacramento.River at the tip of Grand Island.	38.178806	-121.665306	90	90	1/12/2006	5/20/2009
UCD-ATL	812	San Joaquin River, just west of Oulton Point.	38.090306	-121.6405	50	50	2/7/2006	12/12/2007
UCD-ATL	815	San Joaquin, Confluence of Potato Slough.	38.08375	-121.572639	36	36	1/2/2008	5/20/2009
UCD-ATL	902	Old River at mouth of Holland Cut.	38.019195	-121.582194	90	90	1/12/2006	5/20/2009
UCD-ATL	910	San Joaquin River, between Hog and Turner Cut.	38.001806	-121.448694	51	51	1/2/2008	1/2/2008
UCD-ATL	915	Old River-Western arm at railroad bridge.	37.9425	-121.5635	89	89	1/12/2006	5/20/2009
UCD-ATL	CL	Confluence of Lindsey Slough/Cache Slough	38.244222	-121.68875	38	38	1/2/2008	5/14/2009
UCD-ATL	CU	Upper Cache Slough – mouth of Ulatis Creek	38.284083	-121.717861	38	38	6/7/2007	5/20/2009
UCD-ATL	Hood	DWR Water Quality Monitoring Station	38.367667	-121.520444	53	53	5/8/2007	5/12/2009
UCD-ATL	Light 55	Sacramento River Deep Water Channel at Light 55	38.274028	-121.661917	89	89	2/7/2006	5/14/2009
UCD-ATL	R&R	San Joaquin, Rough & Ready Island	37.957908	-121.357763	39	39	1/4/2008	5/12/2009
SRCSD CMP	Freeport	Freeport	38.4855	-121.5091667	44	44	1/11/2001	1/4/2008
SRCSD CMP	River Mile 44	River Mile 44	38.43467	-121.51917	45	45	7/10/2002	6/12/2008
IEP-EMP	C10	San Joaquin River near Vernalis	37.67575	-121.265	311	0	1/21/1975	12/11/1995
IEP-EMP	C10A	San Joaquin River near Vernalis	37.6793	-121.2651	11	11	3/18/2009	1/5/2010
IEP-EMP	C3	Sacramento River @ Greenes Landing	38.34575	-121.5461	332	0	1/22/1975	12/13/1995

Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
IEP-EMP	C3A	Sacramento River @ Hood	38.382	-121.519	11	11	3/18/2009	1/5/2010
IEP-EMP	C7	San Joaquin River @ Mossdale Bridge	37.786	-121.306	307	0	1/21/1975	12/13/1995
IEP-EMP	C9	West Canal @ Clifton Court Intake	37.8298	-121.5574	295	0	1/22/1975	12/11/1995
IEP-EMP	D16	San Joaquin River @ Twitchell Island	38.0969	-121.6691	328	0	1/7/1975	12/15/1995
IEP-EMP	D19	Frank's Tract near Russo's Landing	38.04376	-121.6148	327	10	1/7/1975	1/7/2010
IEP-EMP	D24	Sacramento River below Rio Vista Bridge	38.15	-121.7	340	0	1/7/1975	12/19/1995
IEP-EMP	D26	San Joaquin River @ Potato Point	38.07664	-121.5669	355	11	1/7/1975	1/6/2010
IEP-EMP	D28A	Old River @ Rancho Del Rio	37.97048	-121.573	312	11	2/3/1975	1/7/2010
IEP-EMP	MD10	Disappointment Slough @ Bishop Cut	38.04381	-121.4188	295	0	1/21/1975	12/8/1994
IEP-EMP	MD10A	South channel of Disappointment Slough	38.04381	-121.4188	22	10	1/23/1995	1/6/2010
IEP-EMP	MD6	Sycamore Slough near Mouth	38.1415	-121.4687	98	0	2/4/1975	9/27/1983
IEP-EMP	MD7	South Fork Mokelumne below Sycamore Slough	38.12513	-121.497	122	0	2/4/1975	9/27/1983
IEP-EMP	MD7A	Little Potato Slough @ Terminous	38.11382	-121.498	180	0	1/10/1985	12/14/1995
IEP-EMP	P10	Middle River @ Victoria Canal	37.8912	-121.4894	102	0	3/22/1976	9/20/1982
IEP-EMP	P10A	Middle River @ Union Pt.	37.89126	-121.4883	191	0	10/5/1982	12/13/1995
IEP-EMP	P12	Old River @Tracy Road Br.	37.805	-121.449	247	0	1/22/1975	8/16/1991
IEP-EMP	P12A	Old River @ Oak Island	37.80284	-121.4569	63	0	9/4/1991	12/11/1995
IEP-EMP	P2	Mokelumne River @ Franklin Road bridge	38.25542	-121.4403	29	0	1/21/1975	12/15/1977
IEP-EMP	P8	San Joaquin River @ Stockton aka Buckley Cove	37.97817	-121.3823	332	10	2/3/1975	1/6/2010
DWR-MWQI	B0702000	San Joaquin R. nr. Vernalis	37.67611111	-121.2641667	194	136	7/18/1996	12/15/2009
DWR-MWQI	B9591000	Contra Costa PP Number 01	37.97888889	-121.7008333	92	71	6/6/1996	2/3/2009
DWR-MWQI	B9C74901336	DMC Intake @ Lindemann Rd.	37.81611111	-121.56	16	0	6/13/1996	9/2/1997
DWR-MWQI	B9D43434343	Barker Slough @ Cook Road	38.28416667	-121.8227778	1	0	1/29/1998	1/29/1998
DWR-MWQI	B9D74711184	San Joaquin R. @ Mossdale Bridge	37.78611111	-121.3058333	15	0	6/13/1996	9/2/1997
DWR-MWQI	B9D75351293	Middle R. @ Borden Hwy.	37.89111111	-121.4888889	15	0	6/12/1996	9/3/1997
DWR-MWQI	B9D75351342	Old R. nr. Byron (St 9) (near Hwy 4 Bridge)	37.89111111	-121.5691667	105	84	6/12/1996	2/3/2009

ATTACHMENT 1. Inventory of Co-Occurring Measurements of pH, Water Temperature, Total Ammonia, and (for Estaurine Stations) Salinity

Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
DWR-MWQI	B9D75811344	Old River at Bacon Island	37.96944444	-121.5711111	113	85	6/12/1996	2/3/2009
DWR-MWQI	B9D81561483	Calhoun Cut at Hwy 113	38.26027778	-121.8044444	14	0	1/29/1998	6/24/1998
DWR-MWQI	B9D81651476	Barker Slough Near Pumping Plant	38.27472222	-121.7933333	12	0	3/25/1998	6/24/1998
DWR-MWQI	B9D82071327	Sacramento River at Greene's Ldg.	38.34583333	-121.545	21	0	6/5/1996	5/4/1998
DWR-MWQI	B9D82211312	Sacramento R @ Hood	38.36861111	-121.5205556	134	131	6/10/1998	1/4/2010
DWR-MWQI	KA000000	Clifton Court Intake	37.82978056	-121.5573528	34	34	3/19/2007	12/14/2009
DWR-MWQI	KA000331	Delta P.P. Headworks at H.O. Banks PP	37.80194444	-121.6202778	151	115	6/13/1996	12/16/2009
Estuarine Reaches								
UCD-ATL	323	San Pablo Bay, Rodeo Flats opposite end of rock wall.	38.048306	-122.282806	14	14	1/25/2006	7/27/2006
UCD-ATL	340	Napa River along Vallejo seawall and park.	38.0975	-122.262194	57	57	1/25/2006	5/13/2009
UCD-ATL	405	Carquinez Straight, just west of Benicia army dock.	38.039694	-122.1505	70	70	1/25/2006	5/18/2009
UCD-ATL	504	Suisun Bay, east of middle point.	38.0545	-121.9895	51	51	1/12/2006	12/13/2007
UCD-ATL	508	Suisun Bay, off Chipps Island, opposite Sacramento North ferry slip.	38.0455	-121.918806	87	87	1/12/2006	5/18/2009
UCD-ATL	602	Grizzly Bay, northeast of Suisun Slough at Dolphin.	38.114	-122.046194	89	89	1/25/2006	5/18/2009
UCD-ATL	609	Montezuma Slough at Nurse Slough.	38.167194	-121.938	89	89	1/12/2006	5/18/2009
UCD-ATL	704	Sacramento River, north side across from Sherman Lake.	38.069167	-121.775278	50	50	1/12/2006	12/13/2007
UCD-ATL	804	Middle of Broad Slough, west end.	38.018194	-121.797	53	53	1/12/2006	12/13/2007
UCD-ATL	Napa	Napa River in Napa City @ end of River Park Blvd.	38.277694	-122.282472	28	28	1/1/2008	5/12/2009
UCD-ATL	Suisun Public Dock	Suisun Public Dock	38.236188	-122.037662	2	2	1/1/2008	1/15/2008
UCD-ATL	Suisun Slough	Suisun Slough at Rush Ranch, downstream of Boynton Slough	38.207717	-122.033048	35	35	4/15/2009	5/12/2009
IEP-EMP	D10	Sacramento River @ Chipps Island	38.04631	-121.9183	309	0	1/8/1975	12/18/1995
IEP-EMP	D11	Sherman Lake near Antioch	38.04229	-121.7995	300	0	1/7/1975	12/18/1995
IEP-EMP	D12	San Joaquin River @ Antioch Ship Channel	38.02161	-121.8063	310	0	1/8/1975	12/18/1995
IEP-EMP	D14A	Big Break near Oakley	38.01776	-121.7114	271	0	1/8/1975	12/15/1995

Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
IEP-EMP	D15	San Joaquin River @ Jersey Point	38.053	-121.688	295	0	1/7/1975	12/15/1995
IEP-EMP	D2	Suisun Bay near Preston Point	38.06544	-122.0545	13	0	1/8/1975	12/16/1975
IEP-EMP	D22	Sacramento River @ Emmaton	38.083	-121.739	335	0	1/7/1975	12/19/1995
IEP-EMP	D4	Sacramento River above Point Sacramento	38.06248	-121.8205	343	9	1/7/1975	12/10/2009
IEP-EMP	D41	San Pablo Bay near Pinole Point	38.03022	-122.3729	241	10	2/14/1980	12/14/2009
IEP-EMP	D42	San Pablo Bay near Mare Island	38.05872	-122.2847	44	0	4/8/1976	12/12/1979
IEP-EMP	D6	Suisun Bay @ Bulls Head nr. Martinez	38.04436	-122.1177	324	10	1/8/1975	12/14/2009
IEP-EMP	D7	Grizzly Bay @ Dolphin nr. Suisun Slough	38.11714	-122.0397	331	10	1/8/1975	12/11/2009
IEP-EMP	D8	Suisun Bay off Middle Point nr. Nichols	38.05992	-121.99	315	10	1/8/1975	1/8/2010
IEP-EMP	D9	Honker Bay near Wheeler Point	38.07244	-121.9392	324	0	1/8/1975	12/18/1995
IEP-EMP	S42	Suisun Slough 300' south of Volanti Slough	38.181	-122.046	85	0	2/22/1978	8/3/1984
DWR-MWQI	E0B80261551	Sacramento River @ Mallard Island	38.04361111	-121.9186111	109	85	6/6/1996	1/5/2010

Attachment 2. Formulas Used to Derive Un-ionized Ammonia Fractions and USEPA Ammonia Criteria

Calculating Salinity (ppt) from Electrical Conductivity

$$S = S_{PSS} - \frac{0.0080}{1 + 1.5 \times X + X^2} - \frac{0.0005 \times f(T)}{1 + Y^{0.5} + Y^{1.5}}$$

where,

S = salinity (ppt) (using extension of Practical Salinity Scale to low salinities [0-40])

S_{PSS} = Salinity, using Practical Salinity Scale

$$S_{PSS} = 0.0080 - 0.1692 \times R^{0.5} + 25.3851 \times R + 14.0941 \times R^{1.5} - 7.0261 \times R^2 + 2.7081 \times R^{2.5} + \Delta S$$

$$\Delta S = \left[\frac{T - 15}{1 + 0.0162(T - 15)}\right] \times \left(0.0005 - 0.0056 \times R^{0.5} - 0.0066 \times R - 0.0375 \times R^{1.5} + 0.0636 \times R^2 - 0.0144 \times R^{2.5}\right)$$

$$f(T) = \frac{T - 15}{1 + 0.0162(T - 15)}$$

$X = 400 \times R$

$Y = 100 \times R$

T = temperature (°C)

$$R = \frac{EC_s}{EC_R}$$

EC_s = electrical conductivity of sample (µS/cm)

EC_R = electrical conductivity of seawater reference (58,670 µS/cm)

