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April 21, 2011

Ms. Erin Foresman
U.S. Environmental Protection Agency, Region IX
75 Hawthorne Street, WTR-3
San Francisco, California 94105

Submitted electronically via www.regulations.gov, Docket No. EPA-R09-OW-2010-0976, *and via US mail copy*

Subject: Sacramento Regional County Sanitation District Comments on Water Quality Challenges in the San Francisco Bay/Sacramento--San Joaquin Delta Estuary United States Environmental Protection Agency Unabridged Advanced Notice of Proposed Rulemaking February 2011

Dear Ms. Foresman:

The Sacramento Regional County Sanitation District (SRCSD) appreciates the opportunity to provide comments to the United States Environmental Protection Agency (EPA) on the Advanced Notice of Proposed Rulemaking (ANPR), February 10, 2011 on Water Quality Challenges in the San Francisco Bay-San Joaquin Delta Estuary. SRCSD provides wastewater collection and treatment services to 1.3 million residents of the greater Sacramento area. SRCSD operates its treatment system in compliance with its National Pollutant Discharge Elimination System (NPDES) permit, providing protection of beneficial uses of the Sacramento River and Sacramento-San Joaquin Delta.

SRCSD takes seriously its environmental stewardship role in protecting the environment and our watershed for future generations. We believe environmental stewardship is good business, a public trust responsibility, and key to achieving sustainable outcomes. Motivated by a strong environmental ethic, dedicated staff throughout our organization lead the way in environmental action founded on the latest scientific research. SRCSD is committed to ensuring sound science is a basis for decisions regarding ecosystem protection and water supply in the Delta.

We understand that the purpose of the ANPR is to assess the effectiveness of water quality programs designed to protect the Estuary's aquatic species and to determine if a proposed rule to implement additional regulation is necessary based on available information. This ANPR functionally is a review of the effectiveness of the State Water Resources Control Board and Regional Water Quality Control Board's (Water Boards) water quality programs. We believe the existing regulatory framework, led by the Water Boards, is well suited and has been effective to date in addressing water quality concerns. EPA's role in working together with the on water quality issues is well established. Using this established working relationship, EPA can help with:

Technology in balance with nature

- Synthesis of data and science,
- Pesticides, and
- Contaminants of Emerging Concern

SRCSD believes that EPA can assist the Delta Stewardship Council (Council), through the ANPR, in collecting and synthesizing the best available science regarding water quality in the Delta. We encourage following the Clean Water Act, as it entails a level of rigorous science (including sound statistical and analytical methods), data integrity, peer reviewed research, etc. that will lead to adaptive management.

Solving the Delta ecosystem's problems will require a holistic approach and SRCSD is a strong advocate of using sound science in making integrated environmental decisions. Any focus on water quality should be coupled with a focus on export volumes and timing, hydraulic flow regimes in the Delta, channel hydrology, landscape, and invasive species. EPA is uniquely suited to add value to the Bay Delta proceedings through its authority over pesticides and the development of information and accurate analytical methods for measurement of contaminants of emerging concern (CECs). Rather than undertaking new regulatory initiatives aimed at water quality criteria for specific contaminants, we encourage EPA to assist the State and local agencies to address emerging issues through adaptive management.

The science necessary for setting water quality standards is daunting, and therefore requires expertise from multiple disciplines. Attached to this letter are comments and information developed by a variety of professionals (aquatic biologist, fisheries biologist, environmental engineer, environmental scientist, and toxicologist), based on their knowledge of the up-to-date science regarding water quality and fish corridors in the Delta. The comments are organized by the program areas for public comment, and questions.

If you have any questions about these comments, please contact me, 916-875-9116, somavarapup@sacsewer.com, or Terrie Mitchell, 916-876-6092 or, mitchellt@sacsewer.com.

Sincerely,

Prabhakar Somavarapu

Prabhakar Somavarapu
Director of Policy and Planning

Attachments: 1: SRCSD Comments on the ANPR by Program Area and Question Number
2: State Water Resources Control Board, Delta Flow Criteria Informational Proceeding, Other Stressors Panel, March 24, 2010 with hardcopy
3: Total Nitrogen (TN):Total Phosphorous (TP) Data for Suisun Bay- Engle Unpublished Data

cc: Stan Dean, SRCSD District Engineer
Terrie Mitchell, Legislative and Regulatory Affairs Manager

Attachment 1: SRCSD Comments on the ANPR by Program Area and Question Number

Contaminants

Due to our experience with TMDLs our focus is on question number three. Using ambient water for testing is one way to address interactive effects in a TMDL. Grouping of pollutants under one TMDL based on physical/chemical properties of the constituents is another possible way to incorporate interactive effects.

3. What methods can be used in developing and implementing TMDLs to effectively address or incorporate interactive effects between multiple contaminants and other physical, chemical, and biological stressors on individual water bodies or for water bodies within a watershed?

Interactive effects between multiple contaminants are often only seen when the concentrations tested exceed environmentally relevant concentrations. More and more studies are being conducted to assess the effects of mixtures of chemicals. However, many studies that have been conducted to date have not tested environmentally relevant concentrations. Ambient concentrations are typically well below the ‗no effects' threshold found in experiments.[1] Studies cited in the EPA Technical Support Document (EPA, 1991) found that synergism is a rare occurrence in combined effluent toxicity studies. In most cases it was found:

> *. in the few studies on the growth of fish, the joint effect of toxicants has been consistently less than additive which suggests that as concentrations of toxicants are reduced towards the levels of no effect, their potential for addition is also reduced. There appear to be no marked and consistent differences between the responses of species to mixtures of toxicants[2].*

Using ambient water as the diluent in effluent toxicity testing allows for identification of synergistic, additive or antagonistic characteristics when the effluent mixes with the receiving water, thus can provide a better description of the toxic effects of the discharge in the receiving water. EPA recommends using ambient water for chronic toxicity tests if the objective is to determine additive or mitigating effects of the effluent to already contaminated receiving water as well as estimate the chronic toxicity of the effluent in uncontaminated receiving water.

Chronic toxicity tests were conducted by Buhl (1998) by mixing 11 inorganics (As^{3+}, Cd, Cr^{6+}, Cu, CN, Pb, Hg, Ni, Se^{4+}, Ag, Zn) at a ratio of their proposed Criteria Continuous Concentration (CCC) in an 8-day static-renewal test[3]. The findings were that proposed site-specific CCCs for 11 inorganics are not protective of *Ceriodaphnia dubia* if they occur simultaneously in water. Acute tests were performed on fathead minnow in a 96-hour static-renewal test. The results showed that the proposed site-specific Criteria Maximum Concentration (CMC) for a mixture of these inorganics is protective of fathead minnows. The results of this study suggest that, for more than additive toxicity to occur there must be a coinciding mixture of many chemicals, all at their water quality criterion concentrations, to exhibit such a toxic effect.

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[1] Werner. I. et al., ―Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent- Associated Contaminants on Delta Smelt (2009)", Central Valley Regional Water Quality Control Board and the Sacramento Regional Wastewater Treatment Plant. Final Report.

[2] Alabaster, J., and R. Lloyd, eds. (1982). *Water Quality Criteria for Fish*. 2nd edition. Butterworths, London.

[3] Buhl, K.J. *Toxicity of Proposed Water Quality Criteria-Based Mixtures of 11 Inorganics to Ceriodaphnia Dubia and Fathead Minnow*. USGS, March 1, 1998.

Direct toxicity to Delta species has been evaluated as a potential cause of the Pelagic Organism Decline (POD) for the past several years, although no direct causal relationships have been shown. Werner reported possible synergistic or additive toxicity with ammonia and other contaminants[4]. However, the conditions supporting this possibility were only met at concentrations far exceeding those that are environmentally relevant (i.e. that actually occur) in the Sacramento River and Delta. Repeat testing by Werner in 2008 and 2010 over four other test periods did not show any toxicity to delta smelt from up to 28% effluent. Furthermore, there was no toxicity to delta smelt, *Hyalella,* or fathead minnows in streamside ―in situ" monitoring at Hood and Rough and Ready Island (in the lower Sacramento River confluence with the San Joaquin River) in 2009[5]. Delta smelt survival at Hood was as good as or better than control survival over 7-day exposures on five test events.

Of the current 303(d) listings for Delta waters, other than the Stockton Ship Channel, unknown toxicity and several specific pesticides are the only listed contaminants that pertain directly to aquatic life use impairment. Ambient toxicity testing, by nature, addresses interactive effects, since organisms are exposed to a mixture of potential contaminants in ambient samples. To date, efforts to identify the contaminants causing episodic ―unknown toxicity" in Delta waters has been largely unsuccessful.

Ambient toxicity studies have been, and continue to be performed in the Sacramento Regional Wastewater Treatment Plant (SRWTP) vicinity. Existing toxicity and effluent data support the conclusion that treated wastewater discharged from SRWTP does not pose a potential for toxicity to aquatic organisms in the receiving environment. Infrequent effluent toxicity reported in Whole Effluent Toxicity (WET) tests with SRWTP effluent has only occurred at concentrations exceeding ambient and environmentally relevant conditions in the Sacramento River. In addition, specific effluent-ammonia toxicity tests with delta smelt, *H. azteca*, and copepods have only resulted in toxicity at concentrations that are well above those that occur in the receiving water[6].

The required test species for SRWTP are the water flea, *Ceriodaphnia dubia,* fathead minnow, *Pimephales promelas,* and green alga, *Selenastrum capricornutum*. Performing toxicity testing with these three sensitive species in effluent/receiving water mixtures accounts for synergistic or additive toxicity resulting from the combination of pesticides or other contaminants in the effluent. Therefore, SRCSD is already, in effect, examining the potential for synergistic effects between SWRTP effluent and compounds found in the Sacramento River.

TMDLs and their implementation plans for the Delta could be constructed so as to recognize the similarities in fate and transport of a variety of contaminants and the differences between these conditions and the laboratory studies used in setting numeric water quality limits. For example,

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[4] Werner, I, ―Effects of Ammonia/um and Other Wastewater Effluent Associated Contaminants on Delta Smelt", presented at the 18-19 August 2009 Ammonia Summit at the Central Valley Regional Water Quality Control Board.

[5] Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2010. Acute Toxicity of SRWTP Effluent to Delta Smelt and Surrogate Species. Draft Final Report Submitted to the Central valley Regional Water Quality Control Board on August 23, 2010.

[6] Testimony/Comments of Cameron A. Irvine Regarding Renewal of Waste Discharge Requirements and the NPDES Permit (No. CA0077682) for Sacramento Regional County Sanitation District, Sacramento Regional Wastewater Treatment Plant Before the Central Valley Regional Water Quality Control Board on behalf of the Sacramento Regional County Sanitation District (SRCSD). Page 3.

recent Regional Board studies are focused on developing pesticides TMDL based on 28 high risk pesticides [7]. However, all of these pesticides have the common features of high K_{ow}: low solubility, hydrophobicity, and strong association with silt and sediment. Many pesticides are strongly associated with the particulate fractions in water and may be simultaneously trapped with various source control measures that filter or trap runoff.

As prescribed by the State Board TMDL policy, it is imperative that the implementation plan considers realistic and likely mechanisms to reduce pollutant loads. For example, implementation plans that emphasize source control measures for agricultural runoff will reduce loadings of silt, turbidity, nutrients, and both legacy and current-use pesticides all of which may have individual TMDL target concentrations, but all of which would be improved by the same broad implementation strategies.

TMDL implementation plans that address multiple contaminants should take into account the seasonality of loading of most of these particulate-associated chemicals. Groupings of pollutants under one TMDL is specifically suggested where common factors such as fate, transport, and seasonality govern the pollutants (i.e., a ―common analytical approach")[8].

Ammonia

No independent reviews of the potential impact of ammonia on the Delta have led to a consensus that ammonia, or other nutrients, are a key driver of ecological problems in the Delta, including the pelagic organism decline. The State Water Board examined the issue just last year, convened an ―other stressors" panel in connection with its informational proceeding on Delta flow issues, and concluded only that more study is appropriate.[9] A CD of that informational proceeding is attached for your information. The United States Geologic Survey recently released a report on trends of nutrients in the San Joaquin and Sacramento River basins, that concluded point sources were minor contributors to nutrients in those basins, and provided additional data and analysis for ammonia and nutrients that should be considered in the ANPR.[10]

SRCSD's wastewater treatment plant is commonly cited as the largest contributor of ammonia to the Delta, and therefore we have been performing ammonia monitoring and otherwise studying the issues concerning ammonia for more than three years. To date, there has not been a genuine effort to identify all of the sources of ammonia to the Lower Delta and Suisun Bay. We recommend that a mass balance of ammonia be performed for the Lower Delta and Suisun Bay

[7] CVRWQCB 2009 Central Valley Regional Board Relative-Risk Evaluation for Pesticides Used in the Central Valley Pesticide Basin Plan Amendment Project Area Final Staff Report. February. http://www.waterboards.ca.gov/centralvalley/water_issues/tmdl/central_valley_projects/central_valley_pesticides/risk_evaluation/rre_stff_rpt_feb2009_final.pdf

[8] SWRCB 2011. State Water Resources Control Board. Total Maximum Daily Load (TMDL) Program. http://www.waterboards.ca.gov/water_issues/programs/tmdl/

[9] State Water Resources Control Board (2010) Development of Flow Criteria for the Sacramento-San Joaquin Delta Ecosystem. August 3, 2010 (SWRCB 2010); see also SRCSD's October 2010 Comments and Evidence Letter, pp. 19-20, and attached CD of Other Stressor Panel proceeding, March 24, 2010.

[10] Kratzer, C.R, Kent, R.H., Saleh, D.K, Knifong, D.L., Dileanis,P.D., and Orlando, J.L., 2011, Trends in nutrient concentrations, loads, and yields in streams in the Sacramento, San Joaquin, and Santa Ana Basins, California, 1975-2004: U.S. Geological Survey Scientific Investigations Report 2010-5228, 112p.

that recognizes internal sources of ammonia (such as from sediment/water exchanges, bivalve excretion, decomposition) as well as external inputs.

The following are our findings regarding potential ammonia toxicity and food web impacts.

1. What, if any, information is available on the sources or impacts of total ammonia nitrogen in the Bay Delta Estuary that is not reflected or cited above?

Acute effects concentrations for adult and three-day-old Delta copepods are well above environmentally relevant concentrations of ammonia. Assertions of potential acute toxicity for adult copepods rely on test results obtained using non-representative pH.

In the ANPR water quality findings, a finding from an oral presentation (Teh et al. 2009),[11] that ten percent mortality occurred to both *E. affinis* and *P. forbesi* at ambient concentrations present in the river below the SRWTP, is used to suggest that there is a potential for acute ammonia toxicity for Delta copepods. This interpretation is contrary to the Central Valley Regional Board staff interpretations of these same results. In reviewing these test results, Dr. Chris Foe (Central Valley Water Board) noted that the test pH associated with toxicity in Dr. Teh's experiments (7.2) was not representative of ambient pH levels in the Sacramento River (Foe 2009).[12] In his summary, Dr. Foe states that:

> *"Ten percent mortality occurred to both species at ambient ammonia concentrations present in the river below the SRWTP. <u>However, toxicity was only observed at a lower pH (7.2) than commonly occurs in the River (7.4 to 7.8).</u>**Toxicity was not observed when toxicity testing was done at higher pH levels.**"* (Foe 2009, p. 2; emphasis added)

When environmentally representative pH is considered, test results using adult *E. affinis* and *P. forbesi* do not indicate a potential for acute toxicity in the Sacramento River or the Delta. The LC10s[13] for *E. affinis* and *P. forbesi* at the most environmentally relevant test pH used (pH 7.6) were both about 5 mg N/L total ammonia.[14] This concentration (5 mg N/L) is more than five times higher than the maximum concentrations observed in the Sacramento River during 16 field surveys conducted by the Regional Board from 2009-2010 (Foe et al. 2010).[15] Further, the LC10s are higher than the 99.91 percentile of ammonia concentrations occurring in the Sacramento River 350 feet downstream from the SRWTP diffuser.[16] In other words, for all practical purposes, ambient concentrations of total ammonia in the Sacramento River do not

[11] Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *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.
[12] Foe, C. 2009. *August 2009 Ammonia Summit Summary*. Technical Memo to Jerry Bruns and Sue McConnell, Central Valley Regional Water Quality Control Board, September 24, 2009.
[13] LC10 is the concentration at which it is estimated there is 10% mortality.
[14] LC10s in Teh et al. (2009) were 5.02 and 5.16 mg N/L total ammonia for *E. affinis* and *P. forbesi*, respectively.
[15] Foe, C., A. Ballard, and S. Fong (2010) Nutrient Concentrations and Biological Effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, July 2010.
[16] Larry Walker Associates. 2009 Anti-Degradation Analysis for Proposed Discharge Modification to the Sacramento Regional Wastewater Treatment Plant, DRAFT; prepared for Sacramento Regional County Sanitation District, May 2009.

exceed the lowest acute thresholds (LC10s) thus far reported for adult *E. affinis* or *P. forbesi* for environmentally representative pH conditions.

With respect to the rest of the Delta, there is also no evidence currently supporting a claim of acute toxicity for adult *E. affinis* or *P. forbesi*. None of the ambient total ammonia values measured by the Regional Board at 24 sites throughout the Delta exceeded the environmentally relevant LC10s for the two copepod species (above) during 16 field surveys conducted 2009-2010; most ambient concentrations were more than an order of magnitude lower than the LC10s (Foe et al. 2010)[17]. When expressed as *un-ionized* ammonia, the environmentally relevant LC10s for the two copepod species (0.08 mg N/L un-ionized ammonia for both species at pH 7.6)[18] are well above the 99th percentile (i.e., 0.014 mg N/L un-ionized ammonia) of measured ambient concentrations for the freshwater Delta for 2000-2010 (Figure 1).[19]

With respect to immature life stages of Delta copepods, preliminary evidence regarding acute toxicity at environmentally relevant concentrations of ammonia and environmentally relevant pH is mixed. None of the Regional Board's measurements of total ammonia in the Delta during 2009-2010 (Foe et al. 2010) exceeded a preliminary 96-hour Lowest Observed Effects Concentration (LOEC) for 3-day old nauplii of *P. forbesi* (1.23 mg N/L total ammonia) as reported in a November 10, 2010, letter from Dr. Teh to Dr. Foe,[20] and only one of the ambient un-ionized ammonia measurements in the more extensive dataset illustrated in Figure 1 exceeds this nauplii LOEC when expressed as un-ionized ammonia (0.03 mg N/L un-ionized ammonia at reported test conditions of pH 7.8 and temperature 20°C). A draft final report[21]on 2010 toxicity testing using *P. forbesi* (distributed by the Central Valley Regional Board to the IEP POD Contaminant Work Team for their review on April 14, 2011) includes a preliminary 3-day LOEC for newly hatched nauplii (0.38 mg N/L total ammonia, pH 7.8) that is within range of ambient concentrations of total ammonia in the Sacramento River at Hood.

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[17] Foe et al. (2010), *supra*

[18] Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *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.

[19] Engle, D. (2010) Testimony before State Water Resources Control Board Delta Flow Informational Proceeding. Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web. Testimony submitted to the State Water Resources Control Board, February 16, 2010.

[20] California Regional Water Quality Control Board, Central Valley Region, Order No. R5-2010-0114 NPDES NO. CA0077682, p. J-3.

[21] Teh, S., I. Flores, M. Kawaguchi, S. Lesmeister, and C. The. 2011. Final Report. Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of Pseudodiaptomus forbesi to Ammonia/Ammonium. Draft Report submitted to Chris Foe and Mark Gowdy, State Water Board/UC Davis Agreement No. 06-447-300. Subtask No. 14. March 4, 2011.

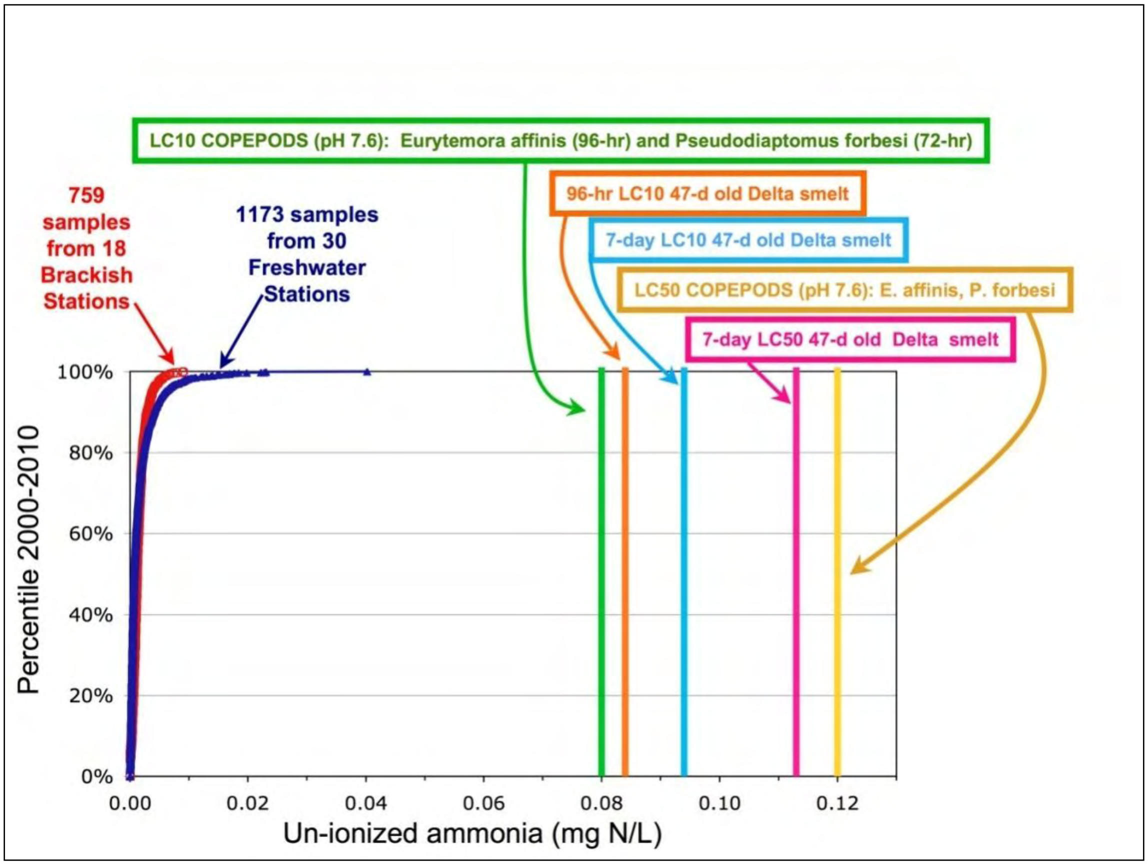

Figure 1. Ranked distribution of ambient concentrations of un-ionized ammonia from estuarine stations (red circles) and freshwater stations (blue triangles) in the upper San Francisco Estuary for 2000-2010. Included are acute effects thresholds for un-ionized ammonia from exposure tests using delta smelt and the adult copepods *Eurytemora affinis and Pseudodiaptomus forbesi*. Preliminary 96-h LC10 for juvenile copepods (3-day-old *P. forbesi* nauplii; 0.030 mg N/L un-ionized ammonia, reported in Nov. 2010. Figure is adapted from Engle (2010).[22]

[22] Engle (2010), *supra, footnote 10*. Figure 3 in Engle (2010) was adapted by adding the LC10 and LC50 for *Pseudodiaptomus forbesi* from Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, CA, August 18-19, 2009.

