EXCEPRTED FROM SRCSD'S OCTOBER 11, 2010 "Comments and Evidence Regarding Tentative NPDES Permit, Time Schedule Order, and Permitting Options Circulated on September 3, 2010"

II. REQUIREMENT FOR NITRIFICATION-PAGES 16-37

A. The Tentative Permit Improperly Finds That Full Nitrification Is Necessary to Protect Aquatic Life Beneficial Uses in the Delta

The Tentative Permit, in conjunction with Attachment K, proposes to adopt very low final effluent limitations for ammonia, without the consideration of dilution, purportedly to protect aquatic life beneficial uses in the Delta. The low limits proposed would require the District to employ advanced treatment at the SRWTP at a level that would require full nitrification of effluent.

In support of the limits, the Tentative Permit makes a number of statements as to why it is appropriate to require full nitrification. However, the findings in the Tentative Permit are not supported by the evidence and would be improper if adopted as proposed. To the extent the Tentative Permit suggests the limits are proper because they *might* be necessary, this is also improper. The Regional Board may of course, reopen a permit if new information becomes available. But there is no justification for full nitrification on the existing evidence.

1. The Evidence Identified in the Tentative Permit Does Not Substantiate the Hypothesis That Ammonia Impacts Pelagic Organism Decline (POD) Species

Attachment K offers 3 potential connections between ammonia in SRWTP effluent and the pelagic organism decline (POD): (1) inhibition of diatom primary production in the Sacramento River, Suisun Bay, and the Delta; (2) causation of acute and/or chronic toxicity to delta smelt and *P. forbesi*, an important food organism for larval and juvenile fish; and (3) contribution to a shift in the algal community from "nutritious species of diatoms" to "less desirable forms like *Microcystis* (blue-green algae)." These hypotheses have been addressed energetically during the last 3 years by researchers funded by the Regional Board, the Interagency Ecological Program (IEP), CalFed, and several stakeholders. None of the studies completed on these topics justify full nitrification at the SRWTP. In fact, several of the studies that have been completed have essentially eliminated concern in one of these areas (e.g., ammonia toxicity to Delta fish species).

a. The Tentative Permit Identifies Significant Uncertainty Associated with Supposed Ammonia Impacts on the Delta Food Web

The Tentative Permit recognizes the significant uncertainty associated with these hypotheses, and by its own terms calls into question the existence of evidence to support full nitrification. For example, the Tentative Permit includes the following statements:

- "The overall impact of the nitrogen uptake inhibition, particularly on Delta smelt food, is not understood. Inhibition of nitrogen uptake in freshwater portions of the Delta has not been proven." (Tentative Permit Options at p. 4.)

- "The causes of low primary production are not understood . . . A combination of . . . factors . . . may contribute to the low diatom abundance now present in the Bay." (Tentative Permit at p. K-5.)

- ". . . chlorophyll-a concentrations decrease as the Sacramento River flows toward the Delta. The decrease in chlorophyll appears to commence above the SRWTP . . . . The SRWTP discharge cannot be the cause of…decline upstream of the discharge point, and may not be contributing to the decline downstream of the discharge point." (Tentative Permit at p. K-6.)

- "Scientists studying the Delta have not reached a consensus on whether ammonia is either inhibiting diatom primary production or shifting algal communities." (Tentative Permit Options at p. 6.)

- ". . . adverse impacts from changed nitrogen:phosphorus ratios in the Delta have not been demonstrated. The overall impact of nitrogen on the Delta is not understood." (Tentative Permit Options at p. 7.)

- "Follow up studies are needed to determine the ecological effect of the change in nutrient concentrations and ratios on the phytoplankton community . . . ." (Tentative Permit at p. K-7.)