SALTWATER FORMULAS

Un-ionized Ammonia in Saltwater

$$f_{NH_3} = \frac{1}{1+10^{\left[pK_a + 0.0324(298-T) + \frac{(0.0415)P}{T} - pH\right]}}$$

where,

f_{NH3} = fraction of un-ionized ammonia

$I = \frac{19.9273 \times S}{1000 - 1.005109 \times S}$ (from EPA 1989, formula 5, p. 2)[1]

$pK_a = 9.245 + 0.116 \times I$

S = salinity (ppt)

T = temperature (°K)

P = pressure (assumed to be 1 atm)

Total Ammonia Saltwater Criterion Maximum Concentration (USEPA 1989, p. 27)

$C_{CMC} = \frac{0.233}{f_{NH_3}}$ (in mg/L as N)

Total Ammonia Saltwater Criterion Continuous Concentration (USEPA 1989, p. 16, 27)

$C_{CCC} = \frac{0.035}{f_{NH_3}}$ (in mg/L as N)

FRESHWATER FORMULAS

Un-ionized Ammonia in Freshwater (USEPA 1999, p. 2)

$$f_{NH_3} = \frac{1}{1 + 10^{pK - pH}}$$

where,

$$pK = 0.09018 + \frac{2729.92}{273.2 + T}$$

T = temperature (°C)

f_{NH3} = fraction of un-ionized ammonia

Total Ammonia Freshwater Criterion Maximum Concentration when salmonid fish are present (USEPA 1999, p. 83)

$$C_{CMC} = \frac{0.275}{1 + 10^{7.204 - pH}} + \frac{39.0}{1 + 10^{pH - 7.204}} \text{ (in mg N/L)}$$

Total Ammonia Freshwater Criterion Continuous Concentration when early life stages of fish are present (USEPA 1999, p. 83)

$$C_{CCC} = \left(\frac{0.0577}{1 + 10^{7.688 - pH}} + \frac{2.487}{1 + 10^{pH - 7.688}} \right) \times MIN\left(2.85, 1.45 \times 10^{0.028(25 - T)}\right) \text{ (in mg N/L)}$$

Attachment 3. Evaluation of ACRs Used to Infer Chronic Toxicity for Delta smelt

Acute-chronic ratios (ACRs) are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive species. For example, hypothetical ACRs for rainbow trout were used in a presentation at the Central Valley Regional Water Quality Control Board (CVRWQCB) Ammonia Summit in August 2009 (Werner 2009, slide10), and in recent reports to the CVRWQCB (Werner et al. 2009a,b), to support an argument that chronic exposure to ambient levels of ammonia in the Delta may cause toxicity for Delta smelt. This logic behind the argument can be summarized as follows:

- Chronic toxicity test results are lacking for Delta smelt.
- Delta smelt appear to be as acutely sensitive to ammonia as rainbow trout (*Oncorhynchus mykiss*).
- Therefore, chronic toxicity values for Delta smelt are probably similar to those for rainbow trout.
- Hypothetical ACRs for rainbow trout are alleged to be in the range 14.6-23.5.
- Therefore, one can divide the LC50 for delta smelt (acute value) by hypothetical ACRs for rainbow trout to estimate the concentration of ammonia that would cause chronic toxicity to Delta smelt
- Some ambient ammonia concentrations in the Delta are higher than the values that result from this exercise.

Below we provide information which shows that the hypothetical ACRs for rainbow trout stated above (14.6 and 23.5) rely on information that was excluded by USEPA in 1999 and 2009 for use in developing the chronic criterion and are not based on evidence for chronic effects of ammonia effects on survival, reproduction, or growth of rainbow trout (USEPA 1999, 2009). Consequently, inferences about chronic toxicity for Delta fish species - such as Delta smelt - based on these ACRs are questionable and should be carefully qualified.

USEPA Position on Valid Chronic Endpoints and Chronic Test Design for Fish, and Interpretation of Chronic Data for Rainbow Trout

In 1999, USEPA used explicit criteria to re-evaluate the available chronic toxicity tests for fish and aquatic invertebrates (USEPA 1999). One result of this analysis was a list of acceptable chronic tests. This list appears as Table 5 ("EC20s from Acceptable Chronic Tests") on page 65 of USEPA (1999), along with Species Mean Chronic Values (SMCV) and Genus Mean Chronic Values (GMCV) where it was appropriate to calculate them. Among the criteria for inclusion in this list were (1) the test had to be a flow-through test (except that static renewal is acceptable for daphnids), (2) test conditions had to include acceptable dissolved oxygen concentrations, and (3) the endpoint(s) of the test had to be survival, growth, and/or reproduction.[1] Where possible, regression analysis was used to generate EC20s for many of the acceptable studies.

In order for a chronic test to be used as part of the basis for a SMCV in USEPA (1999), it had to satisfy the definitions given in the USEPA (1985a) *Guidelines for Deriving Numerical Criteria* for a "life-cycle", "partial-life-cycle", or "early-life-stage" test. These criteria *as they apply to fish* are provided in Table 1 below.

If not meeting the criteria for any of the three test categories in Table 1, USEPA guidelines allow for *potential limited* use of data from two alternative types of tests involving fish:

1. Seven-day tests of survival, reproduction, and/or hatchability, or
2. Ninety-day tests of growth

USEPA requires that such alternative tests using *growth* as an endpoint must last for at least 90 days because reductions in weight gain for fewer than 90 days can be temporary. Per the USEPA (1985a) guidelines, neither of the two alternative types of test above should be used as the basis for a discrete chronic value for a species. However, such tests can be used as evidence for an upper limit for a chronic value (in other words, determinations that the true chronic value is *likely less than* the threshold concentration observed in the test).

The list of acceptable chronic tests for fish and their associated EC20s, and SMCVs and GMCVs (standardized to pH=8 and T=25°C) that resulted from the 1999 vetting process are provided in Table 2 below. Not all of the acceptable chronic tests included in USEPA Table 5 resulted in specific EC20s, or SMCVs. When *none* of the concentrations used in an acceptable chronic test caused significant effects on survival, growth, or reproduction, the highest concentration from the test was entered in USEPA Table 5 as "$>x$" to indicate that underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but higher than the concentration by an unknown amount. Conversely, if *all* of the concentrations used in an acceptable test caused significant effects on survival, growth, or reproduction (i.e., none of the concentrations were "no-effects concentrations", or NOECs), the lowest concentration from the test was entered in the table as "$<x$" to indicate that the underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but less than the concentration by an unknown amount. "Less than" or "greater than" qualifiers were also applied to some of the SMCVs and GMCVs calculated by USEPA.