The state of knowledge regarding algal preferences for ammonium versus nitrate is incorrectly characterized in the ANPR.

The ANPR cites a paper of Dortch (1990)[23] and a case study from the Baltic Sea (Gry et al. 2001)[24] as support for a hypothesis that flagellates and blue-green algae may out-compete diatoms by preferentially using ammonium, compared to other nitrogen sources. However, information in the well cited, detailed review of Dortch (1990) (summarized below) reveals that generalizations about the nitrogen preferences of phytoplankton taxa are inappropriate.

As explained in Dortch (1990), interactions between the uptake and assimilation of ammonium and nitrate by algae are complex, producing a wide range of outcomes that can be demonstrated in growth experiments, including (a) bona fide preference for ammonium (ammonium uptake is faster than nitrate uptake when each is supplied as the sole N source), (b) bona fide preference for nitrate (nitrate uptake is faster than ammonium uptake when each is supplied as the sole N source), (c) ammonium inhibition of nitrate uptake (nitrate uptake is delayed, or slowed, when both compounds are supplied, compared to nitrate uptake when only nitrate is supplied), and (d) nitrate inhibition of ammonium uptake (ammonium uptake is delayed, or slowed, when both compounds are supplied, compared to ammonium uptake when only ammonium is supplied). All of these types of interactions have been documented in the literature – *and individual taxa can exhibit different types of N-uptake behavior in different environmental conditions*.

Although specific ammonium concentrations are sometimes cited as thresholds for inhibition of nitrate uptake by phytoplankton, little is known about how ammonium/nitrate interactions – and thresholds for interactions – differ among taxonomic classes of phytoplankton. There is a large and sophisticated literature concerning interactions between the uptake and assimilation of nitrate and ammonium by marine and freshwater phytoplankton (Dortch, 1990). The literature reviewed by Dortch indicates that *several factors determine which kinds of nitrogen uptake interactions will be observed for a particular phytoplankton taxon under particular environmental or experimental conditions*. The nitrogen status of algal cells (are they N-limited or N-sufficient?), the N exposure history (*preconditioning*) of algal cells (have they been in a high nitrate, high ammonium, or other type of nitrogen environment?), light levels, and water temperature all influence whether ammonium inhibits nitrate uptake at a given place and time in the lab or in nature (Dortch et al., 1991; Lomas & Glibert, 1999)[25]. Such factors play a role in N uptake kinetics because they affect the mechanisms of transport of compounds across cell membranes, ratios of nitrogen compounds inside cells, and intra-cellular or extra-cellular supplies of enzymes, such as nitrate reductase, urease, and amino acid oxidase. In addition, there is growing evidence that many species of marine and freshwater phytoplankton are also able to utilize amino acids, amides, urea, humic substances, and other dissolved organic nitrogen (DON)

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[23] Dortch, Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. Mar. Ecol. Prog. Ser. 61: 183-201.

[24] Gry et al. 2001. Variability in inorganic and organic nitrogen uptake associated with riverine nutrient input in the Gulf of Riga, Baltic Sea. Estuaries 204: 204-14.

[25] Dortch, Q., P. A. Thompson, and P. J. Harrison. 1991. Short-term interaction between nitrate and ammonium uptake in *Thalassiosira pseudonana*: effect of preconditioning nitrogen source and growth rate. Mar. Biol. 110: 183-193.

Lomas, M.W., and P. M. Glibert. 1999. Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Mar. Biol. 133: 541-551.

compounds as sources of nitrogen (Bronk et al., 2007)[26]. DON uptake has been shown to satisfy up to 80% of the total measured N uptake by coastal phytoplankton assemblages.

Enzymatic disruption of nitrate reductase during ammonium assimilation is one of the proposed mechanisms for true ―ammonium inhibition". Dortch (1990) explains that, strictly speaking, ammonium inhibition can be demonstrated only when specific uptake rates for nitrate (V_{NO3}) are measured in the presence *and* absence of ammonium, which is not feasible in field experiments or when ambient water containing both forms of dissolved inorganic nitrogen (DIN) is used to measure V_{NO3} or V_{NH4} in the laboratory setting. Many reports of ammonium inhibition in the literature (<u>including Dugdale et al. 2007 and Wilkerson et al. 2006</u>) result from experiments which are not properly designed to distinguish ammonium *preference* from ammonium *inhibition*. Also, inhibition generally varies inversely with the degree of nitrogen deficiency. In other words, phytoplankton that are not N-limited are less likely to exhibit ammonium inhibition of nitrate uptake. This is potentially an important factor influencing ammonium/nitrate interactions in the Delta, which is not considered a nutrient limited environment.

Other environmental factors which control phytoplankton biomass in the Delta greatly constrain the potential effect of ammonium inhibition on overall productivity.

Historical data indicates that prior to the arrival of the invasive clam *Corbula amurensis*, June-September were the months of highest mean phytoplankton biomass in Suisun Bay and the western Delta (the confluence zone) (Figure 2). Owing to the overwhelming and well-documented impact of benthic grazing by *Corbula* on phytoplankton biomass during the summer and fall in the brackish Delta (Alpine & Cloern 1992, Jassby et al. 2002, Kimmerer 2005, Thompson 2000)[27], a return of historic summer-fall phytoplankton biomass in the brackish Delta is not expected as long as the estuary remains colonized by *Corbula*—regardless of other physical or chemical changes that may occur in the estuary. Currently, the hypothesized potential for increased diatom biomass in the western Delta related to ammonia reduction is primarily constrained to the April-May window when lower benthic grazing rates (clam grazing), increased water temperature, density stratification, appropriate residence times, and other factors occasionally provide windows for bloom development. However, as Figure 2 illustrates, the presumption that a lowering of ammonium levels to levels observed during the 1970s-1980s would substantially restore annual phytoplankton productivity is flawed. Historically, spring blooms contributed only a small portion of annual phytoplankton biomass. Regardless of future changes in ammonium concentrations, grazing by *Corbula* during summer and fall months would still prevent a recovery of annual algal biomass to levels that occurred historically in Suisun Bay in the 1970s and early 1980s.

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[26] Bronk, D. A., J. H. See, P. Bradley, and L. Killberg. 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4: 283-296.

[27] Alpine, A.E., and J.E. Cloern (1992) Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol. Oceanogr. 37:946-955.
Jassby, A.D., J.E. Cloern, B.E. Cole (2002) Annual primary production: patterns and mechanisms of change in a nutrientrich tidal estuary. Limnol Oceanogr 47:698-712.
Kimmerer, W.J. (2005) Long-term changes in apparent uptake of silica in the San Francisco estuary. Limnol Oceanogr 50:793-798.
Thompson, J.K. (2000) Two stories of phytoplankton control by bivalves in San Francisco Bay: the importance of spatial and temporal distribution of bivalves. J Shellfish Res 19:612.

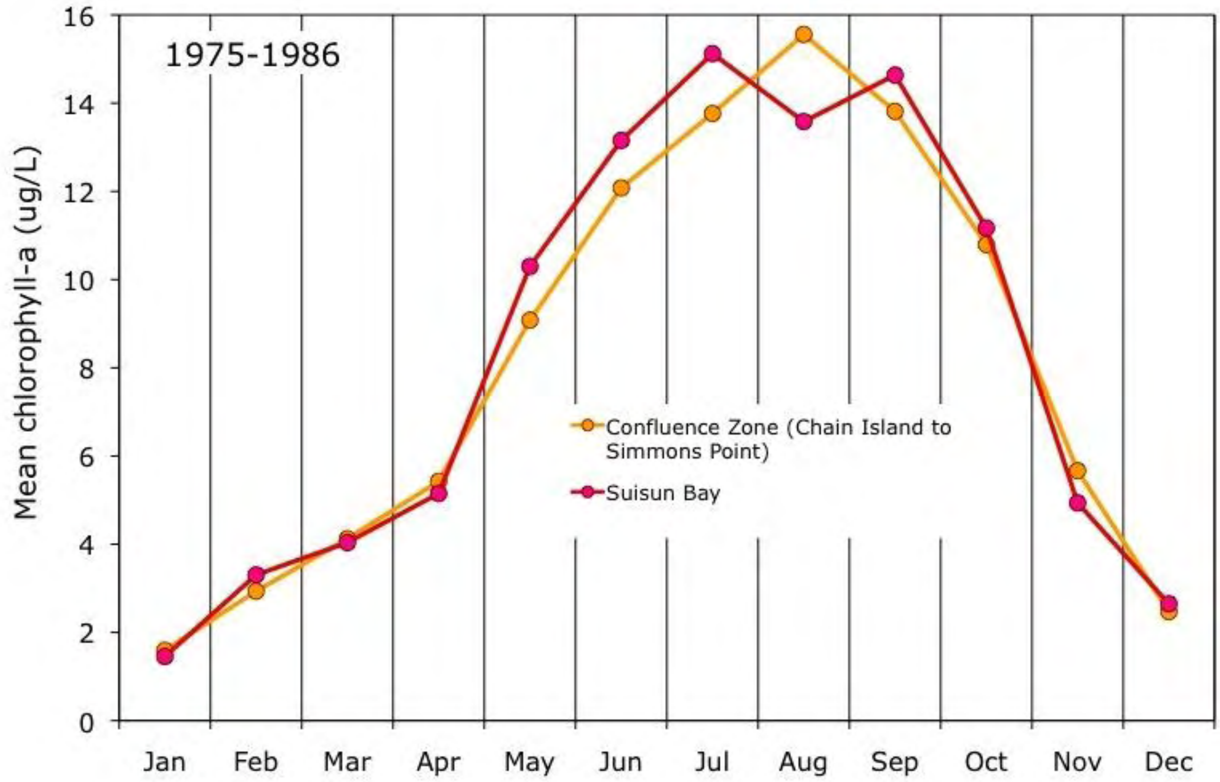

Figure 2. Mean monthly chlorophyll-a concentrations from surface (0.2 m) water samples collected between 1975-1986 at stations used by the IEP, DWR-MWQI, and the USGS. The bulk of annual phytoplankton biomass historically occurred during the same months (June-October) during which *Corbula amurensis* currently controls phytoplankton biomass in the brackish estuary. Figure is from SRCSD (2010).[28]

The water quality findings regarding nutrients cite two publications (Wilkerson et al. 2006[29] and Dugdale et al. 2007)[30] which are commonly cited as evidence that ammonium-induced inhibition of nitrate uptake prevents spring algal blooms from developing in the brackish Delta when conditions are otherwise favorable. However, a critical look at the field data presented in these publications indicates that the ammonium effects observed by these investigators in short, small container experiments do not well predict patterns of phytoplankton biomass in the field. Also, no time series data are presented in either of these publications regarding several environmental parameters (e.g., stratification, benthic grazing rates of clams, herbivorous zooplankton abundance, residence time, Delta outflow) to compare with their records of phytoplankton biomass, although these parameters are critically important to the determination of whether or not conditions are ―favorable" for blooms. In the time series data presented in Wilkerson et al. (2006) and Dugdale et al. (2007), algal blooms were evident in Suisun Bay only twice out of

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[28] Sacramento Regional County Sanitation District Comments on Draft Nutrient Concentration and Biological Effects in the Sacramento-San Joaquin Delta, Central Valley Regional Water Quality Control Board, May 2010. Letter submitted to Chris Foe, Central Valley Regional Water Quality Control Board, June 14, 2010.

[29] Wilkerson, F.P., R.C. Dugdale, V. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. Estuaries and Coasts 29(3):401-416.

[30] 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. Est. Coast. Shelf. Sci. 73:17-29.

five periods when ammonium concentrations fell below 4 µM (Figure 3), and one of the blooms (Spring 2003) failed to yield chlorophyll-a levels above 10 µg/L - a level which is frequently (albeit inappropriately, see below) referenced as a threshold for nutritional adequacy for Delta zooplankton (Müller-Solger et al. 2002).[31]

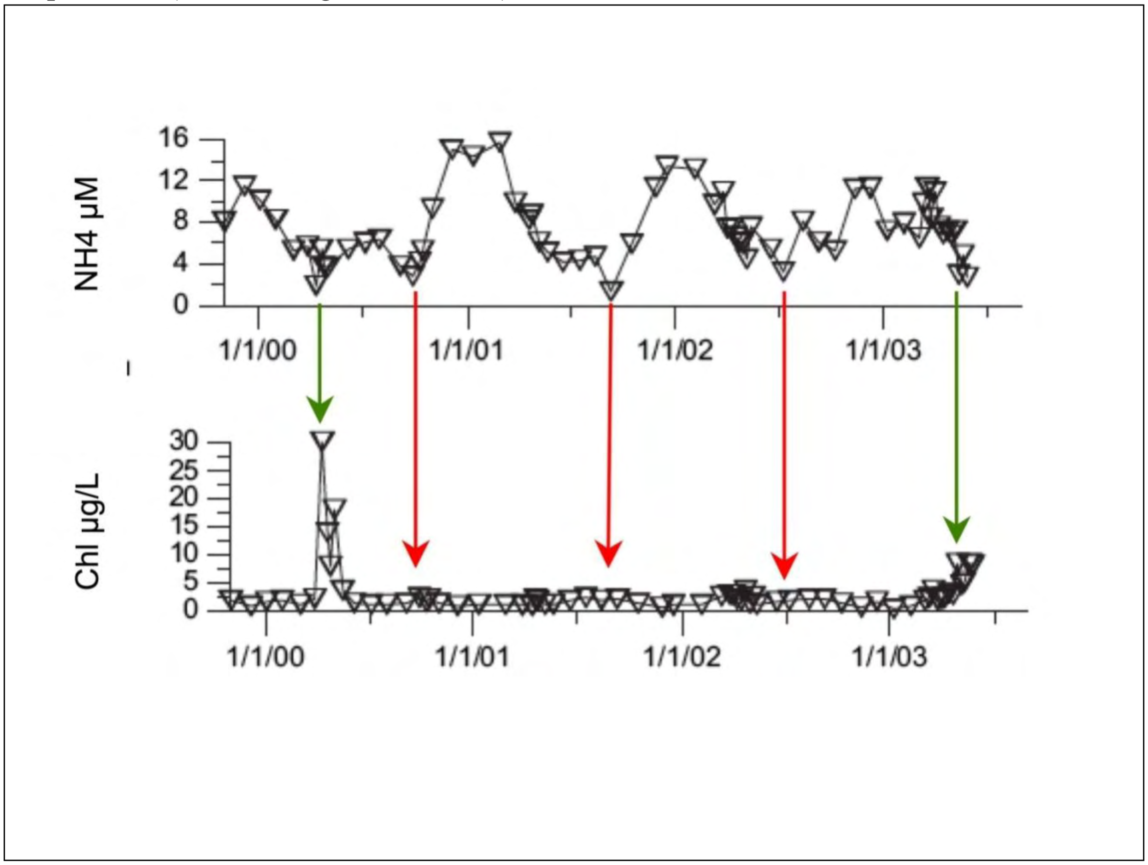

Figure 3. Time series of ammonium and chlorophyll-a from Suisun Bay. Green arrows indicate where ammonium concentrations below a 4 µM threshold were accompanied by increases in chlorophyll-a. Red arrows show periods when similarly low ammonium concentrations were not accompanied by increases in chlorophyll-a. Panels are from Figure 1 in Dugdale et al. (2007); identical time series are presented in Wilkerson et al. (2006).

This lack of consistent correspondence between ammonium concentrations and bloom occurrence amply illustrates that other factors frequently prevent blooms in Suisun Bay, even when ammonium concentrations are below the ―Dugdale" threshold of 4 µM. In fact, considering the documented drawdown of ammonium during the onset of blooms by Wilkerson et al. (2006), time series limited to measurements of ammonium and chlorophyll-a cannot rule out the possibility that low ammonium concentrations *in situ* are the *result* of a bloom triggered by non-nutrient factors, rather than the *cause*.

[31] Müller-Solger, A.B., A. D. Jassby, and D. C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47: 1468-1476.

The same methodological shortcomings apply to the recent field work funded by the San Francisco Regional Board, in which ammonia and chlorophyll-a were measured about twice per month during the spring/summer of 2010 - work which has not been made available in a public report, but which was presented at the Bay-Delta Science Conference September 27-29, 2010.[32] The interpretation of field data for ammonia and chlorophyll-a collected on such a coarse time scale – and the absence of accompanying data for other drivers of phytoplankton biomass - fails to rule out the possibility that other environmental factors initiate blooms in Suisun Bay, and that low ammonium concentrations are a *result* of the blooms (not a requirement for them).

Ammonia concentrations above the postulated inhibition threshold of 4 µM have been shown to stimulate growth of N-Limited Phytoplankton as they enter the Delta in the Sacramento River.

Five-day ―grow-out" experiments were conducted by Parker et al. (2010)[33] using Sacramento River water collected above and below the Sacramento Regional Wastewater Treatment Plant (SRWTP) discharge in November 2008, and March and May 2009. The grow-out experiments were intended to control for the effects of light limitation, but by design also eliminated other environmental factors (e.g., gravitational settling and other *in situ* loss factors) that potentially affect riverine phytoplankton biomass in transport through the Delta. During three out of four of the grow-out experiments, phytoplankton grew *better* in water collected at River Mile 44 (below the SRWTP discharge) than they did in Sacramento River water collected above the discharge, even though the ammonium concentrations at River Mile 44 were well above the postulated ammonium inhibition threshold of 4 µM (see Figure 4).[34]

[32] Marchi A., et al. (unpublished data presented at the Bay-Delta Science Conference, Sacramento, CA, September 27-29, 2010).

[33] Parker, A.E., A.M. Marchi, J. Davidson-Drexel, R.C. Dugdale, and F.P. Wilkerson. 2010. Effect of ammonium and wastewater effluent on riverine phytoplankton in the Sacramento River, CA. Final Report. Technical Report for the California State Water Resources Board, May 29, 2010.

[34] Ammonium concentrations in River Mile-44 water used in the grow-out experiments were: July 2008 - 9.06 µM; November 2008 - 71.87 µM; March 2009 - 12.47 µM; May 2009 - 9.54 µM (Table 19-22 in Parker et al. (2010)).

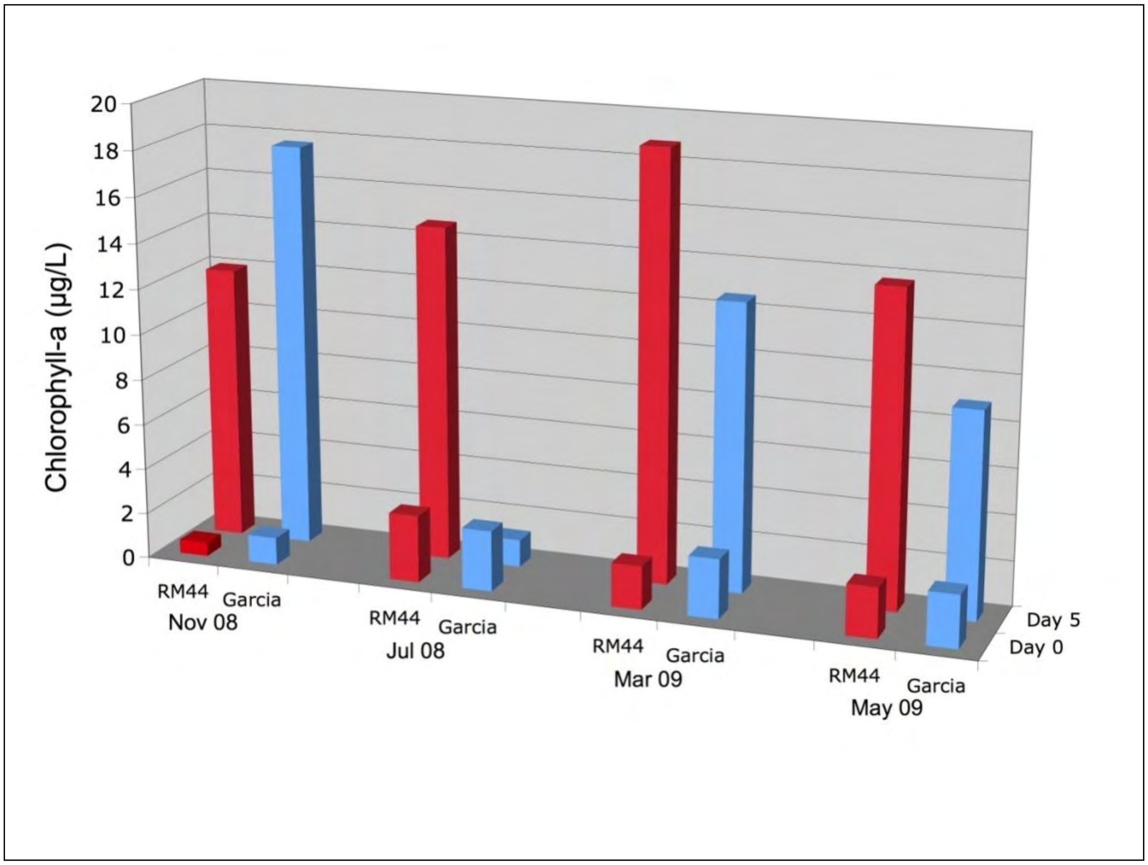

Figure 4. Results of 5-day grow-out experiments using water collected below the SRWTP discharge at River Mile 44 (RM44, red bars) and above the SRWTP discharge (Garcia Bend, blue bars). In three out of four experiments (July 2008, March 2009, May 2009) phytoplankton biomass (chlorophyll-a) was higher after five days in water collected below the SRWTP discharge than in water collected above the discharge. Initial ammonium concentrations in RM-44 water used in the grow-out experiments were: July 2008 - 9.06 µM; November 2008 - 71.87 µM; March 2009 - 12.47 µM; May 2009 - 9.54 µM. Data are from Tables 19-21 in Parker et al. (2010).[35]

These grow-out experiments led Parker et al. to paint a picture of *nitrogen-limited phytoplankton* upstream from the SRWTP, which potentially benefit from the ammonia introduced at the discharge:

> *"Results from experimental grow-outs suggest that after removing light limitation phytoplankton bloom magnitude in the Sacramento River at RM-44 (downstream of SRWTP discharge) and GRC (upstream of SRWTP discharge) is likely determined by dissolved inorganic nitrogen (DIN) availability. Grow-out experiments conducted at RM-44 produced more chlorophyll-a than experimental grow-outs conducted at GRC. Phytoplankton appeared to take advantage of additional DIN, whether supplied as NO3 or NH4 in experiments conducted with water from GRC, or in the form of NH4 supplied in the wastewater effluent (at RM-44) to produce greater biomass*." (Parker et al. 2010, p. 26)

[35] Parker et al. 2010, *supra* note 22

Based on these results, little evidence exists to attribute downstream decreases in chlorophyll-a observed in some field surveys in the Sacramento River to ammonium inhibition, and suggest that it is more appropriate to consider loss factors (e.g., settling) that were nullified by the grow-out tests, but which operate *in situ*.