- "Toxicity impacts from ammonia to more sensitive aquatic life, such as copepods, are continuing to be evaluated . . . current findings need to be confirmed before the information can be used to determine that beneficial uses are impacted." (Tentative Permit Options at p. 6.)

b. Independent Reviews and Reports Do Not Conclude That Ammonia Has Contributed to the POD

The theories regarding ammonia's potential role in the Delta (or San Francisco Estuary (SFE)) ecosystem, and the strength of evidence emerging from the research activities, have been subjected to repeated analysis during the last 3 years through independent review panels, focused workshops, and agency reports. Significantly, none of the independent or agency reviews have reached a determination that ammonia has contributed to the POD. Recent proceedings through which the state of science regarding ammonia and the POD has been considered by independent panels or agency staff, include:

- 2009 SWRCB Comprehensive Review of the Bay-Delta Plan, Water Rights and Other Requirements to Protect Fish and Wildlife Beneficial Uses and the Public Trust

- CalFed Science Program Workshop: The development of a research framework to assess the role of ammonia/ammonium on the Sacramento-San Joaquin Delta and Suisun Bay Estuary Ecosystem, March 10-11, 2009

- IEP/Central Valley Regional Water Quality Control Board "Ammonia Summit" (August 18-19, 2009)

- SWRCB Informational Proceeding to Develop Flow Criteria for the Delta Ecosystem (March 22-24, 2010)

- National Academy of Science Committee on Sustainable Water and Environmental Management in the California Bay-Delta (convened January 2010)

- National Center for Ecological Analysis and Synthesis (NCEAS), Santa Barbara, California (ongoing)

  - Project 12198: Potential role of contaminants in declines of pelagic organisms in the upper SFE

  - Project 12192: Ecosystem analysis of pelagic organism declines in the upper SFE

  - Project 12122: Evaluation of declines of pelagic organisms in the upper SFE

- IEP POD Contaminant Work Team (ongoing), and its Ammonia Subcommittee

Excerpts from work products stemming from the above proceedings, and other communications from recognized Delta experts, reveal the state of science surrounding ammonia in the SFE and the insufficiency of evidence available to affirmatively link SRWTP ammonia discharges to POD.

i. Development of Flow Criteria for the Sacramento-San Joaquin Delta Ecosystem, August 3, 2010[1]

The State Board's recent Delta Flow Criteria report was prepared after submittal of written testimony to the State Board by interested parties, followed by a panel of scientists addressing "other stressors." Thus, the State Board Delta Flow Criteria report reflects a present consideration of the best available scientific understanding of stressors on the Delta ecosystem and was derived from extensive input received from the top scientists in the Delta, various concerns and hypotheses were noted regarding ammonia and nitrogen effects in the Delta. The conclusion from this effort is that more study is needed to test the validity of various hypotheses, as reflected in the following excerpts from that report:

> There is concern that a number of non-303(d)-listed contaminants, such as ammonia . . . could also limit biological productivity and impair beneficial uses.

[1] State Water Resources Control Board (2010) Development of Flow Criteria for the Sacramento-San Joaquin Delta Ecosystem. August 3, 2010 (SWRCB (2010)).

> More work is needed to determine their impact on the aquatic community. (SWRCB 2010, pp. 35-36.)
>
> More experiments are needed to evaluate the effect of nutrients, including ammonia, on primary production and species composition in the Sacramento River and Delta. (SWRCB 2010, p. 93.)
>
> Seven-day flow-through bioassays by Werner et al. (2008, 2009) have demonstrated that ammonia concentrations in the Delta are not acutely toxic to delta smelt. Monthly nutrient monitoring by Foe et al. (2010) has demonstrated that ammonia concentrations are below the recommended USEPA (1999) chronic criterion for the protection of juvenile fish. (SWRCB 2010, p. 36.)
>
> Results from the nutrient monitoring suggest that ammonia-induced toxicity to fish is not regularly occurring in the Delta. (SWRCB 2010, p. 36.)

ii. Nutrient Concentrations and Biological Effects in the Sacramento-San Joaquin Delta, Central Valley Regional Water Quality Control Board, June 2010[2]

This report contains a recommendation for future study to "conduct experiments in the Sacramento River below the City of Rio Vista to determine the effect of ammonia and other nutrients on primary production rates and algal species composition." Statements made in the report clearly indicate that effects of ammonia in the Delta have not been established, and that the benefit of reducing levels of ammonia is uncertain.