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[1] USEPA does not utilize concentrations associated with histopathologic or behavioral endpoints (e.g. swimming speed) for SMCV derivation because they have determined that there is "no justification for equating histopathological effects with effects on survival, growth, and reproduction" (USEPA 1999, p. 45). This position is more fully explained in Appendix 5 in USEPA (1999), and was maintained in the 2009 Draft Update, released on December 30, 2009 (USEPA 2009).

Table 1. USEPA Criteria for Life-Cycle, Partial-Life-Cycle, and Early-Life-Stage Chronic Toxicity Tests for Fish.

Test Type	Fish Test Criteria	Data should Include	Potentially used to Derive:
Life cycle	• Tests must begin with embryos or newly hatched young <48-hrs old • Test must continue through maturation and reproduction • Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids).	• Survival and growth and adults and young • Maturation of males and females • Eggs spawned per female • Embryo viability (salmonids) • Hatchability	Depending on results: • Upper limit for a CV • Lower limit for a CV • CV
Partial life cycle	• Allowed for use with fish that require more than a year to reach sexual maturity. • Test must begin with immature juveniles at least 2 months prior to active gonad development. • Test must continue through maturation and reproduction. • Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids).		
Early life-stage	• Test must begin shortly after fertilization of eggs. • Test must continue through embryonic, larval, and early juvenile development. • Test must continue for 60 day post hatch for salmonids (28-32 days for non-salmonids).	• Survival and growth and adults and young	

Table 2. EC20s and other Toxicity Parameters Accepted by USEPA (1999) from Chronic Tests Meeting USEPA Test Acceptability Criteria for Fish.

Species	EC20s	Species Mean Chronic Value at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Chronic Value (GMCV) at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Acute-Chronic Ratio (GMACR)
Pimephales promelas (fathead minnow)	1.97 2.92 5.12	3.09	3.09	10.9
Catostomus commersoni (white sucker)	>4.79	>4.79	>4.79	<8.4
Ictalurus punctatus (channel catfish)	8.38 9.33 <8.7 to <9.9	8.84	8.84	2.7
Lepomis cyanellus (green sunfish)	7.44 4.88	6.03	2.85	7.6
Lepomis macrochirus (bluegill)	1.35	1.35		
Micropterus dolomieu (smallmouth bass)	3.57 4.01 6.5 4.65	4.56	4.56	7.4
Oncorhynchus clarki (cutthroat trout)	<19.7	Not Available: USEPA determined it was inappropriate to calculate SMCVs for *Oncorhynchus* species (see text).	Not Available	Not Available
Oncorhynchus mykiss (rainbow trout)	>5.4(a) <18.7(b) <1.44(c) 1.34(d)			
Oncorhynchus nerka (sockeye salmon)	<4.16			

(a) based on the highest concentration tested by Thurston et al. (1984)
(b) based on LC50s obtained over 42-days by Burkhalter & Kaya (1977)
(c) based on 73-day LC20 obtained by Solbe & Shurben (1989)
(d) based on test results by Calamari et al. (1977, 1981), interpolated by USEPA to estimate a 72-day LC20

USEPA determined that EC20s from five tests using rainbow trout were from acceptable chronic tests. However, as a group, the EC20s for rainbow trout did not meet USEPA standards for further use in calculating SMCVs, or for use in calculating a GMCV for its genus *Oncorhynchus*:

> “Because of the concerns about some of the tests, the differences among the results, and the fact that some of the results are either “greater than” or “less than” values, even though the various results are included in Table 5, a SMCV is not derived for rainbow trout; instead the results of the chronic tests will be used to assess the appropriateness of the CCC”. (USEPA 1999; p. 60)

No additional chronic test results for rainbow trout were included in the recently released USEPA Draft 2009 Update for the freshwater ammonia criteria (USEPA 2009), in which USEPA again declined to calculate a GMCV for *Oncorhynchus*.

> "As noted in the 1999 AWQC document, five other studies have reported results of chronic tests conducted with ammonia and other salmonids including *Oncorhynchus mykiss* and *Oncorhynchus nerka*. There is a lack of consistency among the chronic values obtained from these tests, and several tests produced "greater than" and "less than" values (Table 5). Consequently, in keeping with the decision made in the 1999 AWQC document, a GMCV is not derived for *Oncorhynchus*. Instead, the results of the chronic tests were used to assess the appropriateness of the CCC." (USEPA 2009, p. 21)

In Appendix 7 of USEPA (1999), Acute-Chronic Ratios (ACRs) were calculated for all EC20s that *were* used to generate SMCVs (from USEPA 1999, Table 5) *and* which could be paired with comparable acute values (LC50s; see more about pairing criteria below). Then, these ACRs were used to calculate Genus Mean Acute Chronic Ratios (GMACR). This analysis resulted in GMACRs for five genera of fish, which are included in Table 2. ***The USEPA-vetted GMACRs for fish occupy the range 2.7-10.9***.

Origin of Postulated ACRs for Rainbow Trout Being Used to Infer Chronic Toxity for Delta Smelt

At the August 2009 Ammonia Summit, Dr. Inge Werner provided two values as the upper and lower limits for the ACR for rainbow trout (14.6-23.5; Werner 2009a, slide 10). The derivation of these values was not a part of Werner's talk at the Ammonia Summit. The same values were presented in the annual reports for 2008 and 2009 for the UC Davis Aquatic Toxicology Lab's Delta smelt ammonia toxicity tests (Werner et al. 2009a, b) as follows (language is from 2009 report; almost identical passage occurs in 2008 report):

> "Exposure duration is an important factor influencing the toxicity of ammonia. Seven-day toxicity tests, as performed in this study, are unable to detect the potential chronic effects of ammonia/um exposure on delta smelt. Acute-to chronic ratios are one method that has traditionally been used to extrapolate between acute and chronic toxicity when procedures for chronic testing are not available. For fish, the US EPA (1999) reports mean acute-to-chronic ammonia/um ratios for warm water fish that range between 2.7 (channel catfish, *Ictalurus punctatus*) and 10.9 (fathead minnow, *P. promelas*). Cold water species such as rainbow trout, with acute ammonia/um sensitivity similar to delta smelt, have a ratio between 14.6 and 23.5, respectively (US EPA, 1999; Passell et al., 2007). If these safety factors were applied to acute effect concentrations for effluent and delta smelt larvae (7-d LC50: 3.92 mg/L)[2] then the resulting threshold concentrations for total ammonia/um would be 0.27 and 0.17 mg/L for the above safety ratios of 14.6 and 23.6, respectively. These chronic effect thresholds are below long-term average concentrations in the Sacramento River below SRWTP." (Werner et al. 2009b, page 33)