Longitudinal studies of the Sacramento River contradict claims that ammonium causes a decrease in phytoplankton biomass or primary production rates, or that it changes the cell size or taxonomic composition of phytoplankton in the river

Multiple longitudinal transects measuring nutrients and algal biomass in the Sacramento River from above Sacramento (I-80 bridge) to Suisun Bay were conducted by Regional Board staff (Foe et al. 2010)[36] and Parker et al. (2009, 2010)[37] in 2008-20010. Both studies revealed that although chlorophyll-a often declines in the downstream direction from the I-80 bridge above Sacramento to Rio Vista, no step decline is associated with ammonium inputs related to the Sacramento Regional Wastewater Treatment Plant (SRWTP). For example, in the data shown in Figure 5, more phytoplankton biomass (green line) was lost from river water *above* the SWRTP discharge than below it; and, most of the decline in diatom biomass (blue bars) occurred *upstream* of the SRWTP—a field result which directly contradicts the ammonium-inhibition hypothesis for the Lower Sacramento River portion of the freshwater Delta. Central Valley Regional Board staff have acknowledged that factors unrelated to the SRWTP discharge are needed to explain 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 the 2008-2009 field studies:

> *"The decrease in chlorophyll a appears to commence above the SRWTP. The average annual decline in pigment between Tower Bridge in the City of Sacramento and Isleton is about 60 percent. The cause of the decline is not known, but has been variously attributed to algal settling, toxicity from an unknown chemical in the SRWTP effluent, or from ammonia. The SRWTP discharge cannot be [the] cause of pigment decline upstream of the discharge point, and may not be contributing to the decline downstream of the discharge point."* CVRWQCB (2010)[38]

[36] Foe, C., A. Ballard, and S. Fong. 2010. Nutrient concentrations and biological effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, Final Report, July 2010.
[37] Parker, A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J. Davidson-Drexel, J. Fuller, and S. Blaser. 2009. *Transport and Fate of Ammonium 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.
Parker et al. 2010, *supra* note 22
[38] California Regional Water Quality Control Board, Central Valley Region, Order No. R5-2010-0114/NPDES NO. CA0077682 page, J-7.

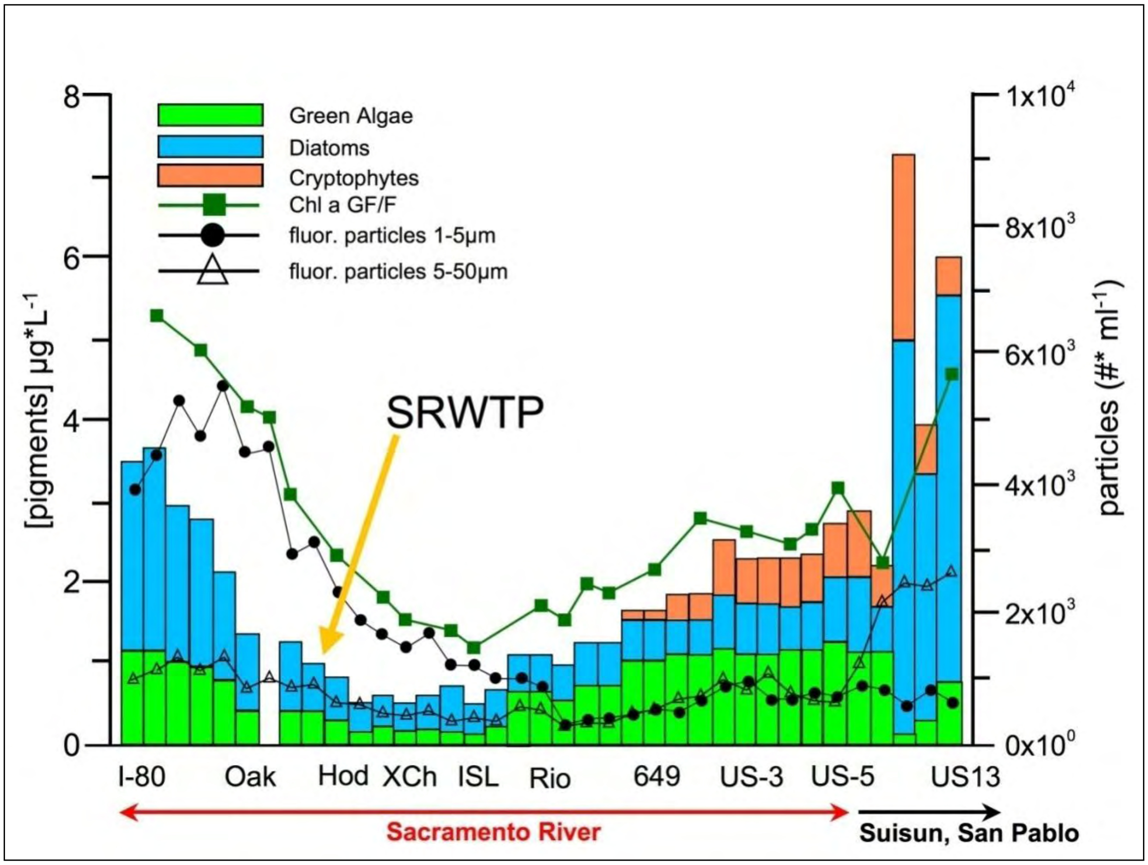

Figure 5. Longitudinal patterns in chlorophyll-a (green squares), biomass of major phytoplankton taxa (colored bars), concentration of small phytoplankton (black circles), and concentration of large phytoplankton (open triangles). Figure is a slight modification from Parker et al. (2009)[39], included in Engle (2010).[40]

Analogous data from Parker et al. (2010)[41] also contradict elements of the ammonium inhibition hypothesis and confirm that the ammonium discharges from the SRWTP cannot explain patterns in phytoplankton biomass, cell size, or taxonomic composition in the Sacramento River. Figure 6 reveals that a downstream decrease in large phytoplankton (assumed by the investigators to be diatoms, shown as light green bars in the figure) is not consistently observed in the river, and when a downstream decrease is observed, it does not begin below the SRWTP discharge. Further, small phytoplankton do not increase in relative abundance below the SRWTP discharge. In fact, the data reveal no consistent longitudinal patterns in the relative abundance of small versus large phytoplankton in the river. In other words, ammonium inputs at the SRWTP discharge do not control the relative abundance of large phytoplankton (presumed to be diatoms) and small phytoplankton. Thus, contrary to the Permit's findings, these field data directly contradict the hypothesis that ammonia will cause small phytoplankton to out-compete

[39] Parker et al. (2009), *supra*, note 26

[40] Engle, D. (2010) Testimony before State Water Resources Control Board Delta Flow Informational Proceeding. Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web. Testimony submitted to the State Water Resources Control Board, February 16, 2010.

[41] Parker et al. (2010), *supra* note 22

large (diatom) phytoplankton.

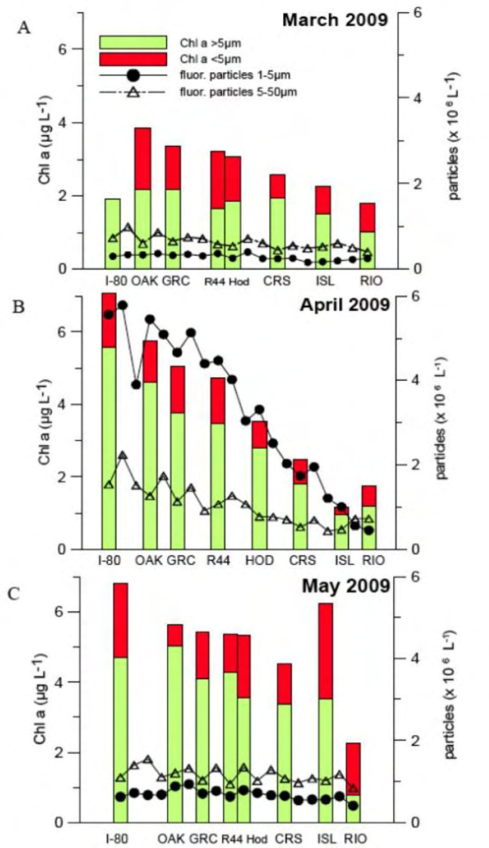

Figure 6. Longitudinal patterns in biomass of large phytoplankton (green bars and open triangles) and small phytoplankton (red bars and closed circles) in the Sacramento River between the I-80 bridge and Rio Vista during Spring 2009; large phytoplankton are presumed by the investigators to include most of the diatoms. Bars indicate biomass as chlorophyll-a; lines indicate cell density measured by fluorescence. Data show that the SRWTP discharge (located between station GRC and R44) does not explain the overall patterns in algal biomass or cell size in the river. Figure is from Parker et al. (2010).[42]

Short-term uptake rate measurements (for carbon, nitrate, and ammonium) made in the same study also contradict elements of the ammonium inhibition hypothesis. Rate measurements in Figure 7 show that primary production rates (brown triangles) do not consistently decline in the downstream direction in the Sacramento River, and when they do, the decline is *not* initiated or intensified after water flows past the SRWTP discharge. The field data also clearly show that ammonium uptake rates (orange symbols) are *not* inversely related to primary production rates (brown triangles) (Parker et al. 2010).[43] These field data directly contradict the hypothesis that ammonium uptake causes a decrease in primary production in the river. These field data clearly

[42] Parker et al. (2010), *supra* note 22
[43] Parker et al. (2010), *supra* note 22

demonstrate that predictions about phytoplankton growth responses and ammonium uptake based on the short-term, small container experiments reported in Wilkerson et al. (2006) and Dugdale et al. (2007) should not be presumed valid outside the laboratory, and cannot be considered evidence of impacts to aquatic life beneficial uses from ammonium in the Delta.

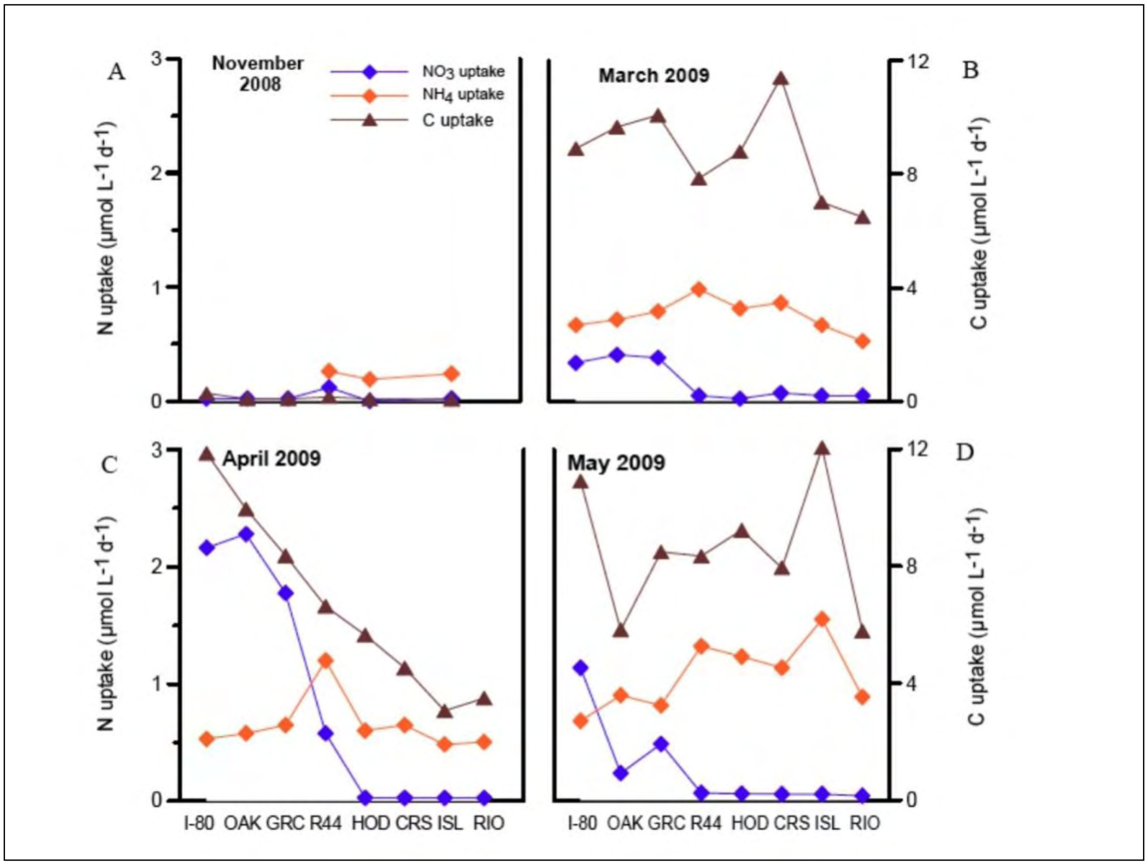

Figure 7. Primary production (C uptake; triangles) and phytoplankton uptake rates of ammonium (orange symbols) and nitrate (blue symbols) made during 24-hr incubations of Sacramento River water collected during four transects between I-80 bridge and Rio Vista. Data do not reveal an inverse relationship between primary production and ammonium uptake. Data further show that longitudinal patterns in primary production are not explained by the SRWTP discharge (located between GRC and R44). Figure is from Parker et al. (2010).[44]

Data from a longer longitudinal transect in the Sacramento River also contradict proposals for an inverse relationship between ammonium uptake and primary production in the Delta. The longitudinal transects by the Parker/Dugdale team during this 2008-2009 Sacramento River project included rate measurements (uptake of carbon, ammonia, and nitrate) at 21 stations starting from I-80 above the city of Sacramento downstream through Suisun Bay and into San Pablo Bay.[45] These rate measurements show a decline in primary production (carbon uptake, indicated by black line in Figure 8) in the upstream reach where nitrate uptake (shown by blue

[44] Parker et al. (2010), *supra* note 22
[45] Parker et al. (2009), *supra*, note 26

bars) exceeded ammonium uptake (shown by red bars). The measurements show that the carbon uptake pattern was independent from the relative contribution of ammonium and nitrate to inorganic nitrogen uptake. Also, in the dataset illustrated in Figure 8, significant *increases* in carbon fixation began in the confluence zone (stations 649 through US3), despite the fact that inorganic nitrogen uptake was dominated by ammonium in that reach. Collectively, these results imply that other factors (probably hydrodynamic factors such as stratification, current speed, residence time) are controlling phytoplankton biomass and primary production in the Sacramento River—not ammonium inhibition.

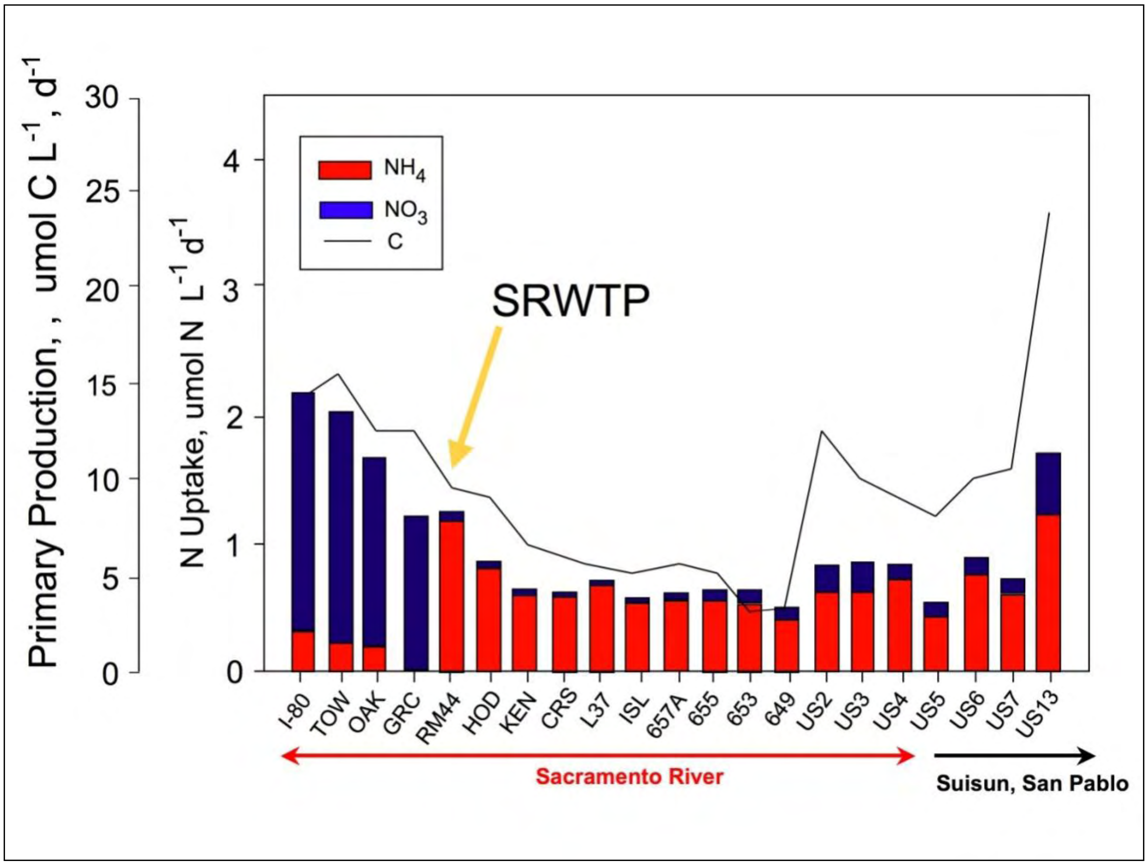

Figure 8. Longitudinal patterns in primary production (black line) and rates of ammonium uptake (red bars) and nitrate uptake (blue bars) in the Sacramento River in March 2009. Data indicate that the location of the SRWTP (and a switch from nitrate to ammonium uptake) does not initiate the decline in primary production in the river, nor does ammonium uptake prevent increases in primary production in the confluence zone (stations 649 through US3).

Evidence From Studies Conducted in the Delta Contradicts the Hypothesis That Ammonia (or Nutrient Ratios Involving Ammonia) Promote Blooms of Microcystis (Blue-Green Algae)

Available research from the Delta argues against a simplistic association between *Microcystis* and nutrient form or concentration. Delta studies conducted by Lehman et al. (2008, 2010)[46] and Mioni (2010)[47] have found no apparent association between ammonium concentrations or NH_4^+:P ratios and either *Microcystis* abundance or toxicity. Instead, it appears from these studies that water temperature is strongly positively correlated with *Microcystis* abundance and toxicity; and, that water transparency, flows, and specific conductivity are also potential drivers of *Microcystis* blooms in the Delta. Specifically, an association between water temperature and *Microcystis* blooms in the Delta is supported by the upward trend in spring-summer mean water temperature in the freshwater Delta between 1996-2005 (Jassby 2008)[48] and would be consistent with observations from other estuaries, where increased residence time (e.g., during drought) and warmer temperatures are acknowledged as factors stimulating cyanobacterial (i.e., *Microcystis*) blooms (Pearl et al. 2009; Pearl & Huisman 2008; Fernald et al. 2007).[49] In addition, there is evidence from other estuaries, and from studies conducted in the Delta (summarized below), that resistance to grazing by molluscs and zooplankton can confer a selective advantage to *Microcystis* and may operate to enhance or prolong *Microcystis* blooms. For example, selective grazing by the non-native Delta copepod *P. forbesi* was recently demonstrated as a viable mechanism for promoting *Microcystis* blooms (Ger et al. 2010).[50]

Information from the Delta and other estuaries indicates that non-nutrient factors are credible alternative explanations for the observed shift in phytoplankton species composition in the Delta.

Physical factors (such as temperature, current speed, residence time, turbulent mixing, stratification, light penetration) may be strongly affecting competitive outcomes between diatoms and other phytoplankton taxa in the Delta; temporal changes in these physical and hydrodynamic factors may be responsible for observed shifts in phytoplankton species

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[46] 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.
[47] Mioni, C.E., and A. Paytan. 2010. *What controls Microcystis bloom & toxicity in the San Francisco Estuary? (Summer/Fall 2008 & 2009)*. Delta Science Program Brownbag Series, Sacramento, CA. May 12, 2010.
[48] Jassby, A. 2008. Phytoplankton in the Upper San Francisco Estuary: recent biomass trends, their causes and their trophic significance. San Francisco Estuary & Watershed Science, Feb. 2008.
[49] Pearl, H.W., K.L. Rossignol, S. Nathan Hall, B.L. Peierls, and M.S. Wetz. 2009. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuaries and Coasts. DOI 10.1007/s12237-009-9137-0.

Pearl, H.W., and J. Huisman. 2008. Blooms like it hot. Science 320:57-58. doi:10.1126/science.1155398.