> The cause of the algal decline [in the Sacramento River] is not known . . . the decline began above the POTW [SRWTP] and continued downstream. (Foe et al. (2010), p. 12.)
>
> No information exists on the effect of ambient ammonia concentrations on algal production downstream of Isleton in the Delta. (Foe et al. (2010), p. 6.)
>
> The study found that ammonia did not inhibit primary production rate measurements in the Sacramento River below the SRWTP when normalized by the amount of chlorophyll present in the bottles. (Foe et al. (2010), pp. 6, 19.)

iii. Regional Board Aquatic Life Issue Paper, April 28, 2010

The Aquatic Life Issue Paper prepared by the Regional Board on April 28, 2010 (p. 7), states:

> . . . many of the key studies (to answer questions relating to SRWTP's discharge, primary productivity and the POD) are not yet complete and will not be available

---

[2] 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). (Foe et al. (2010)).

> in time for consideration by the Central Valley Water Board as part of the SRCSD's NPDES permit renewal. (Aquatic Life Issue Paper, p. 7.)

iv. August 2009 Ammonia Summit Summary [Foe] September 24, 2009[3]

> A combination of the above three factors [ammonia levels that inhibit nitrate uptake by phytoplankton, high filtration rates by *Corbula*, and high turbidity levels] **may explain** the low diatom abundance now present in Suisun Bay. (Foe (2009), p. 2.)

> . . . there is no consensus yet demonstrating that elevated levels of ammonia in the Delta have caused a shift in the algal community from diatoms to less nutritious forms. (Foe (2009), p. 4.)

> . . . no evidence has yet been collected demonstrating that ammonia concentrations are causing beneficial use impairments in the Sacramento River or Delta. (Foe (2009), p. 3.)

Regarding the hypothesis that elevated ammonia levels were responsible for shifting the competitive advantage to blue-green algae such as *Microcystis* in the late summer, "The data collected to date are ambiguous." (Foe (2009), p. 3.)

v. Meyer, Joseph S. et al. (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 the CalFed Science Program, April 13, 2009 (Meyer et al. (2009))

A framework to assess the effects of ammonia in the Delta was developed in 2009 by a panel of independent national experts convened by CalFed. The panel determined that potential drivers for water quality and the structure and function of the Delta ecosystem include climate, water withdrawals, flow modifications, loadings of sediments, nutrients and contaminants, light and food web processes. With regard to the effects of ammonia, the panel stated that alternative hypotheses exist regarding the potential role of ammonium in the Delta, including the hypothesis that: ". . . ammonium enrichment [might not be] a prime factor responsible for . . . trophic and biogeochemical changes [in the Delta]." (Meyer et al. (2009), p. 3.)

The panel report also states that:

> . . . invasions of alien herbivores (e.g. overbite clam (*Corbula amurensis*) and Asian clam (*Corbicula fluminea*) during the past several decades . . . might be the major cause of declining standing stocks of phytoplankton. Finally, export of

---

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

> Delta water, altered hydrologic conditions and temperature increases accompanying recent climate changes might be major physical factors controlling the Bay-Delta estuarine communities (including populations of the POD organisms). (Meyer et al. (2009), p. 3.)

In a document providing comments on the draft panel report and responses to those comments, the subject of the importance of ammonia inhibition in Suisun Bay was directly addressed. In response to a comment by Richard Dugdale that the panel had ignored his unpublished research regarding ammonium suppression of nitrate uptake, the panel stated:

> . . . we do not believe the information provided . . . has conclusively demonstrated that the phenomenon is the dominant driver (i.e. to the exclusion of other factors including grazing and hydrologic variability) of phytoplankton production and composition in the Bay-Delta ecosystem. (Meyer et al. (2009) Responses to Review Comments about Draft Report (dated 20 March 2009): *A Framework for Research Addressing the Role of Ammonia/Ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem* (April 27, 2009) at p. 14.)

vi. Quantifiable Biological Objectives and Flow Criteria for Aquatic and Terrestrial Species of Concern Dependent on the Delta. Draft Report California Department of Fish and Game September 21, 2010 (DFG (2010))

The Delta Reform Act (Senate Bill No. 1 (SB 1) (Stats. 2009 (7th Ex. Sess.) ch 5, § 39)) required the California Department of Fish and Game (DFG) to identify quantifiable biological objectives and flow criteria for the species of concern in the Delta. In its September 2010 draft report, DFG included as a "finding": "1. Ammonia does not appear to be acutely or chronically toxic to delta smelt and other species. More research is needed on the effects of nutrients on Delta ecosystem and its foodweb." (Quantifiable Biological Objectives and Flow Criteria for Aquatic and Terrestrial Species of Concern Dependent on the Delta (Draft Document), DFG (Sept. 21, 2010) at p. 96.)