The passage above can be interpreted to mean that rainbow trout ACRs of 14.6 and 23.5 were derived by USEPA or by Passell et al. (2007). However, neither of these references provide ACRs for rainbow trout. As explained above, in 1999 and 2009, USEPA refused

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[2] This appears to be a mistake in Werner et al. (2009b). 3.92 mg/L was the 7-day LOEC for this test. The LC50 was 5.40 mg/L (see Werner et al. 2009b, p. 15).

to calculate an ACR for rainbow trout - or for even for the genus *Oncorhynchus* - owing to inadequate data. Chronic toxicity tests were not a part of the original work reported in Passell et al. (2007). As clarification, Dr. Werner explained that she had calculated the ACRs for rainbow trout as follows:

> "I used the chronic values for unionized ammonia provided in Table 3 of Passell et al. (0.031 mg/L and 0.05 mg/L), and the species mean acute value from EPA 1999 (given in total ammonia/um)[3] to calculate the corresponding value for unionized ammonia (0.728 mg/L unionized ammonia), then calculated the ratio between them [which] results in 14.56 and 23.5." (I. Werner, pers. comm., Dec. 22, 2009).

Table 3 in Passell et al. (2007) is a collection of acute and chronic values for several fish species from the literature that was included for discussion purposes in the article. In the table, Thurston et al. (1984) and Burkhalter & Kaya (1977) are cited as the original sources of the 0.031 and 0.05 mg/L un-ionized ammonia-N concentrations, respectively. The original sources of the values are not critically evaluated in the article. Below, we discuss the original studies, and associated information about them in USEPA (1999). The results indicate that the chronic concentrations Dr. Werner used to compute ACRs for rainbow trout did not meet USEPA criteria for such use.

<u>Thurston et al. (1984).</u> Thurston et al. (1984) was a 5-year life cycle test which exposed offspring from one pair of rainbow trout, and their F1 and F2 progeny, to the following mean concentrations of un-ionized ammonia in flow-through troughs: 0.001, 0.013, 0.022, 0.044, 0.063, and 0.074 mg N/L. Regarding this study, USEPA (1999) states that "the important data for each life stage are so variable that it is not possible to discern whether there is a concentration-effect curve" (USEPA 1999; p. 58). According to the original article, there was no significant relationship between ammonia concentration and (1) mortality of all three generations, (2) growth of F1 and F2 progeny[4], or (3) egg production. Because none of the exposure levels used by Thurston et al. (1984) caused significant effects on survival, growth or reproduction, the results of this test fell under the "greater than" category of chronic test results in USEPA (1999). In other words, USEPA concluded that the underlying (unknown) chronic value for rainbow trout must be greater than the highest test concentration used in the study (5.4 mg/L total ammonia-N at pH=8, T=25°C).

Passell et al. (2007) do not explain why they identified 0.031 mg/L un-ionized ammonia-N as an appropriate chronic value from Thurston et al. (1984), or why it merited status as one of only two chronic concentrations for rainbow trout to include in their article. Because *none* of the test concentrations in Thurston et al. (1984) resulted in significant effects on survival, growth, or reproduction for 3 generations of fish, no EC20s (or other effects concentrations) are available from this test for approved endpoints. Earlier USEPA criteria documents (Table 2 in both USEPA 1985b, 1989) list 0.031 as a chronic

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[3] The species mean acute value for rainbow trout in USEPA (1999) is 11.23 mg/L total ammonia-N (standardized to pH=8, 25°C).

[4] It was not possible to evaluate growth of the parental fish because they were not weighed at the start of the test.

value for Thurston et al. (1984) which - after comparison of the original article with associated text in USEPA 1984 - appears to have been calculated using a NOEC and LOEC related to epidermal cell changes. However, this interpretation of the results from Thurston et al. (1984), which depends on the use of a non-conventional endpoint, was rejected in both of the most recent USEPA criteria documents (1999, 2009).

<u>Burkhalter & Kaya (1977)</u>. Burkhalter & Kaya (1977) did not report EC20s for rainbow trout. Instead, they reported LC50 results from a 42-day exposure of rainbow trout embryos and sac fry. Because the study did not provide EC20s, the results of this test fell under the "less than" category of chronic test values. In other words, USEPA concluded that the underlying (unknown) chronic value would have been less than the LC50 from their study (18.7 mg/L total ammonia-N at pH=8, T=25°C). However, the value of 0.05 mg/L unionized ammonia-N attributed to Burkhalter & Kaya (1977) in Passell et al.'s table (which was ultimately used by Dr. Werner to generate one of her ACRs for rainbow trout) is *not* that associated with the LC50 from their study (which was 0.25 mg/L unionized ammonia-N). The only available explanation for Passell et al.'s identification of 0.05 mg N/L as a chronic value from Burkhalter & Kaya is that 0.05 mg N/L was the lowest exposure concentration they used, which caused "some retardation of early growth and development" (quote from abstract of Burkhalter & Kaya). However differences in growth rate at this low test concentration (0.05) compared to the control were slight, and disappeared after two weeks of exposure. Because of the short duration of Burkhalter & Kaya's test, it was not considered by USEPA in 1999 as an appropriate test to gauge the effects of ammonia on growth on early life stages of rainbow trout.

As indicated above, Thurston et al. (1984) and Burkhalter & Kaya (1977) are discussed in USEPA (1999) and were two of the rainbow trout studies included in the list of acceptable chronic studies (see EC20 values in Table 2 above). However, as explained above, after re-evaluation of these two studies, USEPA interpreted the results of these two studies as evidence for an EC20 *greater than* 5.4 mg/L total ammonia-N (Thurston et al. study) and *less than* 18.7 mg/L total ammonia-N (Burkhalter & Kaya study; both values standardized to pH=8, 25°C). Taken in isolation from other chronic tests, USEPA's upper and lower limits from these two studies imply that the rainbow trout ACR falls somewhere within the range (0.60-2.08)[5] - which is very different than the one proposed by Dr. Werner (14.6 - 23.5).