Fernald, S.H., N.F. Caraco, and J.J. Cole. 2007. Changes in cyanobacterial dominance following the invasion of the zebra mussel *Dreissena polymorpha*: long-term results from the Hudson River Estuary. Estuaries and Coasts 30:163-170.
[50] Ger, K.A., P. Arneson, C.R. Goldman, and S.J.Teh. 2010. Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Short Communication. J. Plankton Research. doi: 10.1093/plankt/fbq071

composition in the Delta (e.g., fewer diatoms, more blue-greens and flagellates). The influence of flows and residence time on phytoplankton assemblages in estuaries is well-acknowledged in other regions. For example, hydrologic perturbations, such as droughts, floods, and storm-related deep mixing events, overwhelm nutrient controls on phytoplankton composition in the Chesapeake Bay; diatoms are favored during years of high discharge and short residence time.[51] The role of flow and residence time in regulating estuarine phytoplankton composition was summarized by the expert panel convened by CalFed in March 2009 in their final ―*Ammonia Framework"* document:

> *"Diatoms have fast growth rates and may be particularly good competitors during high flows with concomitant short residence times, when their fast growth rates can offset high flushing rates. In moderate flows, chlorophytes and cryptophytes become more competitive, whereas low flows with concomitant longer residence times allow the slower-growing cyanobacteria, non-nuisance picoplankton, and dinoflagellates to contribute larger percentages of the community biomass. These spatially and temporally-variable patterns of phytoplankton composition are typical of many estuaries [e.g., Chesapeake Bay, Maryland; Neuse-Pamlico Sound, North Carolina; Narragansett Bay, Rhode Island; Delaware Bay, Delaware]". (Meyer et al. 2009)*[52]

The idea that flows influence diatom abundance is not new in the Delta. Lehman (1996, 2000)[53] associated a multi-decadal decrease in the proportional biomass of diatoms in the Delta and Suisun Bay to climatic influences on river flow. The Central Valley Regional Board recently found that current speed in the Sacramento River was related to the difference in phytoplankton biomass between Freeport and Isleton (Foe et al. 2010).[54]

Top-down effects on phytoplankton composition, caused by selective grazing by clams and zooplankton, are likely to influence the species composition of phytoplankton in the Delta, and may contribute to the occurrence of *Microcystis*. Clam grazing selectively removes larger particles from the water column (Werner & Hollibaugh 1993);[55] clams may consume a larger fraction of diatoms than smaller plankton taxa such as flagellates. Kimmerer (2005)[56] attributed a step decrease in annual silica uptake after 1986 to efficient removal of diatoms by *Corbula*

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[51] Pearl, H.W., L.M. Valdes, B.L. Peierls, J.E. Adolf, and L.W. Harding, Jr. 2006. Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems. Limnol. Oceanogr. 51(1, part 2): 448-462.
[52] Meyer, J.S., P.J. Mulholland, H.W. Paerl, and A.K. Ward. 2009. A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Final report submitted to CalFed Science Program, Sacramento, CA, April 13, 2009.
[53] 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.

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

[54] Foe, C., A. Ballard, and S. Fong. 2010. Nutrient concentrations and biological effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, Final Report, July 2010.
[55] 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.
[56] Kimmerer, W.J. 2005. Long-term changes in apparent uptake of silica in the San Francisco Estuary. Limnol. Oceanogr. 50: 793-798.

amurensis after its introduction in 1986. Grazing by *Corbicula fluminea* can cause shallow habitats in the freshwater Delta to serve as a net sink for phytoplankton (Lopez et al. 2006, Parchaso & Thompson 2008)[57]; it is possible that diatoms are differentially affected by benthic grazing (e.g., compared to motile or buoyant taxa) in both the brackish and freshwater Delta.

Significantly, benthic grazing has been implicated as a factor favoring *Microcystis* over other phytoplankton, as explained in the CalFed expert panel's ―*Ammonia Framework:"*

> *"However, in places where filter-feeding mussels and clams overlap with habitat suitable for Microcystis (i.e., low salinity), the presence of these invertebrates might enhance bloom formation by selectively rejecting large Microcystis colonies. That grazer selectivity can give Microcystis a grazer-resistant, competitive advantage over other phytoplankton, as Vanderploeg et al. (2001) reported for zebra mussels (Dreissena polymorpha) in the Great Lakes."* (Meyer et al. 2009)[58]

In addition to grazing by mussels and clams, grazing by zooplankton can exert a top-down effect on phytoplankton composition; the literature regarding selective feeding by zooplankton is impractical to review herein. However, in a particularly pertinent example, selective grazing by the Delta copepod *P. forbesi* was recently demonstrated as a viable mechanism for promoting *Microcystis* blooms (Ger et al. 2010).[59]

Experimental data from the Delta contradicts the simplistic assumption that the pelagic food web in the Delta is dependent on diatom biomass.

The widespread assumption that a decline in the relative abundance of diatoms and an increase in other taxa including flagellates, green algae, and cyanobacteria represents a significant degradation of food resources for primary consumers in the Delta (estuarine mesozooplankton, and calanoid copepods in particular) has not been critically examined in policy and regulatory arenas, and is flawed.

At least six different lines of evidence challenge the simplistic ―diatom → copepod → pelagic fish" paradigm that is used to justify much of the attention regarding ammonia and the San Francisco Estuary food web:

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[57] Lopez, C.B., J.E. Cloern, T.S. Shraga, A.J. Little, L.V. Lucas, J.K. Thompson, and J. R. Burau. 2006. Ecological values of shallow-water habitats: implications for the restoration of disturbed ecosystems. Ecosystems 9: 422-440.

Parchaso F., and J. Thompson. 2008. *Corbicula fluminea* distribution and biomass response to hydrology and food: A model for CASCaDE scenarios of change. CALFED Science Conference, Sacramento, CA., October, 2008. Avail at http://cascade.wr.usgs.gov/CALFED2008.shtm.

[58] Meyer, J.S., P.J. Mulholland, H.W. Paerl, and A.K. Ward. 2009. A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Final report submitted to CalFed Science Program, Sacramento, CA, April 13, 2009.

[59] Ger, K.A., P. Arneson, C.R. Goldman, and S.J.Teh. 2010. Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi.* Short Communication. J. Plankton Research. doi: 10.1093/plankt/fbq071

1. **Diatoms can be toxic to copepods.** A large body of literature indicates direct feeding on diatoms can cause reproductive failure in copepods (Ianora & Miralto 2010, and references therein).[60] This potential harmful effect of diatoms on copepods, first described in the early 1990s, prompted an ongoing re-evaluation of the classic paradigm that –diatoms-beget-copepods-beget-fish” and has been the subject of considerable research and special workshops and symposia. The harmful effect is caused by organic compounds (oxylipins), which are released from diatom cells when they are broken during feeding. These compounds then induce genetic defects in copepod eggs. The genetic defects are manifested by a failure of the eggs to hatch or a failure of hatched offspring to develop normally. These toxic effects of diatoms are unrecognized in lab or field studies from the Delta that rely on gut contents, clearance rates, or egg counts to determine the nutritional status of copepods, or to infer the nutritional value of suspended matter, because the harmful compounds involved in diatom toxicity do not affect feeding behavior or the numbers of eggs produced, but instead affect the viability of the eggs that are produced after feeding. There are at least twenty-four (24) recently published experiments indicating harmful effects of diatom grazing for copepod species pertinent to the SFE (i.e., SFE species and their co-familials) (Figure 9).

2. **Delta copepods prefer non-diatom prey.** Published experiments from the Delta show that key Delta copepods (including the ones that delta smelt eat) actually prefer *non*-diatom types of phytoplankton and that much of the time they do not consume phytoplankton at all (preferring instead to consume small heterotrophic organisms in the water column)[61]. These feeding experiments indicate that the principal calanoid copepods in the estuary (*Acartia* spp., *E. affinis*, *P. forbesi*) prefer motile prey over non-motile prey, and prefer heterotrophic prey (e.g., cilliates, heterotrophic dinoflagellates) over phytoplankton (Bollens & Penry 2003, Bouley & Kimmerer 2006, Gifford et al. 2007).[62] Diatoms are not motile, as they lack flagella or other means of locomotion. Thus, Delta copepods do not rely on diatoms as a direct food source, and frequently discriminate against phytoplankton altogether (even during diatom blooms), depending on season and location in the estuary.

3. **The reproductive implications of feeding behavior is virtually unstudied for the copepods of the San Francisco Estuary (SFE).** A recent review of almost 400 research articles regarding the feeding ecology of copepod taxa in the families occurring in the Bay-Delta revealed that only three published studies measured egg production or hatching success for a Delta-pertinent copepod species fed mixtures of diatoms and non-

[60] Ianora, A. and A. Miralto (2010) Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology,* 19, 493-511.
[61] Heterotrophic organisms (such as bacteria and many protozoa) obtain energy by consuming pre-existing organic matter, as opposed to synthesizing organic matter through photosynthesis.
[62] Bollens, Gretchn C. Rollwagen, Penry, Deborah L. Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA).
Bouley, P. and W.J. Kimmerer (2006) Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. *Marine Ecology-Progress Series,* 324, 219-228.
Gifford, S.M., G. Rollwagen-Bollens, and S.M. Bollens. (2007) Mesozooplankton omnivory in the upper San Francisco estuary. *Marine Ecology-Progress Series,* 348, 33-46.

diatoms (Engle 2010).[63] In other words, there is essentially no science which addresses whether observed changes in phytoplankton composition in the Bay Delta Estuary could have had population-level consequences for copepods.

4. **Many non-diatom classes of phytoplankton are highly nutritious.** Non-diatom classes of phytoplankton (including some groups which are now more abundant in the estuary) include species that are considered highly nutritious for zooplankton. Examples include cryptophytes (e.g., *Cryptomonas* and *Rhodomonas* spp.) and many species of green algae (e.g., *Scenedesmus* spp.), which are used as food to rear zooplankton in laboratories.

5. **The interpretation of a specific chlorophyll-a level as an indicator of nutritional sufficiency for Delta copepods is unjustified.** Chlorophyll-a levels below 10 µg/L are frequently cited in Delta literature as evidence that zooplankton in the Delta are food limited (e.g., see Muller-Solger et al. 2002).[64] However, this threshold is based on a set of laboratory growth experiments conducted with a single cladoceran zooplankton species (*Daphnia magna*) and it is unclear whether this threshold is appropriately applied to any of the copepods in this system, especially given the importance of non-phytoplankton particles in the diet of Delta copepods. The heavy reliance of SFE copepods on non-algal foods indicates that detritus-based pathways for energy transfer may contribute more to the pelagic food web in the Delta than has been acknowledged. Such information led the IEP to make the following acknowledgement in its 2007 Synthesis of Results:

> *". . . it is possible that the hypothesis that the San Francisco Estuary is driven by phytoplankton production rather than through detrital pathways may have been accepted too strictly.*" (Baxter et al. 2008)[65]

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[63] Engle, D. (2010) Slides and Oral Remarks Presented in: *Engle, D. (2010) How well do we understand the feeding ecology of estuarine mesozooplankton? A survey of the direct evidence.* 6th Biennial Bay-Delta Science Conference, Sacramento, CA, September 27-29, 2010, 31 pp.

[64] Müller-Solger, A.B., A.D. Jassby, and D.C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47:1468-1476.

[65] 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.

Copepod	Diatom	Egg Prod.	Hatching Success	Normal Nauplii	Complete Develop.
Acartia tonsa	*Thalassiosira weissflogii*	–			–
	Thalassiosira pseudo nana	–			–
	Thalassiosira weissflogii	+	+		
	Chaetoceros affinis	–			
	Phaeodacylum tricornutum	–	–		
Acartia hudsonica	*Skeletonema costatum*	+			
Acartia clausi	*Thalassiosira rotula*	+	–		
Centropages typicus	*Thalassiosira rotula*	–	–		
Temora stylifera	*Thalassiosira rotula*	–		–	–
	Skeletonema costatum			–	–
	Phaeodactylum tricornutum			–	–
	Thalassiosira rotula	+	–		
	Thalassiosira weissflogii	+	–		
	Phaeodactylum tricornutum	–	–		
	Skeletonema costatum	–	–		
	Thalassiosira rotula	+	–		
Temora longicornis	*Thalassiosira rotula*				+
	Thalassiosira weissflogii				+
	Leptocylindricus danicus				+
	Skeletonema costatum				+
	Chaetoceros affinis				–
	Chaetoceros decipiens				–
	Chaetoceros socialis				–
	Thalassiosira rotula				–
	Thalassiosira pseudo nana				–
	Thalassiosira rotula	+	–		
	Thalassiosira weissflogii	+	–		
	Chaetoceros affinis	+	–		
	Leptocylindricus danicus	–	–		
	Skeletonema costatum	–	–		

Figure 9. Reproductive consequences of direct feeding on diatoms for Delta copepod taxa. Experiments listed used copepod species from the Delta or their cofamilials. Positive (green) and negative (red) outcomes are indicated for four measures of reproductive success in feeding experiments: egg production (clutch size), hatching success, normal nauplii, and complete development of nauplii. Data are from the review of Ianora & Miralto (2010)[66] and other published literature reviewed in Engle (2010)[67].

None of the publicly available research from the Delta includes direct evidence that nutrient ratios (NH4:NO3, N:P, etc.) influence the taxonomic composition of phytoplankton in the Delta.

None of the experimental work to date in the Delta provides direct evidence that current N:P or NH4:NO3 ratios in the SFE provide a competitive disadvantage to diatoms and a competitive advantage to blue-green algae and flagellates. None of the publicly available research from the Delta has measured *taxon-specific* growth responses when phytoplankton assemblages were presented with different nutrient ratios in growth media. Microscopic identifications and cell counts, or other direct evidence of species composition, have not been reported for experimental manipulations of the NH4:NO3 ratio (such as the grow-out experiments conducted in Wilkerson et al. 2006, Dugdale et al. 2007, Parker et al. 2010). N:P ratios were not experimentally manipulated or compared to growth rates of different phytoplankton species in any Delta research cited in the ANPR.

There is no scientific evidence or consensus that N:P ratios are currently out of alignment in the Delta, or that lowering the N:P ratio would be beneficial for the Delta. There is no

[66] Ianora and Miralto (2010), *supra*, note 49
[67] Engle (2010), *supra*, note 52

evidence that nitrogen and phosphorus are out of ―stoichiometric” balance in the Delta. Deviations in atomic TN:TP ratios in water samples from the classic ―Redfield Ratio” of 16:1 (named for the oceanographer who determined in 1934 that the mean atomic N:P ratio of marine phytoplankton is 16:1 when neither nutrient limits growth) are often used as a rough indicator of relative N- or P- limitation of phytoplankton growth. Modern surveys indicate that TN:TP <18-22 may indicate N limitation in freshwater and ocean settings; phosphorus limitation is generally not expected unless TN:TP ratios exceed 50:1 (Guilford & Hecky 2000).[68] Boynton et al. (2008)[69] show that TN:TP ratios for 34 coastal, estuarine, and lagoon ecosystems trend somewhat above 16:1. Monthly samples for three IEP Suisun Bay monitoring stations for 2002-2007 provides a mean atomic TN:TP ratio of about 17:1 (16.7:1; Engle *unpublished* data[70]). This ratio is very close to the classic ―Redfield Ratio.” Lower ratios would be considered by many investigators as potential indicators of relative nitrogen deficiency in the water column. Significant concern exists regarding the low productivity of the Delta (Baxter et al. 2007),[71] and currently only a small fraction of in-Delta freshwater phytoplankton production escapes loss processes such as burial, in-Delta grazing, direct export in water diversions, to be transported into the brackish Delta (confluence zone and Suisun Bay) where the early life stages of POD fishes rear (Jassby et al. 2002).[72] Because there is experimental evidence from Parker et al. (2010) that Sacramento River phytoplankton entering the Delta upstream from the SRWTP are nitrogen-limited (see above), it is reasonable to predict that reductions in inorganic nitrogen might lower primary productivity in the Sacramento River.

The relationships between cellular indicators of nitrogen or phosphorus deficiency, inorganic nutrient concentrations, phytoplankton taxonomy and stoichiometry, and TN:TP ratios have not been studied in the SFE. In other words, *bona fide* research which would be required to determine whether current N:P ratios encourage or discourage the growth of particular phytoplankton taxa, or are in any way detrimental to the food web, has not been conducted in the Delta or the rest of the San Francisco Estuary. Central Valley Regional Board staff have acknowledged in 2010 that no science supports a ―target” N:P ratio for the Delta:

> ―*At this time there is no science to support what [N:P] ratio would be appropriate for the Sacramento River and the Sacramento-San Joaquin Delta*.”[73]

There is also no scientific consensus that low N:P ratios favor diatoms over other phytoplankton groups. In fact, low N:P ratios (below the Redfield Ratio) are associated with a shift from diatoms to dinoflagellates in several estuaries[74]—a relationship which is opposite from that proposed for the Delta by some investigators.

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[68] Guildford, S.J., and R.E. Hecky. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnology and Oceanography 45:1213-1223.
[69] Boynton, W.R., J.D. Hagy, J.C. Cornwell, W.M. Kemp, S.M. Greene, M.S. Owens, J.E. Baker, and R.K. Larsen. 2008. Nutrient budgets and management actions in the Patuxent River Estuary, Maryland. Estuaries and Coasts. DOI 10.1007/s12237-008-9052-9.
[70] Data are provided in Attachment 3.
[71] 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.
[72] Jassby, A.D., J.E. Cloern, B.E. Cole. 2002 Annual primary production: patterns and mechanisms of change in a nutrient-rich tidal estuary. Limnol Oceanogr 47:698-712.
[73] Staff Response to Comments, Regional Water Quality Control Board, Central Valley Region Board Meeting – 9 December 2010 Response to Written Comments for Sacramento Regional County Sanitation District Sacramento Regional Wastewater Treatment Plant Tentative Waste Discharge Requirements, p. 31.
[74] Hodgkiss, I.J., and K.C. Ho. 1997. Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147.

Potential negative ramifications of lowering N:P should be considered. For example, the competitive advantage of nuisance species of N-fixing cyanobacteria (e.g., *Aphanizomenon* and *Anabaena*) can increase in estuaries when N:P ratios are reduced if overall nutrient supplies are decreased and if seed populations are present (Piehler et al. 2002);[75] both taxa are present in the upper SFE.[76] Low N:P ratios can also induce blooms of the toxic alga *Microcystis* from resting stages in sediment (Stahl-Delbanco et al. 2003);[77] and, N:P ratios below the Redfield Ratio (i.e., <16:1) increase the risk of toxic red-tides in estuaries (Hodgkiss & Ho 1997).[78]

Table 1. Optimal N:P ratios promoting growth of toxic red tide causing organisms.*

Red Tide Causing Organism	Optimal N:P ratio for Growth	Optimal Ratio is below the Redfield Ratio?
Alexandrium catenella	15-30:1	sometimes
Ceratium furca	12-22:1	sometimes
Skeletonema costatum	15-30:1	sometimes
Gonyaulax polygramma	4-8:1	yes
Gymnodinium nagasakiense	11-16:1	yes
Noctiluca scintillans	8-14:1	yes
Prorocentrum dentatum	6-13:1	yes
Prorocentrum minimum	4-13:1	yes
Prorocentrum sigmoides	4-15:1	yes
Prorocentrum triestrium	8-15:1	yes
Scrippsiella trochoidea	6-13:1	yes

*Data are from Hodgkiss & Ho (1997).

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75 Piehler, M.F., J. Dyble, P.H. Moisander, J.L. Pinckney, and H.W. Paerl. 2002. Effects of modified nutrient concentrations and ratios of the structure and function of the native phytoplankton community in the Neuse River Estuary, North Carolina, USA. Aquatic Ecology 36:371-385.

76 Species belonging to the genera *Anabaena* an *Aphanizomenon* are on the list of species from IEP phytoplankton monitoring data in the upper SFE.

77 Stahl-Delbanco, A., L. Hansson, and M. Gyllstrom. 2003. Recruitment of resting stages may induce blooms of Microcystis at low N:P ratios. J. Plank. Res. 25:1099-1106.

78 Hodgkiss and Ho (1997), *supra, note 63*

Glibert (2010) used an improperly applied statistical transformation (CUSUM) to produce artificial and highly misleading correlations between nutrient parameters and biological parameters in the Delta.

The Water Quality Findings on Nutrients in the ANPR cites Glibert (2010)[79] as evidence that total ammonia nitrogen loadings are correlated with the decline of pelagic fish or copepods in the Delta. Unfortunately, Glibert arrived at her conclusions using an improperly applied statistical transformation (cumulative sums of variability, or CUSUM) to produce artificial and highly misleading correlations between nutrient parameters and biological parameters (phytoplankton, zooplankton, fish abundance).

Glibert's approach is analytically and conceptually flawed, as detailed in Engle & Suverkropp (2010)[80]. Further, the type of correlation analysis used in Glibert's article (using CUSUM values instead of measured values for chemical or biological variables) violates the underlying assumptions for linear regression and produces misleading results, which are not supported by underlying data. Other concerns include the limited geographic extent of the data, possible improper sub-sampling of CUSUM time series, nontransparent data reduction, and omissions of key analyses which were needed to support a claim for a link between nutrient ratios and the food web or which would support alternative hypotheses. Examples of these defects are summarized below:

Inadequate Geographic Coverage. Sweeping generalizations are made in Glibert's paper regarding the estuarine food web and the Pelagic Organism Decline (POD) using data from only one station in the Freshwater Delta (Hood, IEP station C3) and two stations in Suisun Bay (IEP stations D8 and D7).

Violation of Statistical Assumptions. Glibert used a calculation termed *CUSUM* to transform long-term datasets for nutrient concentrations and abundances of selected aquatic organisms, and then performed linear regression using the unordered transformed data for selected pairs of variables. Time series of CUSUM values exhibit features and patterns that diverge in several important ways from those of the underlying measured data and make them inappropriate for standard linear regression. CUSUM series mute seasonal or other short-term variations in a time series (which are important for short-lived organisms like phytoplankton and zooplankton), but exaggerate shifts that occur on long time scales (such as decades). In the statistical literature, CUSUM is primarily used to create charts (or ordered values) for single variables that allow the user to detect change points or determine whether deviations from control points are random or signal a trend. However, the characteristics of CUSUM that lend it useful to change-point analysis and quality control make it completely inappropriate to perform standard linear regression using paired CUSUM values removed from their respective temporal sequences.

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[79] Glibert, P.M. (2010) Long-Term Changes in Nutrient Loading and Stoichiometry and Their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, CA. Rev. Fish. Sci. 18:2, 211-232.