vii. Dr. Jim Cloern, May 2010 Email

Dr. Jim Cloern, a U.S. Geological Survey (USGS) aquatic ecologist with over 30 years of research experience studying primary productivity and other ecological processes in the San Francisco Bay-Delta, in a May 11, 2010, email to San Francisco Bay Regional Board staff and others (previously provided to the Regional Board), questioned whether the reduced uptake of nitrate associated with ammonium inhibition actually translates into a growth rate effect. Dr. Cloern specifically questioned whether ammonium inhibits primary productivity and the importance of this inhibition (if it is occurring) compared to bivalve grazing. Dr. Cloern referred to the panel report from the March 2009 CalFed independent expert workshop as the basis for this question. More importantly, Dr. Cloern stated in the May 11 email that, "*the scientific community is far from consensus on questions related to the ecological significance of ammonium in the Bay-Delta system*."

viii. Dr. Peggy Lehman, March 2010 Email

Dr. Peggy Lehman, a Department of Water Resources expert in phytoplankton ecology in the Delta (in a March 19, 2010, email to Frances Brewster of the State Water Contractors, previously provided to the Regional Board) stated that her sampling of multiple stations in the San Joaquin River in 2000 and 2001 had shown diatoms were common in those samples, despite ammonium levels above the 4 µM threshold that has been proposed by Dugdale and Parker. Dr. Lehman stated that this suggests that ammonium levels may not necessarily have caused the dramatic loss of diatoms that has occurred in the Delta. She also stated that the influence of ammonium on phytoplankton is very complex.

2. Evidence in the Record Demonstrates That Ammonia Is Not Causing Acute or Chronic Toxicity to Delta Fish

As acknowledged by the Regional Board in Attachment K, ample evidence indicates that ambient ammonia concentrations throughout the upper SFE are not high enough to cause acute toxicity to delta smelt or to the wide range of aquatic organisms explicitly protected by current U.S. EPA ammonia criteria. This characterization of ambient conditions applies not only to "POD" years (2002 onward), but also to the entire 35-year period for which long-term monitoring data are available. The characterization also applies to the entire reach of the Sacramento River below the SRWTP discharge (e.g., River Mile 44 and points downstream).

The U.S. EPA acute criterion for ammonia that applies to water bodies with salmonids was specifically derived to protect rainbow trout. Because repeated rounds of testing indicate that delta smelt have similar acute sensitivity to ammonia as rainbow trout (Werner et al. (2008, 2009)),[4] the U.S. EPA acute criterion is appropriately considered protective of delta smelt. Attachment K references two recent studies which indicate that ambient concentrations of ammonia throughout the estuary, including in the Sacramento River below the SRWTP, meet U.S. EPA ammonia criteria:

- Engle (2010)[5] compared U.S. EPA acute and chronic criteria with ambient ammonia concentrations from almost 12,000 grab samples taken throughout the freshwater and brackish estuary from 1974 to the present. The dataset included monitoring results from the IEP, USGS, DWR, USFWS, the District, and the UC Davis Aquatic Toxicology Lab. In this large dataset, ammonia concentrations *never* exceeded the U.S. EPA acute criterion; the chronic criterion was exceeded *only twice* in the available record (one

[4] Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2008. *The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*. Final Report to the Central Valley Regional Water Quality Control Board. September 26, 2008.

Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009. *Acute toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminant on Delta Smelt - 2009*. Final Report to the Central Valley Regional Water Quality Control Board. December 17, 2009.

[5] 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.

sample each in 1976, 1991). Margins of safety were large: the chronic criterion exceeded ambient concentrations by average factors of 40 and 80, in the brackish and freshwater estuary, respectively.