A recent 90-day chronic test measuring the hatching success of newly fertilized eggs from a wild strain of rainbow trout, and subsequent survival and growth of sac fry and swim-up fry (Brinkman et al. 2009), resulted in a chronic value (the geometric mean of the LOEC and NOEC) of 8.06 mg/L total ammonia-N and a 90-day EC20 (based on biomass) of 5.56 mg/L total ammonia-N (standardized to pH 8). This test appears to meet the USEPA criteria for early-life-stage tests for salmonids outlined in Table 1; an ACR for rainbow trout based on the chronic value from this recent test would be about 1.4. However, even if Brinkman et al. (2009) was added to its list of acceptable chronic

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[5] 11.23/18.7 = 0.60; 11.23/5.4=2.08

tests[6], USEPA might still conclude that chronic test data for rainbow trout are too variable, or otherwise insufficient, to calculate SMCVs, or an ACR for the species or the genus.

In general, the approach of pairing acute values and chronic values from different investigations to compute ACRs is not necessarily in agreement with USEPA guidelines. USEPA (1985a), outlines the following steps for producing an ACR from a chronic value:

1. The numerator for the ACR should be the geometric mean of the acute values for that species from all acceptable flow-through acute tests in the same dilution water.
2. For fish, the acute tests should have been conducted with juveniles.
3. The acute tests should have been (a) a part of the same study as the chronic tests, (b) from different studies but from the same laboratory and dilution water, or (c) from studies at different laboratories using the same dilution water.
4. If no such acute tests are available, an ACR should not be calculated.

Conclusion

In summary, *based on the most recent USEPA criteria for chronic test design and endpoints, derivation of ACRs, and interpretation of data from chronic tests for fish*, no information is available to support a proposal that the ACR for rainbow trout occupies the range 14.6-23.5. Derivation of hypothetical ACRs for rainbow trout as high as the ones used at recent meetings and reports is not possible using direct evidence for chronic effects of ammonia on survival, growth, or reproduction and represents a significant departure from current USEPA guidance concerning the use of data from chronic tests for the species. Assertions about chronic toxicity in the Delta that rely on these hypothetical ACRs for rainbow trout should be avoided. At a minimum, such assertions must be carefully qualified as not being based on evidence for population-level effects of ammonia on sensitive fish.

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[6] Brinkman et al. (2009) was published after the Feb. 2009 cut-off for the literature review used for the development of the USEPA 2009 Draft Update of the freshwater ammonia criteria.

REFERENCES CITED

Note: References below were provided as pdfs on the CD-ROM which accompanied written testimony submitted to the SWRCB by Sacramento Regional County Sanitation District.

Brinkman, S.F., J.D. Woodling, A.M.Vajda, and D.O. Norris. 2009. *Chronic Toxicity of Ammonia to Early Life Stage Rainbow Trout*. Trans. Amer. Fish. Soc. 138: 433-440.

Burkhalter, D.E., and D.M. Kaya. 1977. *Effects of Prolonged Exposure to Ammonia on Fertilized Eggs and Sac Fry of Rainbow Trout (Salmo gairdneri).* Trans. Amer. Fish. Soc. 106*:* 470-475*.*

Calamari, D., R. Marchetti, & G. Vailati. 1977. *Effects of Prolonged Treatments with Ammonia on Stages of Development of Salmo gairdneri*. Nuovi Ann. Ig. Microbiol. 28: 333-345. (not on CD, but discussed in USEPA 1999)

Calamari, D., R. Marchetti, & G. Vailati. 1981. *Effects of Long-term Exposure to Ammonia on the Developmental Stages of Rainbow Trout (Salmo gairdneri Richardson)*. Rapp. P.-v/ Reun. Cons. Int. Explor. Mer. 178:81-86. (not on CD, but discussed in USEPA 1999)

Passell, H.D., C.N. Dahm, & E.J. Bedrick. 2007. *Ammonia Modeling for Assessing Potential Toxicity to Fish Species in the Rio Grande, 1989-2002*. Ecol. Appl. 17: 2087-2099.

Solbe, J.F.L.G., & D.G. Schurben. 1989. Toxicity of Ammonia to Early Life Stages of Rainbow Trout (*Salmo gairdneri*). Water Res. 23:127-129.

Thurston, R.V., R.C. Russo, R.J. Luedtke, C.E. Smith, E.L. Meyn, C. Chakoumakos, K.C. Wang, & C.J.D. Brown. 1984. *Chronic Toxicity of Ammonia to Rainbow Trout*. Trans. Amer. Fish. Soc. 113:56-73.

USEPA. 1985a. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. United States Environmental Protection Agency.

USEPA. 1985b. *Ambient Water Quality Criteria for Ammonia - 1984*. EPA 440/5-85-001. United States Environmental Protection Agency, January 1985.

USEPA. 1989. *Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989.* EPA 440-5-88-004. United States Environmental Protection Agency, April 1989.

USEPA. 1999. *1999 Update of Ambient Water Quality Criteria for Ammonia*. United States Environmental Protection Agency, December 1999. EPA 822-R-99-014

USEPA. 2009. *Draft 2009 Update Aquatic Life Ambient Water Quality Criteria for Ammonia-Freshwater.* EPA 822-D-09-001. United States Environmental Protection Agency, December 2009.