[80] Engle, D. and C. Suverkropp. 2010. Memorandum: Comments for Consideration by the State Water Resources Control Board Regarding the Scientific Article *Long-term Changes in Nutrient Loading and Stoichiometry and their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, California* by Patricia Glibert. 17 pp. July 29, 2010.

Accordingly, the simple CUSUM correlations that represent the basis for Glibert's conclusions violate virtually every assumption of a standard correlation analysis. CUSUM series are inherently serially correlated, heteroscedastic and non-normally distributed, and the residuals of CUSUM correlations are non-independent.[81] Further, not all of the datasets used by Glibert are appropriate for customary uses of CUSUM. Autoregressive time series such as flow data are not appropriate for CUSUM change-point analysis. CUSUM change point analysis also assumes that underlying data are homoscedastic and often assumes that data are normally distributed. Glibert did not test raw data for autocorrelation, normality, or equal variance prior to the CUSUM transformation. Another requirement of CUSUM analysis is that time series being compared must start and stop at the same point in time. However, Glibert's correlations appear to be performed by pairing CUSUM series generated by underlying data spanning different time periods.

Artificial Relationships and Inflated R^2 Values. The CUSUM transformation results in a very limited range of serially correlated data structures, which (if linear regression is performed for pairs of CUSUM series) leads to ―correlations" with impressively inflated R^2 values that are largely artificial and cannot be interpreted in the same way as standard parametric correlation or regression analysis. Equally important, statistically significant relationships that *are* present in underlying data can be disguised when CUSUM time series are compared instead of real world measurements.

Nutrient Ratios were Not Compared to Biota at the Bottom of the Food Web. Despite widespread public perception to the contrary, Glibert failed to relate trends in nutrient ratios to those of phytoplankton or copepods in her article. Several obvious pairings of environmental variables were omitted from Glibert's portfolio of CUSUM correlations, including those that were needed for her to claim that nutrient ratios and phytoplankton taxa were statistically related. For example, CUSUM regressions between nutrient *ratios* (TN:TP, NO_3:NH_4, or DIN:DIP) and phytoplankton indices (chlorophyll-a or abundances of individual taxonomic groups) were not included in her analysis. Also, CUSUM trends in nutrient ratios were not directly compared to those for copepod abundance. NO_3:NH_4 trends were not compared to *any* of the biological trends (phytoplankton, copepods, clams, or fish); they were only compared to trends in Delta outflow. As a consequence, Glibert's publication did not make the case (even accepting its flawed statistical approach) that N:P ratios and phytoplankton composition are statistically related to each other, nor that N:P ratios are related to other abundances of other organisms (copepods) near the base of the pelagic food web in the Delta. In addition, the Glibert article reviewed no direct experimental evidence from the SFE or other systems that supports her conclusions regarding nutrient ratios and estuarine phytoplankton composition.

Glibert's selection of environmental parameters was biased, and did not include water exports. Glibert did not utilize data for export volumes as an independent variable in any of her CUSUM correlations. However, Figure 10 shows that when subjected to the same analysis used in Glibert's paper, annual water exports perform as well as ammonia concentrations in explaining trends in the summertime abundance of delta smelt. In addition to water export volumes, many other widely accepted alternative potential drivers of the changes in plankton

[81] See Engle & Suverkropp (2010), *supra*, note 69, for more detail.

composition or biomass and fish abundance in the SFE (and in estuaries, generally)—which would have been testable using her CUSUM methodology—were omitted from Glibert's analysis and from discussion in her article. Due to the peculiarity of the CUSUM transformation, it is likely that a wide variety of non-nutrient environmental factors (essentially any factors which have trended over time in the SFE in concert with changes in fish abundance such as clam abundance, invasive aquative macrophyte abundance, other invasive species abundances, turbidity, water exports, etc.) could be shown to be highly correlated with pelagic fish abundance using CUSUM correlations. Although Glibert's CUSUM correlations between fish abundance and ammonia are convenient for focusing attention on ammonia (as opposed to other potential drivers of the food web or the POD), they ultimately signify little with respect to the relative importance of multiple environmental factors, which have changed over recent decades in the SFE.

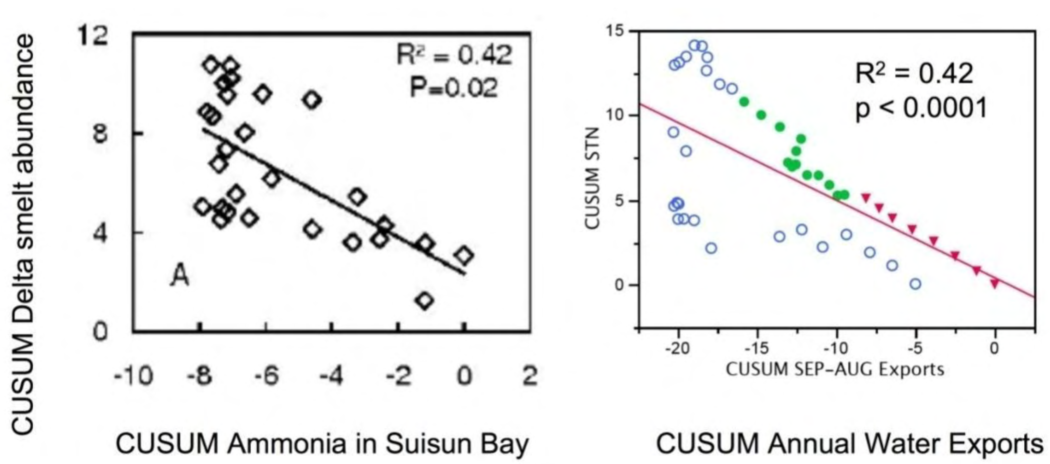

Figure 10. Comparison of correlations using CUSUM ammonia (Suisun Bay) or CUSUM annual Delta water exports (SWP, CVP, and Contra Costa Canal combined) as the independent variables (x-axis) and CUSUM values for the delta smelt Summer Townet Index as the dependent variable (y-axis). Correlation using ammonia is from Glibert (2010) and used data for 1975-2005. Correlation using annual water exports is from Engle & Suverkropp (2010)[82]; color coding for subsets of the CUSUM series is as follows: open blue circles for pre-Corbula years (1956-1986), solid green circles for post-*Corbula* years 1987-1999, red triangles for POD years 2000-2007. Details regarding underlying analyses are in Engle & Suverkropp (2010). The correlation coefficient (R^2 value) is the same for both regressions (0.42); both regression lines are significant. Figure is a combination of Figures 3 and 4 in Engle & Suverkropp (2010).

[82] Engle & Suvercropp (2010), supra, note 69

Pesticides

Pesticides may be discharged to Publically Owned Treatment Works (POTW) in conjunction with both indoor and outdoor pesticide applications. POTWs are not designed to treat pesticides. Pesticides can potentially interfere with treatment plant operation, ability to recycle reclaimed water and biosolids, and compliance with NPDES permit effluent limits. When surface water bodies become impaired due to pesticides, POTWs discharging to the water bodies can be impacted through requirements established as part of TMDLs for those water bodies.

When a pesticide is used indoors, it can be discharged to a sewer, either because the use produces wastewater (e.g., human head lice shampoos and pet flea shampoos), or because an indirect pathway for sewer discharge exists (e.g., the treated surface is eventually cleaned with water or a pesticide-impregnated garment is laundered). Some outdoor uses of pesticides also lead to sewer discharges of the pesticides (e.g., filter backwash from swimming pools containing antimicrobial agents). Since POTWs are not designed to treat pesticides, treatment plant effluent and biosolids may contain the pesticide. Such pesticide releases can cause aquatic toxicity and exceedances of NPDES permit effluent limits.

EPA has authority to regulate pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). EPA‘s previous environmental risk assessments for pesticide registration and reregistration have not adequately evaluated and mitigated potential water quality impacts for pesticide discharges into sewers. Pesticide water quality impacts should be properly evaluated and mitigated through EPA‘s pesticide registration processes instead of regulating dischargers under the Clean Water Act. This is a pathway through which EPA could greatly contribute to helping reduce water quality impacts not only to the Delta but the rest of the United States.

1. What, if any, additional scientific information is available on (a) the effects of pesticides in stormwater discharges, or (b) the potential interactive effects of combinations of pesticides on aquatic resources in the Bay Delta Estuary?

(b) Interactive effects of combinations of pesticides

The most important thing to consider when assessing interactive effects of combinations of pesticides on aquatic resources is the ambient concentrations being combined. Studies cited in the EPA Technical Support Document (EPA, 1991) found that synergism is a rare occurrence in combined effluent toxicity studies. In most cases it was found:

> *"…in the few studies on the growth of fish, the joint effect of toxicants has been consistently less than additive which suggests that as concentrations of toxicants are reduced towards the levels of no effect, their potential for addition is also reduced. There appear to be no marked and consistent differences between the responses of species to mixtures of toxicants."*

Presently, most guidelines and standards for pesticides in surface waters are based on single toxicant studies. More and more studies are being conducted to assess the effects of mixtures of chemicals. However, many studies that have been conducted to date do not test environmentally relevant concentrations. Ambient concentrations are typically well below the ‗no effects' threshold found in experiments.[83]

To account for potential additivity between two pesticides, the Central Valley Basin Plan established that the wasteload allocation for diazinon and chlorpyrifos shall not exceed the sum of 1. This potential additivity is also accounted for in SRCSD's NPDES permit:

Sum = —— ——

Where:

C_D = diazinon effluent concentration
C_C = chlorpyrifos effluent concentration
WQO_D = diazinon water quality objective
WQO_C = chlopyrifos water quality objective

Understanding the environmental relevance of toxicity data from various sources is important for properly interpreting the significance of any results. Pyrethroids have been shown to be present in urban storm runoff and in some effluent samples from treated wastewater discharged to the Sacramento-San Joaquin Delta [84]. Runoff from urban storm drains can be toxic to sensitive species (i.e., *Hyalella azteca*) at ambient concentrations during rain events. Conversely, pyrethroid concentrations in treated wastewater from SRWTP have only been found to cause toxicity in effluent concentrations that were not environmentally relevant, i.e. that do not occur in the Sacramento River below the SRWTP discharge. SRWTP effluent is diluted, on average, 50:1 in the Sacramento River. Daily dilutions have always been greater than 20:1 in the past decade. Given this dilution effect, and the measured concentrations in SRWTP effluent, concentrations of pyrethroids in the Sacramento River can be calculated (Table 2). These estimated river concentrations were below the effect concentrations reported by Weston and Lydy (2010) and almost all were below the very conservative draft and final water quality criteria (WQC) developed for the Central Valley Regional Water Quality Control Board[85].

Accounting for actual dilution in the Sacramento River reduced pyrethroids from SRWTP effluent to concentrations well below these acute and chronic WQC in all cases, except for cypermethrin exceeding a draft chronic criterion in one of the six samples. The basis for this draft chronic WQC for cypermethrin is suspect due to uncertainties in its derivation. Chronic WQC derived for the Regional Water Board were typically calculated by dividing the median 5th percentile of the acute toxicity data by an acute-to-chronic ratio (ACR) developed from paired

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[83] Werner. I. et al., ―Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent- Associated Contaminants on Delta Smelt (2009)", Central Valley Regional Water Quality Control Board and the Sacramento Regional Wastewater Treatment Plant. Final Report.

[84] Weston, D.P. and M.J. Lydy. 2010. Urban and Agricultural Sources of Pyrethroid Insecticides to the Sacramento-San Joaquin Delta of California. Environ. Sci. Technol. 44 (5), pp. 1833-1840.

[85] Central Valley Regional Water Quality Control Board, Final Bifenthrin, Cyfluthirn, and Lambda-Cyhalothrin Criteria Report. March 2010. http://www.waterboards.ca.gov/centralvalley/water_issues/tmdl/central_valley_projects/central_valley_pesticides/criteria_method/index.shtml

acute and chronic toxicity values in the dataset; or using a default value for datasets lacking appropriate data. In the case of cypermethrin and cyfluthrin, the median 1st percentile (50% confidence limit) of the acute toxicity data was divided by the ACR. ACR values for the five pyrethroids with WQC ranged from 4.73 to 12.4 (the default value) with the exception of cypermethrin (ACR= 949). The cypermethrin ACR of 949 is based on one study that reported chronic effects to *Daphnia magna* at significantly lower concentrations than the other studies in the dataset. Cypermethrin ACRs for two other species, one copepod and one fish, were 2.1 and 2.3. There is also uncertainty in the reported concentrations from this study that were based on nominal concentrations rather than measured values, the lack of reporting control data, and the failure to report the statistical methods upon which significant differences were based. Given the highly conservative and uncertain nature of the draft cypermethrin chronic WQC, the relevance of this one exceedance is highly uncertain.

As demonstrated in the above analysis, there is very little potential for toxicity in the Sacramento River due to pyrethroids discharged in SRWTP effluent. Despite the potential for pyrethroid toxicity at extremely low levels, pyrethroid concentrations detected in SRCSD effluent are insufficient to cause toxicity to the most sensitive species in the Sacramento River. Accounting for dilution of the effluent at the time of sampling indicates that concentrations would be well below known effect levels. These low ambient concentration estimates are supported by the absence of observed pyrethroid toxicity in ambient samples from the Sacramento River in recent studies (Weston and Lydy 2010) and by the lack of acute or chronic toxicity to *H. azteca* in 51 samples from the Hood sampling station – located downstream of SRWTP on the Sacramento River[86].

Furthermore, due to the conservativeness of the methods used to derive the Regional Water Board WQC for pyrethroids, including the lack of bioavailability considerations, it is recommended that they not be used or considered as criteria. Exceedances of these values would indicate that additional information or study may be needed to determine if measured pyrethroid levels are of a concern.

Table 2. Estimated Pyrethroid Concentrations in the Sacramento River downstream from the SRWTP discharge based on measured SRWTP Effluent Concentration

Sample Date	1/27/2008	5/27/2008	7/15/2008	9/19/2008	11/2/2008	2/17/2009	Published EC50 (Weston and Lydy, 2010)	Proposed WQC*		Status
Conditions	WET	DRY	DRY	DRY	WET	WET		ACUTE[1]	CHRONIC[2]	

[86] Werner I., D. Markiewicz, L.A. Deanovic, R.E. Connon, S. Beggel, S.J. Teh, M. Stillway, and C. Reece. 2010b. Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010. Final Report.

Sample Date	1/27/2008	5/27/2008	7/15/2008	9/19/2008	11/2/2008	2/17/2009	Published EC50	Proposed WQC*		Status
Dilution Ratio (# : 1)	94.0	47.7	59.6	39.2	33.6	95.4				
bifenthrin	0	(0.057)	0	0	0	0	3.3	4	0.6	Final
lamda-cyhalothrin	0.06	0	0.06	0	0	0	na	1	0.5	Final
esfenvalerate	0	0	0	0.094	0	0	na	na	na	-
delatamethrin	0	0	0	0	0	0	na	na	na	-
permethrin	0.07	0	0.20	0.44	0	0.10	na	10	2	Draft
cyfluthrin	(0.018)	0	0	0	0	0	na	0.3	0.05	Final
cypermethrin	0	0	0	0	0	0.18	1.9	1[a]	0.003[b]	Draft
fenpropathrin	0	0	0	0	0	0	1.7	na	na	-
Summed Pyrethroids	*0.15*	*(0.057)*	*0.26*	*0.53*	*0*	*0.28*	- -	-	-	-

Notes:
Concentrations in ng/L
Values in parentheses were based on qualified results.
Values in green highlight were below toxicity values and proposed chronic WQC.
Values in yellow were below toxicity values, but above a draft chronic WQC.
* Proposed water quality criteria developed by UC Davis for the CVRWQCB.
[1]Acute criteria derived by dividing the median 5th percentile (50% confidence limit) of the acute data by two, unless otherwise indicated.
[2]Chronic criteria derived by dividing the median 5th percentile (50% confidence limit) of the acute data by the ACR, unless otherwise indicated.
[a] Acute criteria derived by dividing the median 1st percentile (50% confidence limit) of the acute data by two to be protective of sensitive species.
[b] Chronic criteria derived by dividing the median 1st percentile (50% confidence limit) of the acute data by the ACR to be protective of sensitive species.
ACR = acute-to-chronic ratio (derived using paired acute and chronic toxicity values)
EC50 = concentration causing an effect (i.e., paralysis) in 50 percent of the test organisms.
na = not available
WQC = water quality criteria

2. What, if any, actions should EPA take under its authority to improve the effectiveness of regulating pesticide contamination of the Bay Delta Estuary watershed?

Current rules do not allow EPA to obtain all the data needed to ensure that pesticides are registered in a manner protective of water quality. The actions EPA should take to improve the effectiveness of pesticide regulation are:

- Require internal coordination efforts between FIFRA and Office of Water,
- Use its authorities under FIFRA to regulate pesticide sales and use,
- Properly implement EPA's registration processes,
- Focus on the more sensitive species and exposure endpoints as part of the pesticide registration and registration review processes,
- Impose more stringent conditions on issuing waivers for aquatic toxicity data for pesticide registration and registration review.
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FIFRA provides for federal regulation of pesticide distribution, sale, and use. All pesticides distributed or sold in the United States must be registered (licensed) by EPA. Before EPA may register a pesticide under FIFRA, an applicant must show that using the pesticide according to specifications ―will not generally cause unreasonable adverse effects on the environment.‘‘ FIFRA defines the term ‗unreasonable adverse effects on the environment‘‘ to mean: ‗(1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or (2) a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under section 408 of the Federal Food, Drug, and Cosmetic Act.‘‘

Additionally, the Endangered Species Act (ESA) of 1973 prohibits any action that can adversely affect an endangered or threatened species or its habitat. In compliance with this law, EPA is required to ensure that use of the pesticides it registers will not harm these species or habitat critical to endangered species survival.

To the extent necessary to prevent unreasonable adverse effects on the environment, the EPA may, by regulation, limit the distribution, sale, or use in any State of any pesticide that is not registered under this Act and that is not the subject of an experimental use permit under section 5 or an emergency exemption under section 18.

From a POTW perspective, the Clean Water Act does not give regulatory authority to local agencies to control the source of a pesticide. EPA is using a ―harmonization" process to evaluate the registration of bifenthrin, which involves the Offices of Pesticide Programs, Water, and Wastewater Management to work together during the review process to consider water issues related to this pesticide. EPA should continue to take approaches that require internal coordination efforts that bring stakeholders into the process at the appropriate time.

POTWs need assistance from EPA and the Food and Drug Administration (FDA‘s) to protect surface water and biosolids from pesticides. Under California law (similar to laws in most other states), POTWs cannot regulate the sale or use of pesticides. POTWs have limited practical ability to keep residents and small businesses from discharging ordinary consumer products, like pyrethroids, to their indoor drains. For these reasons, attempts to address pesticide discharges through Clean Water Act-based regulation of POTW effluent and biosolids will not lead to water quality improvement but will unfairly burden local wastewater agencies. The only practical and cost effective means of controlling pesticide discharge is for the federal government to use its authorities under FIFRA and Federal Food, Drug, and Cosmetic Act (FFDCA) to regulate pesticide sales and use.

FIFRA provides for federal regulation of pesticide distribution, sale, and use. EPA or FDA must register all pesticides distributed or sold in the United States. Under FIFRA, EPA has a statutory responsibility to ensure that pesticides are safe and effective for their intended uses and to prevent unreasonable adverse effects to man, other animals, and the environment from their usage (7 U.S.C. §136(bb), §136a(a), §136a(d)(2); §136d(b)). The risk benefit standard in FIFRA requires EPA ensure that pesticides are used in such a manner that mitigation under the Clean Water Act is minimal or unnecessary. Properly implemented, EPA‘s registration processes can ensure that water quality standards are met and the Bay Delta Estuary aquatic habitat is protected.

Although pediculicide uses of pesticides, such as pyrethroid head lice treatments, are not currently subject to regulation under FIFRA, they were subject to such regulation until November 5, 1979, when EPA acted to exempt pediculicides from the requirements of the FIFRA (44 Federal Register, 63749). Since pediculicides are considered to be drugs, they are also subject to the FFDCA. The regulation of these products under both the FIFRA and the FFDCA was considered duplicative. In announcing the exemption, ―EPA and FDA concluded that the dual review of pesticide/new drug products offered solely for human use represents an expensive duplication of time and resources for both the Agencies and the sponsors of these products without any significant increase in benefits to public health and/or the environment. It is further concluded that regulations of these products solely by FDA under the FFDCA would adequately serve the intent of FIFRA."

Regulation under the FIFRA and the FFDCA is no longer duplicative. Since 1979, the degree of regulation under FIFRA has changed considerably, most notably with passage of the Food Quality and Protection Act of 1996 (FQPA). This statute requires EPA to review all pesticide registrations on at least a fifteen-year cycle (7 U.S.C. §136a(g)(1)(A)). The goal of this requirement is to ensure that all pesticides continue to meet up-to-date standards for safety, public health, and environmental protection. EPA has the authority to require data and take action if needed between registration cycles (7 U.S.C. §136a(c)(2)(B); §136a-1(d)(3)). No similar provisions exist under the FFDCA. Additionally, EPA has emergency suspension authority, which means a pesticide registration can be canceled immediately if there is an emergency, imminent threat to public health or the environment (7 U.S.C. §136d(c)). This appears to be a much more direct and powerful tool to regulate pesticides when compared to the FDA‘s authority to simply require an Environmental Assessment in such circumstances. It is SRCSDs position that EPA should reassert its control over pediculicides under FIFRA.

EPA should also update and revise data requirements for the registration and registration review of pesticides under FIFRA. The data requirements are intended to ensure that EPA has all the information necessary to evaluate the environmental and human health risks of pesticides. Current rules do not allow EPA to obtain all the data needed to ensure that pesticides are registered in a manner protective of water quality.

Although aquatic life toxicity testing is required in the pesticide registration and registration review processes, data using the more pesticide-sensitive species and endpoints are generally lacking. For example, a review of registrant generated invertebrate sensitivity data will reveal the majority of their testing is being conducted using the less sensitive *Daphnia* genera as opposed to the more sensitive *Ceriodaphnia dubia*. It is impractical, if not impossible, to conduct laboratory toxicity testing on every relevant species. Therefore, the limited testing conducted as part of the pesticide registration and registration review processes should focus on the more sensitive species and exposure endpoints in order to be a useful surrogate representative of the diverse ecosystem.

Requiring, at a minimum, chronic toxicity species sensitive screening consisting of the fathead minnow (*Pimephales promelas*) seven-day survival and growth test, *Ceriodaphnia dubia* seven-day survival and reproduction test, and four-day green algae cell density test is not overly burdensome or financially costly. In fact, the majority of the NPDES dischargers are required to conduct similar screenings annually. Chronic toxicity testing with these three species is conducted nationally and internationally. These methods have been fully evaluated and promulgated in 40 CFR Part 136 and are a required monitoring component of nearly all U.S. dischargers. The cost associated with such a screen ranges from $3,000 to $4,500. In addition to this minimum testing, toxicity testing with other species should also be considered on a case-by-case basis. For example, it has been well established in the literature that the amphipod *Hyalella* is particularly sensitive to pyrethroids.

EPA should also consider imposing more stringent conditions on issuing waivers for aquatic toxicity data for pesticide registration and registration review. For example, EPA should withhold registration decisions until required data is submitted and evaluated. By registering pesticides without required aquatic toxicity data, EPA cannot ensure that the pesticide does not pose an unreasonable adverse risk to the environment.

Failure to require such minimal aquatic toxicity testing has shifted the burden and financial responsibility of detecting environmentally harmful pesticide concentrations to NPDES permit holders and other dischargers. Through the ―no toxics in toxic amounts" provision of the Clean Water Act, dischargers must demonstrate that effluents and receiving waters are not exhibiting toxicity using the previously mentioned species and endpoints. Having access to reliable acute and chronic toxicity results using these same methods, species, and procedures provided at the time of pesticide registration or registration review will allow dischargers to more effectively ―rule in" or ―rule out" currently used pesticides when chronic toxicity triggers and/or limits are exceeded.

EPA should also evaluate potential impacts from synergists and multiple active ingredient pesticide formulations during pesticide registration and registration review. Currently these impacts are not evaluated.

When potential water quality impacts are identified during registration or registration review for a pesticide, EPA should implement adequate risk management strategies. EPA should also require risk management strategies for all potential exceedances of water quality criteria (or equivalent values calculated for the purpose of the risk assessment) and all expected incidents of non-compliance by NPDES permit holders.

If risk management strategies include phase-out of the use of a pesticide, EPA needs to develop procedures to ensure the phase-out itself does not lead to adverse water quality impacts. These impacts can be caused by replacement of the phased-out pesticide with another pesticide causing water quality problems. Improper disposal of phased-out pesticides is also a serious concern. For example, during the phase-out of most urban uses of diazinon, a POTW experienced a toxicity incident in ambient water that appeared to be caused by illegal disposal of a diazinon-based pesticide.

EPA has already taken important steps towards protecting water quality through its various registration processes; however, EPA can further integrate urban water quality protection more effectively into its pesticide review programs. Coordination between EPA's Offices of Pesticide

Programs, Water, and Wastewater Management in reviewing pesticide data needs is essential to Clean Water Act implementation; it also provides an appropriate method of meeting FIFRA's goal of preventing unreasonable adverse impacts from pesticide use.

3. How can the process for establishing numeric water quality criteria be streamlined while maintaining technical integrity?

Over the years, various pesticides have been implicated and identified as the source of water quality impairments. With protective aquatic life water quality criteria established for only a few of these compounds, the majority of these pesticide impairments were identified through regulatory-mandated acute and chronic toxicity testing programs. The costs to POTWs associated with these impairments have exceeded millions of dollars. As detailed in the response to question 2 above, the water quality impacts of pesticides should be properly evaluated and mitigated during EPA's registration processes thus preventing water quality impacts and making mitigation under the Clean Water Act minimal or unnecessary.

EPA should be very cautious about streamlining how to establish numeric criteria as the streamlining itself could threaten the technical integrity of the criteria development process, especially where little data exists. Any process that relies on large safety factors to account for a paucity of supporting data should be avoided.

4. What are the benefits and constraints of using fish tissue in place of, or in addition to, water column concentrations when establishing water quality criteria for pesticides?

Using fish tissue to establish water quality criteria can be beneficial when assessing human health or wildlife effects, as bioaccumulation and bioconcentration can be more directly taken into account. Concentrations of pesticides in the fish that people are eating is a concern that relates directly to human health risk and should be addressed. However, for assessing the health of the Bay Delta Estuary, water column concentrations are important for assessing potential toxicity to fish and other aquatic organisms through water column exposure and for source tracking. There is a wealth of information available describing effects to various aquatic organisms based on surface water concentrations, while only limited data are available describing effect concentrations in tissues, and such concentrations are organism and tissue specific. Therefore, it is most useful to have water quality (and sediment) data for assessing the potential for adverse effect to biota. To gain a broad picture of the effects of pesticides on ecosystem health, all pathways of exposure (water column, sediment and biota) should be assessed through the registration and registration review under FIFRA.

5. Are there testing protocols that would effectively and efficiently identify synergistic toxic effects in the Bay Delta Estuary?

Performing toxicity testing with ambient waters directly tests for synergistic toxic effects in the Bay Delta Estuary for the selected test organisms. Ambient waters contain mixtures of chemicals at environmentally relevant concentrations. Therefore, the results of toxicity tests provide at least a snapshot of synergistic or additive effects in the samples taken.

The uncertainties related to the lack of realistic environmental exposure in laboratory-based toxicity testing could be addressed by conducting *in situ* toxicity testing in the Delta. This approach balances the controls of standard laboratory testing with environmentally realistic field exposures where the organisms are exposed to natural diurnal changes in temperature, light, and flow through water quality variations in the various site media (i.e., surface water, sediment-water interface, surficial sediment, or pore water)[87]. These *in situ* exposure approaches provide unique assessment information that is complementary to traditional laboratory-based toxicity testing and reduce the uncertainty of extrapolating from the laboratory to field. Native test organisms and standard method test organisms have been used successfully with *in situ* exposure methods to assess the potential for adverse effects to species of interest.

The relative toxicity from multiple stressors in ambient surface water or sediment samples can, in some cases, be determined using toxicity identification evaluation methods (EPA 1992, 1993a, 1993b, 2007). Toxicity identified during standard toxicity tests can be fractionated and then reconstructed for various toxicants. Novel methods need to be employed for some contaminant classes such as pyrethroids[88] (Wheelock et al. 2004; Amweg and Weston 2007; Weston and Amweg 2007) in addition to the general tools provided in the EPA Guidance (EPA 1992, 1993a, 1993b)[89].

6. What, if any, specific combinations of contaminants are of particular concern in the Bay Delta Estuary?

Researchers prepared a primary list of potentially important contaminants based on the relative risk evaluation for pesticides used in the Central Valley Pesticides Basin Plan Amendment

[87] Burton, G. A., M.S. Greenberg, C.D. Rowland, C. A. Irvine, D.R. Lavoie, J.A. Brooker, L. Moore, D.F.N. Raymer, and R. Williams. 2005. *In situ* exposures using caged organisms: a multi-compartment approach to detect toxicity and bioaccumulation. Environmental Pollution. 134:133-144.

[88] Wheelock, C.E., J. L. Miller, M.J. Miller, S.J. Gee, G. Shan, and B.D. Hammock.. 2004. Development of toxicity identification evaluation procedures for pyrethroid detection using esterase activity.Environmental Toxicology and Chemistry, Vol. 23, No. 11, pp. 2699–2708,
Amweg E.L. and D.P. Weston. 2007. Whole-sediment toxicity identification evaluation tools for pyrethroid insecticides: I. piperonyl butoxide addition. Environmental Toxicology and Chemistry, Vol. 26, No. 11, pp. 2389–2396.
Weston, D.P. and E.L. Amweg. 2007. Whole-sediment toxicity identification evaluation tools for pyrethroid insecticides: II. Esterase addition. Environmental Toxicology and Chemistry, Vol. 26, No. 11, pp. 2397–2404.

[89] United States Environmental Protection Agency (EPA). 1992. Toxicity Identification
Evaluation: Characterization of Chronically Toxic Effluents, Phase I, EPA 600/6-91/005F, May.
United States Environmental Protection Agency (EPA). 1993a. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity, Second Edition, EPA 600/R-92/080, September.
United States Environmental Protection Agency (EPA). 1993b. Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity, Second Edition, EPA 600/R-92/081, September.
United States Environmental Protection Agency (EPA). 2007. Sediment Toxicity Identification Evaluation (TIE) Phases I, II, and III Guidance Document EPA/600/R-07/080 Office of Research and Development. Washington, DC

project area[90]. This evaluation examined 28 high risk and 10 moderate risk pesticides used in the Sacramento River and San Joaquin River watersheds. Only 10 chemicals were considered to have sufficient data in the database to allow further analysis, as listed below:

- Chlorpyrifos
- Diazinon
- Diuron
- Bifenthrin
- Esfenvalerate/fenvalerate
- Lambda-cyhalothrin
- Permethrin
- s-metolachlor
- Propanil
- Copper

The presence and magnitude of chemicals on this list should be considered for additional testing in ambient waters. As mentioned above, the results of toxicity tests performed on ambient waters already provide an integrated account of any synergistic or additive effects.

7. Should EPA and our state partners move away from evaluating isolated aquatic species for one or two pollutants, and towards evaluations of water conditions more representative of the actual aquatic conditions in the Bay Delta Estuary? How might this be done?

Performing three-species chronic toxicity testing on upstream water, downstream water, and effluent accounts for any synergistic or additive toxicity resulting from combined contributions of contaminants. Such testing accounts for multiple contaminants, at environmentally relevant concentrations. The results of such testing near the SRCSD effluent discharge has helped to address concerns regarding the potential effects of Sacramento River water being discharged into the Bay Delta Estuary.

As commented previously, the uncertainties related to the lack of realistic environmental exposure in laboratory-based toxicity testing could be addressed by conducting *in situ* toxicity testing in the Delta. This approach balances the controls of standard laboratory testing with environmentally realistic field exposures where the organisms are exposed to natural diurnal changes in temperature, light, and flow through water quality variations in the various site media (i.e., surface water, sediment-water interface, surficial sediment, or pore water)[91].

Contaminants of Emerging Concern

The ANPR includes a discussion of contaminants of emerging concern (CECs), including pharmaceuticals, personal care products, solvent stabilizers, flame retardants, pesticides, and other commonly used commercial and industrial compounds. SRCSD encourages EPA to approach the issue of CECs in a cooperative manner with the regulated community. State and national associations in the wastewater industry, such as Tri-TAC (a technical advisory committee representing the League of California Cities, California Association of Sanitation

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[90] Johnson, M.L., I. Werner, S. Teh, F. Loge. *Evaluation of Chemical, Toxicological, and Histopathologic Data to Determine their Role in the Pelagic Organism Decline.* April 20, 2010. http://www.waterboards.ca.gov/centralvalley/water_issues/tmdl/central_valley_projects/central_valley_pesticides/risk_evaluation/rre_stff_rpt_feb2009_final.pdf

[91] Burton et al. (2005), supra note 85

Agencies(CASA), and California Water Environment Association(CWEA)) and the National Association of Clean Water Agencies along with research associations such as the Water Environment Research Foundation and Water Education Foundation, have led a number of groundbreaking efforts regarding CECs, including monitoring, research on effects, and source control campaigns. As mentioned in the ANPR, the State Water Board has also been active in addressing CECs, including convening an expert panel to make recommendations on CEC monitoring in recycled water and an expert panel on CECs in the ocean, estuaries and wetlands. We strongly urge EPA to build on these efforts as it moves forward in addressing CECs, and help provide the sound scientific basis for adaptive management in the Delta regarding CECs.

Answers to several of the questions relating to CECs are provided below.

1. What, if any, additional information is available regarding the effects of CECs on aquatic resources in the Bay Delta Estuary?

The risks posed by the presence of contaminants of emerging concern (CECs) to aquatic organisms and to humans are largely unknown, in part because ambient concentrations are difficult to detect and in part due to the lack of demonstrated effects at ambient levels. Science is only in the initial stages of study to gain a full understanding of the health and environmental impacts of CECs[92,93].

Of the limited number of studies conducted on the effects of CEC on human health, no studies have effectively linked low concentrations of CECs to adverse health effects in humans. To date, no studies in the U.S have effectively tied changes in the fish populations to wastewater treatment plant effluents. Many data gaps currently exist that researchers are attempting to address including: linking measures of exposure with adverse (and beneficial) effects; linking adverse effects observed in the laboratory with adverse effects in the field; linking adverse effect at the cellular and organ level to adverse effects in the whole organism; linking adverse effects in individual organisms to adverse effect in populations, and evaluating the effect of mixtures of low-concentration microconstituents.

The Society of Environmental Toxicology and Chemistry (SETAC) supports several independent groups of global technical experts such as the Pharmaceutical Advisory Group and Nanotechnology Advisory Group. Such resources would be valuable for reviewing and helping to identify current trends, findings, and leading research in these CEC topic areas.

2. What, if any, specific information exists to identify the sources and nature of discharges of CECs into the Bay Delta Estuary?

Various quantitative assessments have been performed to document the presence of CECs in wastewater treatment plant effluent and receiving waters. One of the most significant amongst the recent scientific studies that have looked at the presence of CECs in water bodies is ―A

[92] EPA, 2008. Aquatic Life Criteria for Contaminants of Emerging Concern, Part I, General Challenges and Recommendations (Draft), OW/ORD Emerging Contaminants Work Group, June 2008.
[93] WERF. WERF, 2005. Technical Brief: Endocrine Disrupting Compounds and Implications for Wastewater Treatment. 2005. 04-WEM-6.

National Reconnaissance" conducted by the United States Geological Survey (USGS)[94]. In this study, water samples from 139 surface water bodies were tested for the presence of 95 CECs. Water bodies that were susceptible to the presence of CECs due to contamination from human, agricultural, or industrial wastewater effluent were chosen for this study. One or more CECs were found in 80% of the water bodies, indicating the widespread presence of CECs in surface water in the United States. The most widely occurring and persistent CECs detected in the USGS Study were the insect repellent, DEET, and the pesticide atrazine.

Principal sources of microconstituents include the following:[95]

- Natural products from the environment
- Household products
- Pesticides
- Industrial chemicals
- Air emissions

3. What, if any, monitoring mechanisms or methodologies are available to assist in identifying CECs?

CECs can be detected at levels ranging from 10 to 50 ng/L, depending on the structure of the compound, using gas chromatography/tandem mass spectrometry (GC/MS/MS); high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) techniques; isotope dilution mass spectrometry; or gas chromatography/electron capture detection (GC/ECD). As with all laboratory analytical techniques, these methods require a high level of expertise, careful attention to detail, and rigorous quality assurance/quality control (QA/QC) measures. A majority of these methods have been developed in research laboratories and have not been officially approved by EPA or other regulatory entities for routine monitoring or regulatory purposes. However, there are two methods for CEC detection that have been developed by the EPA, but are not yet included in EPA regulations[96]. These are (1) Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS; and (2) Method 1698: Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS.

Bioassay methods (e.g. ELISA) can be used to screen for the presence of low levels of classes of compounds. These methods are inexpensive compared GC/MS and LC/MS, but are nonspecific and would need to be supplemented with conventional analytical methods when used.

Another monitoring mechanism under consideration is the development of bioanalytical screening techniques that include CECs currently not identified but potentially present (―unknown" chemicals). If these screening techniques were fully developed, they could be used

[94] Kolpin, D.W., Furlong. E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., and H.T. Buxton (2002). ―Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance." Environmental Science & Technology **36**(6): 1202-1211.

[95] Byers, H.; J.E. Denver; A. Goodman; J. Witherspoon (2007). "Technical Practice Update: Sources of Microconstituents and Endocrine Disrupting Compounds." Prepared for the Water Environment Federation. Order No: P07018E. July.

[96] Environmental Protection Agency (2008). ―Clean Water Act Analytical Methods; Other Methods." <http://www.epa.gov/waterscience/methods/method/other.html> Accessed on 9/30/2008.

to evaluate whether water has chemicals that produce biological responses such as estrogen receptor binding. If so, further tests would be performed to identify the responsible chemical or chemicals.

Since the passage of the Food Quality Protection Act and the Safe Drinking Water Act Amendments in 1996 that required EPA to screen pesticide chemicals for their potential to produce estrogenic effects in humans, EPA has added androgenic and thyroid system effects and fish and wildlife as receptors under their Endocrine Disruptor Screening Program (EDSP). The EPA initiated this program to develop and validate methods or assays to identify and characterize endocrine activity of pesticides, commercial chemicals, and environmental contaminants. While the EDSP process is not yet ready for widespread use, this is a thorough and scientifically supported process of method development that will lead to a promulgated method for EDC screening and testing. Such a unified approach is needed to ensure consistency of application and for generating data that can be useful for data comparisons[97]. These assays are to be used in a two-tiered screening and testing process in which chemicals that have the potential to interact with the endocrine system are identified in Tier 1 and the endocrine-related effects caused by each chemical as well as information about effects at various doses will be determined in Tier 2. There are currently 12 Tier 1 assays at the validation and peer review stage and 5 Tier 2 assays at the development or pre-validation stage.

According to the EDSP website endocrine disruption screening is currently proceeding on three fronts:

- Developing and validating Tier 2 tests,
- Selecting chemicals for screening and testing, and
- Implementing the policies and procedures the EPA will use to require screening.

The initial list of chemicals to be screened was announced on April 15, 2009 and the first test orders were issued on October 29, 2009. The EDSP website also provides the status of the test order responses for each chemical. A second list of 134 chemicals and substances was identified for Tier 1 screening on November 17, 2010. At this time, EPA also made available the ―Draft Policies and Procedures for Screening Safe Drinking Water Act Chemicals" which describes the EPA's draft policies and procedures for requiring Tier 1 screening of substances for which EPA may issue testing orders. EPA cautioned that chemicals on the Tier 1 screening lists were selected on the basis of exposure potential only and therefore, should not be construed or characterized as known or likely endocrine disruptors.

At this time, EPA also does not provide information on how to measure specific EDC concentrations, nor does EPA identify any trigger levels, or actions to take if a potential endocrine disrupting chemical is detected.

A State Water Board's expert panel supports a risk-based approach for evaluating the potential for adverse effects from CECs in their ―Monitoring Strategies for Chemicals of Emerging Concern (CECs) in Recycled Water". This approach recommends monitoring (i.e., measured

[97] USEPA, Endocrine Disruptor Screening Program, http://www.epa.gov/endo/index.htm

environmental concentration or MEC) and interpreting these monitoring data through chemical specific comparisons to concentrations known to cause adverse effects (i.e., monitor triggering level or MTL). This method should be supported (when MECs represent environmentally relevant concentrations) because chemical specific assessments are necessary to identify toxicity drivers and to direct plans to reduce those toxicants[98].

An alternative risk-based approach for identifying the sources and nature of discharges of CECs into the Delta is by developing methods for measuring CEC in wastewater. For example, the Southern California Coastal Water Research Project (SCCWRP) is in final year of a five-year study to develop analytical methods for CECs[99]. The project goal is to develop and evaluate analytical methods for detection and quantification of specific classes of emerging contaminants in various matrices (e.g., water, sediment, and biological tissues) at environmentally relevant levels. The data generated would be used to assess the sources, environmental distribution, and potential for chemically mediated effects due to CECs within the Southern California Bight. An initial focus is on the more hydrophobic contaminant classes including current-use pesticides, brominated flame retardants, and commercial phenolic compounds. SCCWRP is also studying the occurrence and fate of CECs in the coastal environment. Under this study, SCCWRP has identified and measured several classes of CECs in POTW effluent, receiving seawater, marine sediment, and fish[100].

SRCSD urges EPA to support approaches such as this that consider the environmental relevance of CECs in the environment along with fate and transport. Discharged concentrations of CECs may not be representative of the concentrations occurring in the environment after degradation, settling, and biotransformation.

4. What, if any, methods are most effective to minimize introduction of CECs into the Bay Delta Estuary?

The list of CECs is complex and large; therefore, it is difficult to make a general statement about the removal of CECs in wastewater treatment processes[101]. WERF has shown that most CECs are substantially (80-90%), but not completely, removed by biological wastewater treatment plants[102]. However, treatment plants, with few exceptions, are not designed to remove any specific compounds, such as pharmaceuticals and personal care products (PPCPs) or endocrine disrupting compounds (EDCs). Removal of PPCPs, EDCs, or other CECs is incidental to the

[98] State Water Resources Control Board, 2010. Monitoring Strategies for Chemicals of Emerging Concern (CECs) in Recycled Water Recommendations of a Science Advisory Panel. Final Report. Prepared by P.Anderson, N. Denslow, J.E. Drewes (Chair), A. Olivieri, D. Schlenk, and S. Snyder for the State Water Resources Control Board, Sacramento, CA. June 25.

[99] Sothern California Coastal Water Research Project, 2010-2011 Research Plan, Approved by SCCWRP Commission - June 2010, http://www.sccwrp.org/Documents/ResearchPlan.aspx#3._Emerging_Contaminants

[100] Southern California Coastal water Research Project, Advisory Panel for CECs in Coastal and Marine Ecosystems Section 1 Background and Objectives http://www.sccwrp.org/researchAreas/Contaminants/ContaminantsOfEmergingConcern/EcosystemsAdvisoryPanel.aspx

[101] Anderson, Paul (2008). ―Technical Brief: Trace Organic Compounds and Implications for Wastewater Treatment." Water Environment Research Foundation (WERF) CEC3R07.

[102] Anderson, Paul (2005). Technical Brief: Endocrine Disrupting Compounds and Implications for Wastewater Treatment. WERF. 04-WEM-6

removal of degradable organic material in the wastewater. Certain compounds like triclosan (antibacterial agent), galaxolide and musk ketone (musks), DEET (insecticide), benzophenone (UV filter/sunscreen), and TCEP (flame retardant) have been found to be persistent in wastewater treatment plants[103].

Below are key findings from recent research on the effectiveness of treatment processes for removal of PPCPs and EDCs from water and wastewater:[104] Solids retention time (SRT) employed in an activated sludge treatment plant has a pronounced effect on the removal of some PPCPs/EDCs. In general, longer SRTs appear to result in more effective removal of key PPCPs/EDCs. An SRT greater than 5 days was required to consistently achieve 80 percent removal of most PPCPs/EDCs. Pure oxygen activated sludge (as employed by SRWTP) may be more effective than conventional activated sludge at SRTs less than 5 days.