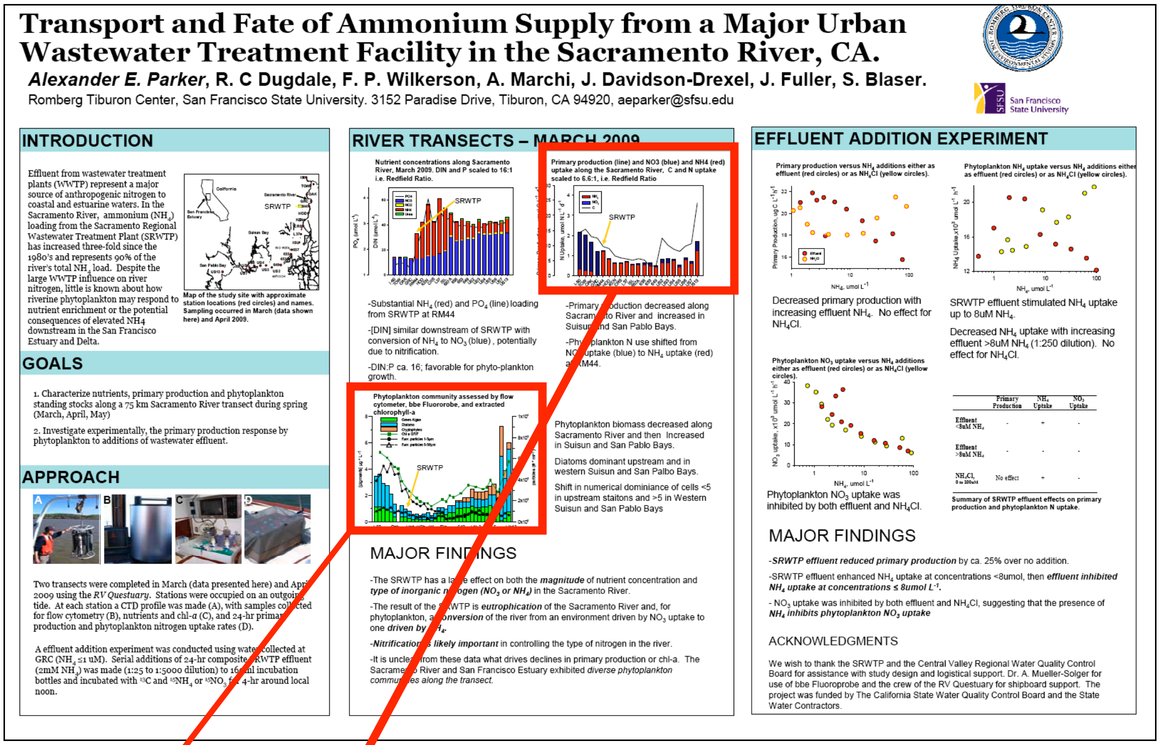
Werner, I. 2009. *Effects of Ammonia/um and Wastewater Effluent Associated Contaminants on Delta Smelt.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Werner , I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009a. *The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt.* Draft Final Report to the Central Valley Regional Water Quality Control Board. January 28, 2009.

Werner , I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009b. *Acute toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminant on Delta Smelt - 2009.* Draft Report to the Central Valley Regional Water Quality Control Board. October 30, 2009.

Attachment 4. Figures from Parker et al. 2009b

Note: pdf of complete poster is included on the CD Rom of SRCSD exhibits



Transport and Fate of Ammonium Supply from a Major Urban Wastewater Treatment Facility in the Sacramento River, CA.

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INTRODUCTION

Effluent from wastewater treatment plants (WWTP) represent a major source of anthropogenic nitrogen to coastal and estuarine waters. In the Sacramento River, ammonium (NH_4) loading from the Sacramento Regional Wastewater Treatment Plant (SRWTP) has increased three-fold since the 1980's and represents 90% of the river's total NH_4 load. Despite the large WWTP influence on river nitrogen, little is known about how riverine phytoplankton may respond to nutrient enrichment or the potential consequences of elevated NH4 downstream in the San Francisco Estuary and Delta.

Map of the study site with approximate station locations (red circles) and names. Sampling occurred in March (data shown here) and April 2009.

GOALS

1. Characterize nutrients, primary production and phytoplankton standing stocks along a 75 km Sacramento River transect during spring (March, April, May)
2. Investigate experimentally, the primary production response by phytoplankton to additions of wastewater effluent.

APPROACH

Two transects were completed in March (data presented here) and April 2009 using the *RV Questuary*. Stations were occupied on an outgoing tide. At each station a CTD profile was made (A), with samples collected for flow cytometry (B), nutrients and chl-*a* (C), and 24-hr primary production and phytoplankton nitrogen uptake rates (D).

A effluent addition experiment was conducted using water collected at GRC (NH_4 ≤1 uM). Serial additions of 24-hr composite SRWTP effluent (2mM NH_4) was made (1:25 to 1:5000 dilution) to 160ml incubation bottles and incubated with ^{13}C and $^{15}NH_4$ or $^{15}NO_3$ for 4-hr around local noon.

RIVER TRANSECTS – MARCH 2009

Nutrient concentrations along Sacramento River, March 2009. DIN and P scaled to 16:1 i.e. Redfield Ratio.

-Substantial NH_4 (red) and PO_4 (line) loading from SRWTP at RM44

-[DIN] similar downstream of SRWTP with conversion of NH_4 to NO_3 (blue) , potentially due to nitrification.

-DIN:P ca. 16; favorable for phyto-plankton growth.

Primary production (line) and NO3 (blue) and NH4 (red) uptake along the Sacramento River, C and N uptake scaled to 6.6:1, i.e. Redfield Ratio

-Primary production decreased along Sacramento River and increased in Suisun and San Pablo Bays.

-Phytoplankton N use shifted from NO_3 uptake (blue) to NH_4 uptake (red) at RM44.

Phytoplankton community assessed by flow cytometer, bbe Fluororobe, and extracted chlorophyll-a

Phytoplankton biomass decreased along Sacramento River and then Increased in Suisun and San Pablo Bays.

Diatoms dominant upstream and in western Suisun and San Palbo Bays.

Shift in numerical dominiance of cells <5 in upstream staitons and >5 in Western Suisun and San Pablo Bays

MAJOR FINDINGS

-The SRWTP has a large effect on both the ***magnitude*** of nutrient concentration and ***type of inorganic nitrogen (NO_3 or NH_4)*** in the Sacramento River.

-The result of the SRWTP is ***eutrophication*** of the Sacramento River and, for phytoplankton, a ***conversion*** of the river from an environment driven by NO_3 uptake to one ***driven by NH_4***.

-***Nitrification is likely important*** in controlling the type of nitrogen in the river.

-It is unclear from these data what drives declines in primary production or chl-a. The Sacramento River and San Francisco Estuary exhibited *diverse phytoplankton communities along the transect.*

EFFLUENT ADDITION EXPERIMENT

Primary production versus NH_4 additions either as effluent (red circles) or as NH_4Cl (yellow circles).

Decreased primary production with increasing effluent NH_4. No effect for NH_4Cl.

Phytoplankton NH_4 uptake versus NH_4 additions either as effluent (red circles) or as NH_4Cl (yellow circles).

SRWTP effluent stimulated NH_4 uptake up to 8uM NH_4.

Decreased NH_4 uptake with increasing effluent >8uM NH_4 (1:250 dilution). No effect for NH_4Cl.

Phytoplankton NO_3 uptake versus NH_4 additions either as effluent (red circles) or as NH_4Cl (yellow circles).

Phytoplankton NO_3 uptake was inhibited by both effluent and NH_4Cl.

	Primary Production	NH_4 Uptake	NO_3 Uptake
Effluent <8uM NH_4	-	+	-
Effluent >8uM NH_4	-	-	-
NH_4Cl, 0 to 100uM	No effect	+	-

Summary of SRWTP effluent effects on primary production and phytoplankton N uptake.