- Trickling filters, which have short hydraulic retention times, are relatively less effective for PPCPs/EDCs removal compared to activated sludge.
- The membrane bioreactor (MBR) process, which operates at long SRTs, is typically somewhat more effective than conventional activated sludge for removing some PPCPs/EDCs.
- Some compounds (e.g. TCEP, galaxolide, and musk ketone) are poorly removed (<50%) by all forms of biological treatment.
- Return flows from biosolids handling facilities can contain significant loads of microconstituents, suggesting the biosolids treatment is only partially successful in removing microconstituents adsorbed onto biosolids.
- UV irradiation at disinfection doses is not effective for removal of PPCPs/EDCs, but irradiation at high-energy oxidative doses can be highly effective, especially when combined with peroxide treatment.
- Disinfection with free chlorine can remove some target compounds, depending on structure, including most natural and synthetic estrogenic hormones, which are the compounds of greatest concern because of their biological potency.

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[103] Stephenson, Roger (2007). ―Fate of Pharmaceuticals and Personal Care Products Through Municipal Wastewater Treatment Processes" Water Environment Research Foundation 03-CTS-22UR)

[104] Scruggs, C. (2007). ―Technical Practice Update: Effects of Treatment on Microconstituents." Prepared for the Water Environment Federation. May. Order No: P07014E.
Anderson, P. *supra* note 98
Snyder, S.A.; E.C. Wert; H. Lei; P. Westerhoff; Y. Yoon (2007). ―Removal of EDCs and Pharmaceuticals in Drinking Water and Reuse Treatment Processes." Prepared for the American Water Works Association Research Foundation.
Sedlak, D.L; K. Pinkston; C. Huang (2005). ―Occurrence Survey of Pharmaceutically Active Compounds." Prepared for the American Water Works Association Research Foundation.
Drewes, J.E.; J.D.C. Hemming; J.J. Schauer; W.C. Sonzogni (2006). ―Removal of Endocrine Disrupting Compounds in Water Reclamation Processes." Prepared for the Water Environment Research Federation. Project 01-HHE-20T.

- Disinfection with chloramines is much less effective for removal of PPCPs/EDCs than disinfection with free chlorine. Chloramination can also form NDMA when precursors are present.
- Ozone is more effective than free chlorine at removal of PPCPs/EDCs and can effectively remove most target compounds.
- Advanced oxidation processes (AOPs) (e.g. ozone/peroxide and UV/peroxide) are highly effective at removal of most target compounds, but little is known regarding treatment byproducts.
- Activated carbon is highly effective for removal of most target compounds.
- Soil Aquifer Treatment of wastewater is highly effective for removal of most target compounds. Riverbank filtration (i.e. pumping groundwater adjacent to rivers) is an effective method of water supply treatment for removal of PPCPs/EDCs, as well as bacteria virus and parasites.
- Engineered treatment wetlands are largely ineffective for removal of PPCPs/EDCs.
- Reverse osmosis and nanofiltration are highly effective for removal of PPCPs/EDCs, but ultrafiltration and microfiltration are largely ineffective. Reverse osmosis and nanofiltration will concentrate microconstituents in the reject brine, which requires additional treatment. The costs for implementation of this technology by POTWs are exorbitant, in terms of both capital, operational and energy costs.
- A multiple barrier, treatment train approach that combines various advanced processes (e.g. reverse osmosis, AOP, ozonation, activated carbon) is the most effective for removing trace concentrations of PPCPs/EDCs. Again, the costs to implement this treatment technology are exorbitant.
- It is unlikely that removal of all contaminants to levels below detection will ever be achievable, given the ability of analytical methods to detect microconstituents at ultra-trace levels.

SRWTP currently uses a high purity oxygen activated sludge biological treatment process. By 2020, that system most likely will be replaced with a nitrifying-dentrifying air activated sludge biological treatment process employing SRTs in the range of 5 days +. UV irradiation at disinfection doses will be pilot tested for potential SRWTP use by 2020. UV irradiation in combination with peroxide may also be pilot tested. The pilot study treatment objective is for disinfection, not for CEC degradation. Chlorine disinfection is currently employed at SRWTP. Due to the presence of ammonia, all chlorine is most likely currently converted to chloramines.

Chlorine disinfection will be pilot tested as a disinfection alternative combined with the nitrifying/denitrifying air activated sludge biological treatment process and filtration for potential SRWTP use by 2020. Disinfection byproduct formation caused by free chlorine reactions with naturally occurring organic matter will probably result in free chlorine. Ozone disinfection will be pilot tested as a disinfection alternative for possible SRWTP use by 2020. Ozonation will also be pilot tested as pre-treatment before biologically active filtration to assess its affect on virus reduction and CEC destruction. Pre-filtration ozonation will be pilot tested in combination with disinfection alternatives (UV irradiation, chlorine, ozone).

The American Water Works Association Research Foundation report on Removal of EDCs and Pharmaceuticals in Drinking and Reuse Treatment Processes notes that presence of a compound does not necessarily mean that is detrimental to the environment[105]. The toxicological significance of trace occurrence of various microconstituents should be determined to establish a scientific basis for establishing sensible monitoring requirements, treatment goals, and regulatory limits. Furthermore, there are many sources and pathways for microconstituents in the natural environment. As safe levels of various microconstituents are determined through research, the relative costs and benefits of any potential reductions at wastewater treatment plants should be compared with the control and treatment of other sources.

The sources and levels of CECs make them difficult to control or monitor. Pollution prevention efforts include pharmaceutical take-back programs, which are in place in Australia, Europe, and some parts of the United States. These programs allow consumers to return unused prescription and non-prescription pharmaceuticals at take-back locations. The collected pharmaceuticals are then disposed of by incineration, thus preventing them from entering waterbodies. Outreach and education about CECs can also be used as a pollution prevention tool[106]. Numerous Delta counties operate take-back programs such programs exist in the Bay Area; however, in January 2011 the San Francisco Board of Supervisors rescinded the Safe Drug Disposal Ordinance back to the public policy committee.[107] Many local agencies have a problem with establishing drug take back programs that include taking back controlled substances, which the Federal Drug Enforcement Agency has jurisdiction over. EPA could work with the Federal Drug Enforcement Agency to make drug take back programs more accessible.

Fish Migration Corridors

Fish migration corridors are affected by many factors, both physical and chemical, that can interact with each other. Hydraulic alterations are a major factor in the suitability of the Bay-Delta as a corridor for salmon. Biological criteria that more directly measure fish migration and spawning success could be developed and used to measure success of restoration. Any change in system hydrology could affect the physical, chemical, and biotic processes, and thus can affect related temperature and dissolved oxygen (DO) conditions in the San Joaquin River. SRCSD does not support development of additional solutions for DO impairments in the Stockton Deep Water Ship Channel (DWSC) or any other Delta locations until the current studies for TMDL development are completed and a determination of long-term solutions can be made.

1. What role, if any, do gradients in physical and chemical constituents of water play in the suitability of the Bay Delta Estuary and San Joaquin River migratory corridor for salmon?

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[105] Stephenson, R. and Oppenheimer, J. (2007). ―Fate of Pharmaceuticals and Personal Care Products Through Municipal Wastewater Treatment Processes" Water Environment Research Foudation 03-CTS-22UR.
[106] For instance, http://www.nodrugsdownthedrain.org/
[107] http://www.baybio.org/about/presidents-desk/sf-board-of-supervisors-takes-back-drug-take-back-program.

Based on the numerous factors contributing to fish migration barriers in the San Joaquin River system, the EPA should consider both the unique physical and chemical factors contributing to fish migration barriers in this watershed, in terms of their individual effects and the interactions of these factors, during any rulemaking process. The San Joaquin River Restoration Program (SJRRP) has identified a comprehensive list of stressors and limiting factors facing Chinook salmon immigration in the San Joaquin River, which include: (1) inadequate flows and high Delta export rates, (2) high water temperatures, (3) physical barriers and flow diversion, (4) Delta water quality, and (5) in-river harvest[108]. In addition predation occurs at manmade structures.

Alterations in the hydrology and water quality of the Bay Delta Estuary and San Joaquin River have had substantial effects, in terms of both physical and chemical gradients, on migration corridors for anadromous salmonids. The alterations in hydrology resulting from the Central Valley Project (CVP) and State Water Project (SWP) operations, as well as the numerous diversions and discharges throughout the system, have created adverse conditions for migrating anadromous salmonids through a combination of factors that include, but are not limited to, flow reductions, increased temperatures, decreased DO conditions, low-flow barriers and complete de-watering of substantial reaches of the San Joaquin River, contamination loading, and disruption of homing cues resulting from large-scale exchange of water sources (e.g., Sacramento River water being drawn into the lower reaches of the San Joaquin River channel).

Hallock et al. reported that a temperature of 69.8°F and DO concentrations less than 5 mg/l created a thermal barrier to immigration of adult fall-run Chinook salmon in the Delta at Stockton[109]. These authors concluded that avoidance by adult Chinook salmon occurred at water temperatures exceeding approximately 66°F when DO concentrations were less than 5 mg/l. This study and others show that low DO concentrations can substantially affect immigrating Chinook salmon behavioral responses to various water temperatures. Where low DO was not a problem, Dunham reported that water temperatures approaching 76°F in the lower Klamath River had no observable effect on the upstream migration of adult Chinook salmon[110]. Marine reported that adult Chinook salmon can tolerate short-term and transient temperature exposures to temperatures of 77–80.6°F during spawning migrations[111]. These findings indicate that the EPA's temperature criterion for fish migration (18°C/64°F) is quite conservative and should also

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[108] SJRRP (San Joaquin River Restoration Program). 2010. *Conceptual Models of Stressors and Limiting Factors for San Joaquin River Chinook Salmon; Fisheries Management Plan: A Framework for Adaptive Management in the San Joaquin River Restoration Program*. November. www.restoresjr.net/program_library/02-Program_Docs/index.html

[109] Hallock, R.J., R.F. Elwell, and D.H. Fry, Jr. 1970. Migrations of Adult King Salmon *Oncorhynchus Tshawytscha* in the San Joaquin Delta as Demonstrated by the Use of Sonic Tags. California Department of Fish and Game *Fish Bulletin* No. 151.

[110] Dunham, L.R. 1968. Recommendations on thermal objectives for water quality control policies on the interstate waters of California. A report to the State Water Resources Control Board. Cal. Dept. Fish and Game, *Water Proj. Br. Rept.* 7.

Boles, G.L., S.M. Turek, C.D. Maxwell, and D.M. McGill. 1988. *Water Temperature Effects on Chinook Salmon (Oncorhynchus tshawytscha) with Emphasis on the Sacramento River: a literature review*. California Department of Water Resources, Northern District, Red Bluff, CA.

[111] Marine, K.R. 1992. *A background investigation and review of the effects of elevated water temperature on reproductive performance of adult chinook salmon (Oncorhynchus tshawytscha).* Prepared for East Bay Municipal Utility District.

consider a water body‘s seasonal DO regime, seasonal temperature regime, and the portion of the river channel that exceeds the thresholds for fish migration.

Finally, the effects of large-scale water diversions that result in Sacramento River water being drawn into the lower reach of the San Joaquin River may disrupt the olfactory senses of anadromous salmonids, thereby interfering with their ability to find their way back to their natal stream during their spawning immigrations. Numerous researchers (e.g., Hara et al. 1965; Ueda et al. 1967) have demonstrated that the homing ability of salmonids rely largely on olfactory senses[112]. Juvenile anadromous salmonids are believed to undergo a process known as ―sequential imprinting” during their emigration from natal streams to the ocean, a mechanism that allows returning adults to find their way from the ocean, despite the high degree of dilution that water from their natal stream may undergo in large systems (e.g., the San Joaquin River). Sequential imprinting is believed to be the result of surges in thyroxine development occurring at river confluences, or ―olfactory waypoints”, encountered by juvenile salmonids emigrating from natal streams to the ocean. When adults return to freshwater during their spawning migrations, it is believed that they rely on olfactory waypoints to guide them when they encounter confluences, rather than relying strictly on the scent of their natal stream. This concept is exemplified by Dittman et al., who observed that coho salmon reared to adulthood in a hatchery did not return when released approximately 1 km downstream, even though the majority of members of the same cohort that were released as smolts did return[113]. Other studies have similarly observed that juvenile salmonids released in the ocean, in lieu of allowing them to migrate downstream on their own accord, exhibited atypically high degrees of straying[114]. These studies suggest that alterations in San Joaquin River hydrology, including the intrusion of the lower 40-km stretch of the river by water derived from the Sacramento River, could disrupt imprinting in juvenile fishes, thereby reducing the likelihood that they would be able to rely on olfactory cues to return to their natal streams as adults. Consequently, the EPA should consider the uniqueness of this San Joaquin River situation during any rulemaking process that could be applied to other water bodies.

2. What are the best measures of success for restoration of a migratory corridor? Could these measures be incorporated into new or revised biological criteria protecting the fish migration designated use?

The ultimate measure of success for restoration of a migratory corridor is that fish are able to pass physical barriers and avoid predation as juveniles., with minimal delay or exposure to potentially detrimental effects that could reduce reproductive success (e.g., increased egg mortality rates), ultimately reaching their spawning habitat. Restoration monitoring efforts that

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[112] Hara, T.J., K. Ueda, and A. Gorbman. 1965. Electroencephalographic studies of homing salmon. *Science* 149:884–885.

Ueda, K., T.J. Hara, and A. Gorbman. 1967. Electroencephalographic studies on olfactory discrimination in adult spawning salmon. *Comp. Biochem. Physiol.* 21:133–143.

[113] Dittman, A.H., T.P. Quinn, and G.A. Nevitt. 1996. Timing of imprinting to natural and artificial odors by coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* 53:434–442.

[114] Schroeder, R.K., R.B. Lindsay, and K.R. Kenaston. 2001. Origin and straying of hatchery winter steelhead in Oregon coastal rivers. *Transactions of the American Fisheries Society* 130:431–441.

involve the monitoring of fish movement and behavior are difficult and time-consuming; therefore, more practical measures of success would include monitoring the physical or water quality conditions that occur in a given water body and comparing that to values cited in the scientific literature to determine whether or not the conditions are suitable for fish passage by maintaining suitable zones of passage in which a portion(s) of the channel cross-section, sufficient to allow unimpeded passage by anadromous fishes, is below threshold values for temperature and DO that could cause blockage. In addition, such zones of passage should provide sufficient flow in the channel to ensure that low-flow barriers are not present. In the case of anadromous salmonids migrating through the Delta and its two primary tributaries, the San Joaquin and Sacramento rivers, passage success would thus be assumed by maintaining water quality conditions that are suitable across a sufficient portion of the channel for the successful passage of fishes and are also sufficiently protective that any associated delays in spawning do not result in substantial pre-spawn egg mortality resulting from prolonged exposure to elevated temperatures.

Alternatively, the direct counting of adult salmonids passing specified locations in the river system could potentially be used to determine the degree of immigration success and thus whether a migration corridor has been successfully restored. Rather than defining water quality and flow criteria only, biological criteria that more directly measure fish migration and spawning success could be developed and used. Such biological criteria would be a reflection of the effects of all stressors on migration and spawning, even if we do not completely understand the role that each individual stressor plays. If the biological result is favorable, and can be developed into biological criteria, then the variable flow, temperature, and water quality criteria can also be deemed acceptable. This would be analogous to the benthic macroinvertebrate metrics that are currently used to assess the effects of flow, temperature, and water quality on the ecological health of creeks and streams. However, as noted above monitoring efforts that involve the monitoring of fish movement and behavior are difficult and costly to implement and, ultimately, produce data that is difficult to interpret relative to the role than an individual stressor plays.

As discussed above, migration can be blocked or delayed if the combination of elevated water temperatures and reduced DO concentrations exceed threshold levels for a given species and lifestage. In addition to the direct thermal effects on migrating fish, elevated water temperatures can lead to thermally induced egg losses, if adult female fish are exposed to elevated temperatures for a sufficient duration during their spawning migrations. Consequently, by maintaining temperature conditions below the thresholds and durations that cause substantially elevated egg mortality rates, and/or provide adequate zones of passage, fish migration corridors would be considered restored and the Migration beneficial use would be considered adequately protected.

3. Should temporal characteristics be included in the definition of the physical and/or chemical properties of a migration corridor based on a reference condition? If so, how? What frequency and duration of such a corridor is required for salmonids? How might these characteristics change with the impacts of climate change?

The temporal characteristics of a migration corridor can, and should be, defined by considering both short-term duration and seasonality. Parameters for protecting migrating fishes and maintaining migration corridors should provide a seasonal component that considers the most sensitive species that are likely to be migrating seasonally. As such, criteria developed to protect Chinook salmon migration should be applied, as appropriate, during the fall-early winter adult immigration and during the winter-spring juvenile emigration period. Summer conditions are not as important for salmonid migration.

Climate change may alter both river temperature and flow conditions, relative to historical conditions, which can in turn change DO levels and other physical and chemical conditions. In situations where migrations corridors are blocked as a result of elevated temperatures and decreased DO levels under current conditions, the effects of climate change may include an increase in the duration in which water quality conditions exceed the threshold levels that cause such blockages and delays and, under the most extreme conditions, lead to an increase in the frequency of blockage conditions, particularly where marginal conditions already occur. However, it is important to note that, because DO concentrations play such an important role in the creation of blockage conditions for migrating salmonids, and due to the numerous factors that can affect DO concentrations in aquatic ecosystems, the effects of climate change on DO concentrations in the Delta and San Joaquin and Sacramento rivers and the resultant effect on fish migration corridors cannot be reliably estimated.

As for frequency of suitable migration corridors, we would want to achieve suitable migration corridors in all years, or as many years as possible. As for duration, suitable corridors do not necessarily have to exist at all times throughout the migrations season, but should exist for a substantial portion of the migration season. If suitable conditions ―open up" periodically, they ideally need to occur early-, mid-, and late-season, such that early-, mid-, and late-season migrants are successful, rather than just early migrants at the expense of mid- and late-season migrants. The latter is detrimental to the genetic diversity and resiliency of anadromous salmonid populations.

4. Would establishing a migratory corridor for upmigrating adult Chinook salmon succeed in improving adult migration success if temperatures upstream of Vernalis are unchanged? If so, how? How might actions to establish a migratory corridor in the south Delta also moderate temperature and/or dissolved oxygen problems in the San Joaquin River?

In order to truly be effective and achieve the underlying goal of restoring and enhancing anadromous salmonid populations in the San Joaquin River watershed, establishing migration corridors for adult immigrating Chinook salmon should be examined from a more comprehensive population restoration perspective. Improving passage conditions in the San Joaquin River likely involves improving not only temperature and DO conditions, but also flow (e.g., attraction flows) and instream habitat conditions throughout the watershed, particularly during the key fall-early winter adult immigration period and during the winter-spring juvenile emigration period. Moreover, establishing or improving migration corridors in the San Joaquin River should be undertaken with clear goals and objectives in mind that prioritize improving access of anadromous fishes to San Joaquin River tributaries that provide suitable habitat for spawning, incubation, and early rearing. Establishing or improving migration corridors to reaches of rivers that have unsuitable or marginal habitat for spawning, egg incubation, and early rearing could create an ―ecological sink" in which fish are attracted, and in some cases, potentially falsely attracted from other water bodies (e.g., the Sacramento River system), to river reaches providing little or no suitable habitat. Consequently, fish that would otherwise have spawned elsewhere end up perishing without successfully spawning and increasing population recruitment.

Any change in system hydrology could affect the physical, chemical, and biotic processes, and thus can affect related temperature and DO conditions in the San Joaquin River. There are multiple and complex cause and effect relationships among these variables in aquatic ecosystems. The DO TMDL for the Stockton DWSC identified the principal causes of DO impairment as loads of oxygen demanding substances from upstream sources, and DWSC geometry and reduced flow through the DWSC which affect the DO and assimilative capacity of the system[115]. However, the TMDL report also suggests that the specific cause and effect relationships are not well understood and, thus, among the primary implementation requirements of the TMDL were additional studies regarding upstream loading sources and effects of current DWSC geometry on DO and assimilative capacity conditions[116].

Based on the identified need for additional studies to resolve the *existing* DWSC DO impairment, it is apparent that it is unlikely that there is sufficient data or analytical techniques or modeling available at this time to predict how improving migratory conditions in the South Delta might change the lower San Joaquin River-Delta temperature and DO conditions. That said, moving greater volumes of water through the system has the potential to improve both temperature and DO conditions in this portion of the system where flows have been low and, thus, water exchange rates have also been low.

5. Are additional efforts to improve dissolved oxygen regimes in the Delta necessary to provide an adequate migratory corridor for San Joaquin salmonids? If so, what should those efforts include?

As a means to improve DO conditions in the DWSC, the Central Valley Regional Water Board implemented a DO TMDL and began implementation of a series of studies intended to determine the cause of the low DO conditions. The studies required by the TMDL of upstream loading sources were determined to be somewhat inconclusive, and other downstream studies needed to understand how oxygen demanding substances and their precursors impact DO levels once they enter the tidal section of the San Joaquin River starting near Mossdale have not yet been completed. Efforts to collect the necessary data to develop improved analytical and modeling methods that can be used to define the *existing* relationships of temperature/DO variables to salmon migration success should be supported. Efforts to address the existing DWSC DO

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[115] Central Valley RWQCB. 2010. Staff Report. Stockton Deep Water Ship Channel Dissolved Oxygen Basin Plan Amendment Control Program – Status Report on Reconsideration of the Prohibition of Discharge, Allocations and Implementation Provisions. (January 28, 2010).

[116] Central Valley RWQCB. 2005. Amendments to the Water Quality Control Plan for the Sacramento River and San Joaquin River Basins for the Control Program for Factors Contributing to the Dissolved Oxygen Impairment in the Stockton Deep Water Ship Channel, Final Staff Report. February 28.

impairment with implementation of temporary and cost-effective aeration devices to attenuate the problem have been shown to be moderately effective and are supported, to the extent that such actions provide time to collect data and predictive methods for determining definitive long-term solutions to the migration problem. SRCSD does not support development of additional solutions for DO impairments in the DWSC until the current studies are completed and a determination of long-term solutions can be made.

6. What other information is available on barriers to salmon migration in the Bay Delta Estuary and San Joaquin River watershed?

The San Joaquin River has been the subject of much attention by fisheries biologists in the Central Valley. Biologists from NMFS, California Department of Fish and Game (CDFG), Department of Water Resources (DWR), and other agencies can provide a large amount of information pertaining to barriers to salmon migration in this watershed. As discussed above, the SJRRP has published and compiled numerous documents pertaining to the various factors affecting anadromous salmonids in the San Joaquin River system, much of which is available for download from its website.