MAJOR FINDINGS

-***SRWTP effluent reduced primary production*** by ca. 25% over no addition.

-SRWTP effluent enhanced NH_4 uptake at concentrations <8umol, then ***effluent inhibited NH_4 uptake at concentrations ≤ 8umol L^{-1}.***

- NO_3 uptake was inhibited by both effluent and NH_4Cl, suggesting that the presence of ***NH_4 inhibits phytoplankton NO_3 uptake***

ACKNOWLEDGMENTS

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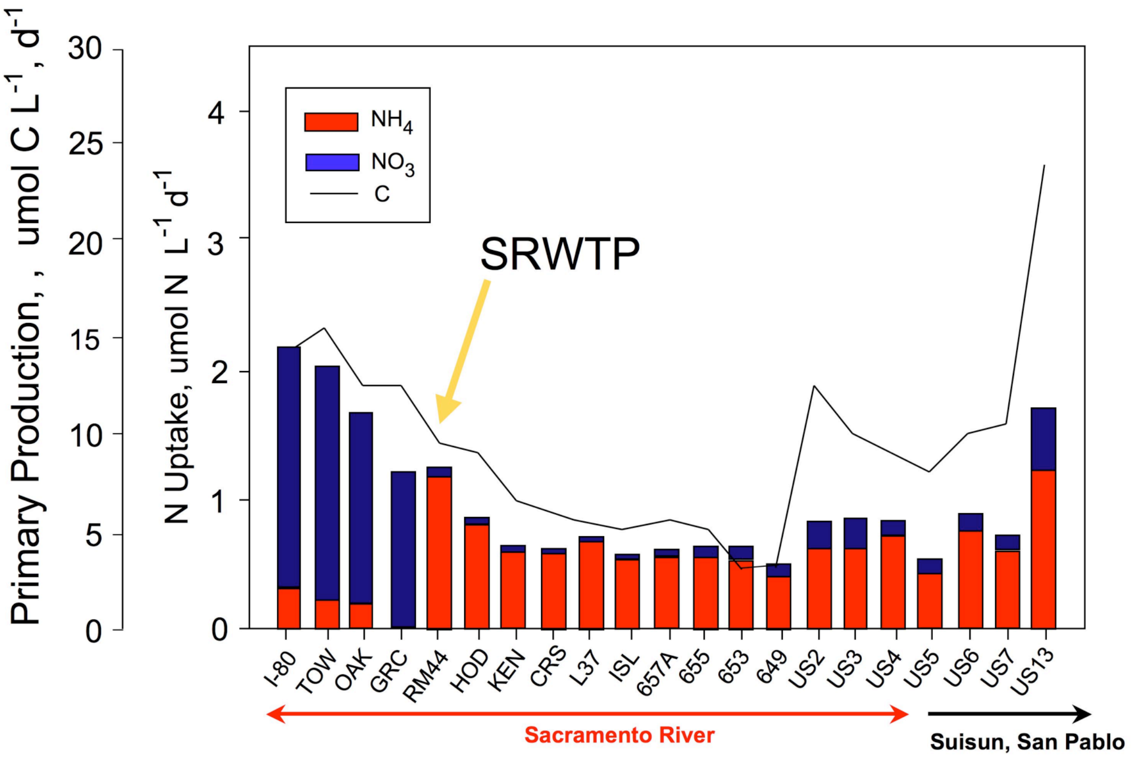
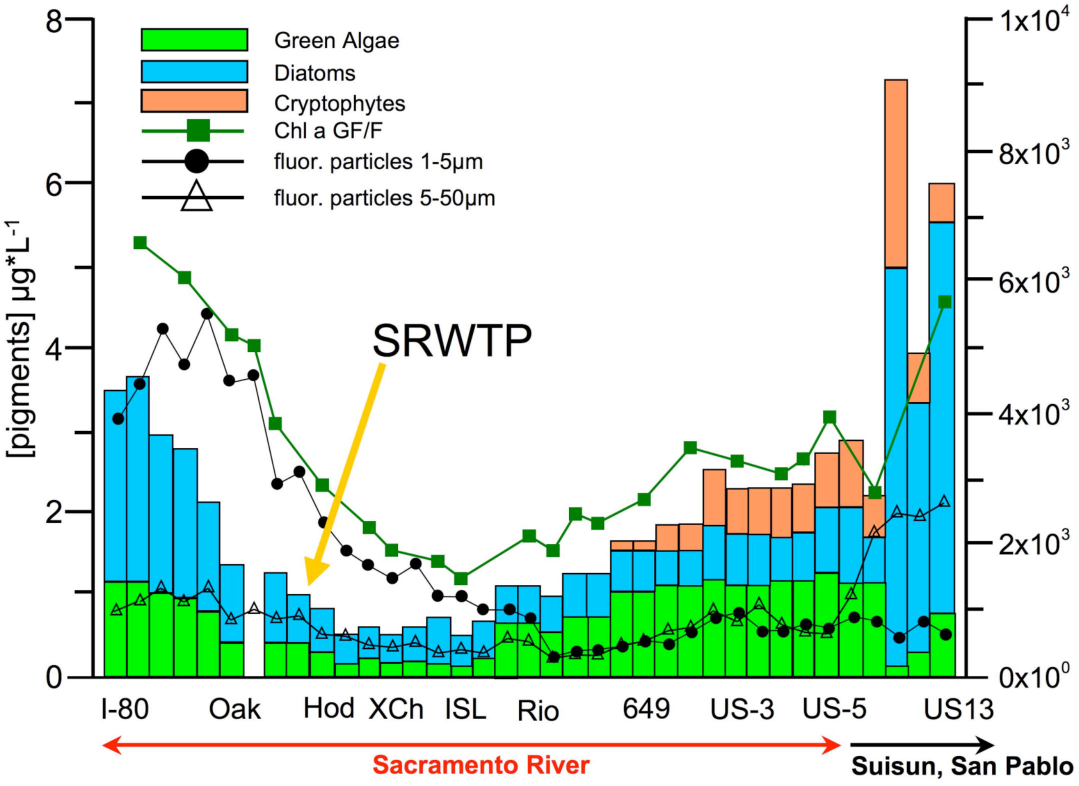
Figures enlarged on next 2 pages.

ATTACHMENT 4. Figures from Parker et al. 2009b



(arrows below station codes were added to original figure)



(arrows below station codes were added to original figure)