Attachment 2

State Water Resources Control Board, Delta Flow Criteria Informational Proceeding, Other Stressors Panel, March 24, 2010 submitted via US Mail

Please note that this will be in the form of a CD delivered along with a hard copy.

Attachment 3: TN:TP Data for Suisun Bay- Engle Unpublished Data

IEP Station Code	Station Name	Sample Date	TN (mg/L)	TP (mg /L)	Mass Ratio (N:P)	molar ratio (N:P)
D7	Grizzly Bay @ Dophin nr. Suisun Slough	1/8/02	1.48	0.15	9.87	21.82
D6	Suisun Bay @ Bulls Head nr. Martinez	1/9/02	1.30	0.15	8.67	19.17
D8	Suisun Bay off Middle Pt. nr. Nichols	1/9/02	1.15	0.16	7.19	15.89
D7	Grizzly Bay @ Dophin nr. Suisun Slough	2/6/02	0.94	0.14	6.71	14.85
D6	Suisun Bay @ Bulls Head nr. Martinez	2/7/02	1.00	0.10	10.00	22.11
D8	Suisun Bay off Middle Pt. nr. Nichols	2/7/02	0.95	0.12	7.92	17.51
D7	Grizzly Bay @ Dophin nr. Suisun Slough	3/6/02	1.17	0.14	8.36	18.48
D8	Suisun Bay off Middle Pt. nr. Nichols	3/7/02	0.86	0.12	7.17	15.85
D6	Suisun Bay @ Bulls Head nr. Martinez	3/7/02	0.83	0.12	6.92	15.30
D7	Grizzly Bay @ Dophin nr. Suisun Slough	4/5/02	0.73	0.11	6.64	14.68
D6	Suisun Bay @ Bulls Head nr. Martinez	4/8/02	0.79	0.11	7.18	15.88
D8	Suisun Bay off Middle Pt. nr. Nichols	4/8/02	0.82	0.15	5.47	12.09
D7	Grizzly Bay @ Dophin nr. Suisun Slough	5/3/02	0.91	0.18	5.06	11.18
D8	Suisun Bay off Middle Pt. nr. Nichols	5/6/02	0.77	0.12	6.42	14.19
D6	Suisun Bay @ Bulls Head nr. Martinez	5/6/02	0.68	0.11	6.18	13.67
D7	Grizzly Bay @ Dophin nr. Suisun Slough	6/4/02	0.91	0.14	6.50	14.37
D6	Suisun Bay @ Bulls Head nr. Martinez	6/5/02	0.78	0.11	7.09	15.68
D8	Suisun Bay off Middle Pt. nr. Nichols	6/5/02	0.81	0.12	6.75	14.93
D7	Grizzly Bay @ Dophin nr. Suisun Slough	7/17/02	0.92	0.16	5.75	12.72
D8	Suisun Bay off Middle Pt. nr. Nichols	7/18/02	1.12	0.13	8.62	19.05
D6	Suisun Bay @ Bulls Head nr. Martinez	7/18/02	0.82	0.14	5.86	12.95
D7	Grizzly Bay @ Dophin nr. Suisun Slough	8/15/02	0.85	0.21	4.05	8.95
D8	Suisun Bay off Middle Pt. nr. Nichols	8/16/02	0.78	0.14	5.57	12.32
D6	Suisun Bay @ Bulls Head nr. Martinez	8/16/02	0.85	0.16	5.31	11.75
D7	Grizzly Bay @ Dophin nr. Suisun Slough	9/13/02	1.10	0.20	5.50	12.16
D6	Suisun Bay @ Bulls Head nr. Martinez	9/16/02	0.73	0.15	4.87	10.76
D8	Suisun Bay off Middle Pt. nr. Nichols	9/16/02	0.46	0.12	3.83	8.48
D7	Grizzly Bay @ Dophin nr. Suisun Slough	10/11/02	0.80	0.18	4.44	9.83
D6	Suisun Bay @ Bulls Head nr. Martinez	10/15/02	0.74	0.14	5.29	11.69
D8	Suisun Bay off Middle Pt. nr. Nichols	10/15/02	0.70	0.15	4.67	10.32
D7	Grizzly Bay @ Dophin nr. Suisun Slough	11/13/02	0.85	0.09	9.44	20.89
D6	Suisun Bay @ Bulls Head nr. Martinez	11/14/02	0.82	0.10	8.20	18.13
D8	Suisun Bay off Middle Pt. nr. Nichols	11/14/02	0.86	0.12	7.17	15.85
D7	Grizzly Bay @ Dophin nr. Suisun Slough	12/11/02	1.07	0.15	7.13	15.77
D6	Suisun Bay @ Bulls Head nr. Martinez	12/12/02	0.93	0.12	7.75	17.14
D8	Suisun Bay off Middle Pt. nr. Nichols	12/12/02	0.99	0.13	7.62	16.84
D7	Grizzly Bay @ Dophin nr. Suisun Slough	1/10/03	1.34	0.16	8.38	18.52
D8	Suisun Bay off Middle Pt. nr. Nichols	1/13/03	1.14	0.10	11.40	25.21
D6	Suisun Bay @ Bulls Head nr. Martinez	1/13/03	0.84	0.10	8.40	18.58
D7	Grizzly Bay @ Dophin nr. Suisun Slough	2/7/03	0.81	0.16	5.06	11.20
D6	Suisun Bay @ Bulls Head nr. Martinez	2/10/03	0.70	0.08	8.75	19.35
D8	Suisun Bay off Middle Pt. nr. Nichols	2/10/03	0.62	0.08	7.75	17.14
D7	Grizzly Bay @ Dophin nr. Suisun Slough	3/12/03	0.83	0.09	9.22	20.39
D8	Suisun Bay off Middle Pt. nr. Nichols	3/13/03	0.73	0.08	9.13	20.18
D6	Suisun Bay @ Bulls Head nr. Martinez	3/13/03	0.57	0.08	7.13	15.76
D7	Grizzly Bay @ Dophin nr. Suisun Slough	4/10/03	0.69	0.10	6.90	15.26
D8	Suisun Bay off Middle Pt. nr. Nichols	4/11/03	0.77	0.10	7.70	17.03
D6	Suisun Bay @ Bulls Head nr. Martinez	4/11/03	0.72	0.10	7.20	15.92
D7	Grizzly Bay @ Dophin nr. Suisun Slough	5/9/03	0.72	0.11	6.55	14.47
D8	Suisun Bay off Middle Pt. nr. Nichols	5/12/03	0.48	0.07	6.86	15.16
D6	Suisun Bay @ Bulls Head nr. Martinez	5/12/03	0.60	0.09	6.67	14.74
D7	Grizzly Bay @ Dophin nr. Suisun Slough	6/9/03	0.66	0.12	5.50	12.16
D6	Suisun Bay @ Bulls Head nr. Martinez	6/10/03	0.57	0.10	5.70	12.60
D8	Suisun Bay off Middle Pt. nr. Nichols	6/10/03	0.44	0.11	4.00	8.85

Attachment 3: TN:TP Data for Suisun Bay- Engle Unpublished Data

D7	Grizzly Bay @ Dophin nr. Suisun Slough	7/21/03	0.63	0.15	4.20	9.29
D8	Suisun Bay off Middle Pt. nr. Nichols	7/22/03	0.60	0.10	6.00	13.27
D6	Suisun Bay @ Bulls Head nr. Martinez	7/22/03	0.64	0.12	5.33	11.79
D7	Grizzly Bay @ Dophin nr. Suisun Slough	8/6/03	1.10	0.16	6.88	15.20
D8	Suisun Bay off Middle Pt. nr. Nichols	8/7/03	0.46	0.09	5.11	11.30
D6	Suisun Bay @ Bulls Head nr. Martinez	8/7/03	0.64	0.13	4.92	10.89
D7	Grizzly Bay @ Dophin nr. Suisun Slough	9/3/03	0.72	0.17	4.24	9.37
D8	Suisun Bay off Middle Pt. nr. Nichols	9/4/03	0.60	0.12	5.00	11.06
D6	Suisun Bay @ Bulls Head nr. Martinez	9/4/03	0.64	0.14	4.57	10.11
D7	Grizzly Bay @ Dophin nr. Suisun Slough	10/17/03	0.74	0.11	6.73	14.88
D6	Suisun Bay @ Bulls Head nr. Martinez	10/20/03	1.03	0.14	7.36	16.27
D8	Suisun Bay off Middle Pt. nr. Nichols	10/20/03	0.84	0.12	7.00	15.48
D7	Grizzly Bay @ Dophin nr. Suisun Slough	11/18/03	0.92	0.12	7.67	16.95
D6	Suisun Bay @ Bulls Head nr. Martinez	11/19/03	1.11	0.12	9.25	20.46
D8	Suisun Bay off Middle Pt. nr. Nichols	11/19/03	0.93	0.12	7.75	17.14
D7	Grizzly Bay @ Dophin nr. Suisun Slough	12/17/03	1.86	0.12	15.50	34.28
D6	Suisun Bay @ Bulls Head nr. Martinez	12/18/03	1.15	0.10	11.50	25.43
D8	Suisun Bay off Middle Pt. nr. Nichols	12/18/03	1.07	0.11	9.73	21.51
D7	Grizzly Bay @ Dophin nr. Suisun Slough	1/16/04	1.10	0.13	8.46	18.71
D6	Suisun Bay @ Bulls Head nr. Martinez	1/21/04	0.88	0.10	8.80	19.46
D8	Suisun Bay off Middle Pt. nr. Nichols	1/21/04	0.89	0.12	7.42	16.40
D8	Suisun Bay off Middle Pt. nr. Nichols	2/19/04	0.94	0.11	8.55	18.90
D6	Suisun Bay @ Bulls Head nr. Martinez	2/19/04	1.15	0.14	8.21	18.16
D7	Grizzly Bay @ Dophin nr. Suisun Slough	2/23/04	0.95	0.18	5.28	11.67
D7	Grizzly Bay @ Dophin nr. Suisun Slough	3/16/04	1.15	0.11	10.45	23.12
D6	Suisun Bay @ Bulls Head nr. Martinez	3/17/04	0.76	0.09	8.44	18.67
D8	Suisun Bay off Middle Pt. nr. Nichols	3/17/04	0.66	0.09	7.33	16.22
D7	Grizzly Bay @ Dophin nr. Suisun Slough	4/13/04	0.76	0.14	5.43	12.00
D6	Suisun Bay @ Bulls Head nr. Martinez	4/14/04	1.17	0.12	9.75	21.56
D8	Suisun Bay off Middle Pt. nr. Nichols	4/14/04	0.60	0.10	6.00	13.27
D7	Grizzly Bay @ Dophin nr. Suisun Slough	5/12/04	0.70	0.10	7.00	15.48
D6	Suisun Bay @ Bulls Head nr. Martinez	5/13/04	0.81	0.09	9.00	19.90
D8	Suisun Bay off Middle Pt. nr. Nichols	5/13/04	0.61	0.11	5.55	12.26
D7	Grizzly Bay @ Dophin nr. Suisun Slough	6/8/04	0.83	0.12	6.92	15.30
D6	Suisun Bay @ Bulls Head nr. Martinez	6/9/04	0.91	0.10	9.10	20.12
D8	Suisun Bay off Middle Pt. nr. Nichols	6/9/04	0.85	0.11	7.73	17.09
D7	Grizzly Bay @ Dophin nr. Suisun Slough	7/7/04	0.91	0.19	4.79	10.59
D8	Suisun Bay off Middle Pt. nr. Nichols	7/8/04	0.87	0.12	7.25	16.03
D6	Suisun Bay @ Bulls Head nr. Martinez	7/8/04	0.90	0.15	6.00	13.27
D7	Grizzly Bay @ Dophin nr. Suisun Slough	8/23/04	0.81	0.14	5.79	12.79
D8	Suisun Bay off Middle Pt. nr. Nichols	8/24/04	0.75	0.11	6.82	15.08
D6	Suisun Bay @ Bulls Head nr. Martinez	8/24/04	0.83	0.14	5.93	13.11
D7	Grizzly Bay @ Dophin nr. Suisun Slough	9/22/04	0.89	0.13	6.85	15.14
D8	Suisun Bay off Middle Pt. nr. Nichols	9/23/04	0.58	0.11	5.27	11.66
D6	Suisun Bay @ Bulls Head nr. Martinez	9/23/04	0.54	0.13	4.15	9.19
D7	Grizzly Bay @ Dophin nr. Suisun Slough	10/20/04	1.47	0.10	14.70	32.51
D6	Suisun Bay @ Bulls Head nr. Martinez	10/21/04	1.06	0.10	10.60	23.44
D8	Suisun Bay off Middle Pt. nr. Nichols	10/21/04	0.68	0.11	6.18	13.67
D7	Grizzly Bay @ Dophin nr. Suisun Slough	11/19/04	0.94	0.09	10.44	23.10
D6	Suisun Bay @ Bulls Head nr. Martinez	11/22/04	0.82	0.08	10.25	22.67
D8	Suisun Bay off Middle Pt. nr. Nichols	11/22/04	0.87	0.09	9.67	21.38
D7	Grizzly Bay @ Dophin nr. Suisun Slough	12/20/04	1.42	0.17	8.35	18.47
D8	Suisun Bay off Middle Pt. nr. Nichols	12/21/04	1.44	0.12	12.00	26.54
D6	Suisun Bay @ Bulls Head nr. Martinez	12/21/04	1.07	0.11	9.73	21.51
D7	Grizzly Bay @ Dophin nr. Suisun Slough	1/19/05	1.20	0.08	15.00	33.17
D6	Suisun Bay @ Bulls Head nr. Martinez	1/20/05	0.97	0.10	9.70	21.45
D8	Suisun Bay off Middle Pt. nr. Nichols	1/20/05	1.12	0.15	7.47	16.51
D7	Grizzly Bay @ Dophin nr. Suisun Slough	2/16/05	1.12	0.12	9.33	20.64
D6	Suisun Bay @ Bulls Head nr. Martinez	2/17/05	0.87	0.09	9.67	21.38

Attachment 3: TN:TP Data for Suisun Bay- Engle Unpublished Data

D8	Suisun Bay off Middle Pt. nr. Nichols	2/17/05	1.00	0.11	9.09	20.10
D7	Grizzly Bay @ Dophin nr. Suisun Slough	3/21/05	1.11	0.13	8.54	18.88
D6	Suisun Bay @ Bulls Head nr. Martinez	3/22/05	1.10	0.10	11.00	24.33
D8	Suisun Bay off Middle Pt. nr. Nichols	3/22/05	0.98	0.12	8.17	18.06
D7	Grizzly Bay @ Dophin nr. Suisun Slough	4/18/05	0.76	0.10	7.60	16.81
D8	Suisun Bay off Middle Pt. nr. Nichols	4/19/05	0.75	0.09	8.33	18.43
D6	Suisun Bay @ Bulls Head nr. Martinez	4/19/05	0.75	0.38	1.97	4.36
D7	Grizzly Bay @ Dophin nr. Suisun Slough	5/18/05	0.65	0.15	4.33	9.58
D6	Suisun Bay @ Bulls Head nr. Martinez	5/19/05	0.69	0.10	6.90	15.26
D8	Suisun Bay off Middle Pt. nr. Nichols	5/19/05	0.53	0.08	6.63	14.65
D7	Grizzly Bay @ Dophin nr. Suisun Slough	6/15/05	0.51	0.10	5.10	11.28
D6	Suisun Bay @ Bulls Head nr. Martinez	6/16/05	0.54	0.08	6.75	14.93
D8	Suisun Bay off Middle Pt. nr. Nichols	6/16/05	0.50	0.08	6.25	13.82
D7	Grizzly Bay @ Dophin nr. Suisun Slough	7/13/05	0.59	0.11	5.36	11.86
D6	Suisun Bay @ Bulls Head nr. Martinez	7/14/05	0.70	0.11	6.36	14.07
D8	Suisun Bay off Middle Pt. nr. Nichols	7/14/05	0.57	0.09	6.33	14.01
D7	Grizzly Bay @ Dophin nr. Suisun Slough	8/10/05	1.26	0.18	7.00	15.48
D6	Suisun Bay @ Bulls Head nr. Martinez	8/11/05	0.66	0.09	7.33	16.22
D8	Suisun Bay off Middle Pt. nr. Nichols	8/11/05	0.44	0.08	5.50	12.16
D7	Grizzly Bay @ Dophin nr. Suisun Slough	9/23/05	0.72	0.16	4.50	9.95
D8	Suisun Bay off Middle Pt. nr. Nichols	9/26/05	0.58	0.10	5.80	12.83
D6	Suisun Bay @ Bulls Head nr. Martinez	9/26/05	0.66	0.12	5.50	12.16
D7	Grizzly Bay @ Dophin nr. Suisun Slough	10/24/05	1.05	0.15	7.00	15.48
D8	Suisun Bay off Middle Pt. nr. Nichols	10/25/05	0.85	0.11	7.73	17.09
D6	Suisun Bay @ Bulls Head nr. Martinez	10/25/05	0.73	0.12	6.08	13.45
D7	Grizzly Bay @ Dophin nr. Suisun Slough	11/21/05	1.17	0.12	9.75	21.56
D8	Suisun Bay off Middle Pt. nr. Nichols	11/22/05	1.08	0.10	10.80	23.88
D6	Suisun Bay @ Bulls Head nr. Martinez	11/22/05	0.94	0.15	6.27	13.86
D7	Grizzly Bay @ Dophin nr. Suisun Slough	12/21/05	1.02	0.10	10.20	22.56
D8	Suisun Bay off Middle Pt. nr. Nichols	12/22/05	1.00	0.10	10.00	22.11
D6	Suisun Bay @ Bulls Head nr. Martinez	12/22/05	1.10	0.13	8.46	18.71
D7	Grizzly Bay @ Dophin nr. Suisun Slough	1/23/06		0.10		
D6	Suisun Bay @ Bulls Head nr. Martinez	1/24/06	0.75	0.09	8.33	18.43
D8	Suisun Bay off Middle Pt. nr. Nichols	1/24/06	0.66	0.08	8.25	18.24
D7	Grizzly Bay @ Dophin nr. Suisun Slough	2/21/06	0.57	0.08	7.13	15.76
D6	Suisun Bay @ Bulls Head nr. Martinez	2/22/06	0.70	0.07	10.00	22.11
D8	Suisun Bay off Middle Pt. nr. Nichols	2/22/06	0.51	0.06	8.50	18.80
D7	Grizzly Bay @ Dophin nr. Suisun Slough	3/22/06	0.43	0.04	10.75	23.77
D8	Suisun Bay off Middle Pt. nr. Nichols	3/23/06	0.49	0.04	12.25	27.09
D6	Suisun Bay @ Bulls Head nr. Martinez	3/23/06	0.57	0.07	8.14	18.01
D7	Grizzly Bay @ Dophin nr. Suisun Slough	4/21/06	0.43	0.08	5.38	11.89
D6	Suisun Bay @ Bulls Head nr. Martinez	4/24/06	0.54	0.04	13.50	29.85
D8	Suisun Bay off Middle Pt. nr. Nichols	4/24/06	0.45	0.07	6.43	14.22
D7	Grizzly Bay @ Dophin nr. Suisun Slough	5/19/06	0.54	0.08	6.75	14.93
D6	Suisun Bay @ Bulls Head nr. Martinez	5/22/06	0.38	0.05	7.60	16.81
D8	Suisun Bay off Middle Pt. nr. Nichols	5/22/06	0.45	0.06	7.50	16.59
D6	Suisun Bay @ Bulls Head nr. Martinez	6/26/06	0.57	0.08	7.13	15.76
D8	Suisun Bay off Middle Pt. nr. Nichols	6/26/06	0.46	0.10	4.60	10.17
D7	Grizzly Bay @ Dophin nr. Suisun Slough	6/27/06	0.57	0.13	4.38	9.70
D7	Grizzly Bay @ Dophin nr. Suisun Slough	7/17/06	0.71	0.10	7.10	15.70
D6	Suisun Bay @ Bulls Head nr. Martinez	7/18/06	0.72	0.10	7.20	15.92
D8	Suisun Bay off Middle Pt. nr. Nichols	7/18/06	0.54	0.10	5.40	11.94
D6	Suisun Bay @ Bulls Head nr. Martinez	8/16/06	0.58	0.10	5.80	12.83
D8	Suisun Bay off Middle Pt. nr. Nichols	8/16/06	0.45	0.08	5.63	12.44
D7	Grizzly Bay @ Dophin nr. Suisun Slough	8/18/06	0.66	0.27	2.44	5.41
D7	Grizzly Bay @ Dophin nr. Suisun Slough	9/13/06	0.62	0.10	6.20	13.71
D8	Suisun Bay off Middle Pt. nr. Nichols	9/14/06	0.70	0.10	7.00	15.48
D6	Suisun Bay @ Bulls Head nr. Martinez	9/14/06	0.64	0.11	5.82	12.87
D7	Grizzly Bay @ Dophin nr. Suisun Slough	10/12/06	0.77	0.11	7.00	15.48

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D8	Suisun Bay off Middle Pt. nr. Nichols	10/16/06	0.77	0.09	8.56	18.92
D6	Suisun Bay @ Bulls Head nr. Martinez	10/16/06	0.79	0.12	6.58	14.56
D7	Grizzly Bay @ Dophin nr. Suisun Slough	11/13/06	0.83	0.09	9.22	20.39
D8	Suisun Bay off Middle Pt. nr. Nichols	11/14/06	0.84	0.06	14.00	30.96
D6	Suisun Bay @ Bulls Head nr. Martinez	11/14/06	0.81	0.10	8.10	17.91
D7	Grizzly Bay @ Dophin nr. Suisun Slough	12/11/06	1.11	0.09	12.33	27.27
D6	Suisun Bay @ Bulls Head nr. Martinez	12/12/06	1.07	0.09	11.89	26.29
D8	Suisun Bay off Middle Pt. nr. Nichols	12/12/06	1.02	0.10	10.20	22.56
D7	Grizzly Bay @ Dophin nr. Suisun Slough	1/12/07	1.57	0.11	14.27	31.56
D8	Suisun Bay off Middle Pt. nr. Nichols	1/16/07	1.16	0.09	12.89	28.50
D6	Suisun Bay @ Bulls Head nr. Martinez	1/17/07	0.97	0.10	9.70	21.45
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