- Regional Board staff conducted ambient water sampling at 21 sites in the freshwater Delta between March 2009-February 2010 (Foe et al. (2010)).[6] None of their measurements of ammonia exceeded the U.S. EPA acute or chronic criterion. In addition, Regional Board staff screened their ambient data using an ultra-conservative, hypothetical chronic criterion for delta smelt, which they created by using the highest of 3 Acute to Chronic Ratios (ACRs) (20.7, 9.7, 6.5) for fathead minnow contained in U.S. EPA (1999)[7]. Although such use of an ACR of 20.7 conflicts with the U.S. EPA interpretation of fathead minnow data[8], and although U.S. EPA does not use ACRs for single species to derive chronic criteria[9], the hypothetical chronic criterion so derived was not exceeded by any of the ambient concentrations measured in the Regional Board study.

In Attachment K, the Tentative Permit references an opinion expressed by Werner et al. (2008, 2009) that repeated excursions of pH above 8.0 in the Delta equate to a potential for chronic toxicity for delta smelt. This gross generalization is not evaluated using ambient data in Werner et al. (*ibid.*), and does not constitute a valid basis for inferring chronic toxicity in the estuary. Because total ammonia concentrations and water temperature vary widely within pH strata across the estuary, ambient pH alone is an inappropriate basis for gauging whether un-ionized ammonia concentrations are of concern. Plots of pH versus un-ionized ammonia for both the brackish estuary and the freshwater Delta for the years 2000-2010 (SRCSD (2010))[10] indicate that un-ionized ammonia concentrations span the full range of ambient values (low to high) when pH >8.0.

---

[6] 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.

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

[8] U.S. EPA used the geometric mean of all three available ACRs (20.7, 9.7, 6.5) to characterize the acute:chronic sensitivity of fathead minnow (Pimephales), not the highest of the available ACRs (20.7). This was done because U.S. EPA considered the test that yielded the ACRs of 20.7 to be flawed (see U.S. EPA 1999 pp. 53-54). The resulting Genus Mean ACR (GMACR) for fathead minnow is 10.86.

[9] Five GMACRs for fish genera have survived vetting by the U.S. EPA and were published in both the 1999 (see reference above) and 2009 (U.S. EPA, Draft 2009 Update Aquatic Life Ambient Water quality Criteria for Ammonia – Freshwater. EAP-822-D-09-001. December 2009) U.S. EPA ammonia criteria documents (*Pimephales* - 10.86, *Catostomus* - <8.33, *Ictaluris* - 2.712, *Lepomis* - 7.671, *Micropterus* - 7.688). All five GMACRs are used by U.S. EPA in the derivation of the chronic ammonia criterion - not just the GMACR for fathead minnow.

[10] 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 (SRCSD (2010).)

3. Hypothesized Benefits of Ammonia Reduction in Terms of Increased Diatom Biomass in Suisun Bay Are So Uncertain As to Make a Requirement for Full Nitrification Unreasonable

The Tentative Permit alleges that ammonium inhibition of nitrate uptake reduces the frequency of diatom blooms in Suisun Bay. The Tentative Permit provides no direct evidence regarding how often this alleged impact occurs, for how long, why it is a problem, how it affects the food web, or whether it affects fish species—all information needed to describe how it might impair the aquatic life beneficial use. Due to the overwhelming impact of benthic grazing by the invasive clam *Corbula amurensis* on phytoplankton biomass during the summer and fall in Suisun Bay (Alpine & Cloern 1992, Jassby et al. 2002, Kimmerer 2005, Thompson 2000)[11], no serious student of the upper SFE would expect a return of historic summer-fall phytoplankton biomass in the brackish Delta as long as the estuary remains colonized by *Corbula*—regardless of other physical or chemical changes that may occur in the estuary. Consequently, postulated dividends of increased diatom biomass related to ammonia reduction are logically currently constrained primarily to the April-May window, when lower benthic grazing rates, increased water temperature, increased thermal stratification, and other factors occasionally provide windows for bloom development. However, what seems lost from Regional Board discussions about Suisun Bay is that—historically—*the spring period (Apr-May) was never when the bulk of annual phytoplankton biomass occurred in Suisun Bay*. Instead, prior to the arrival of the clam in 1987, June-September were the months of highest mean phytoplankton biomass in Suisun Bay and the confluence zone (SRCSD 2010, Figure 2). Consequently—even if ammonium reductions led to more frequent spring blooms in Suisun Bay—grazing by *Corbula* during summer and fall months will still prevent a recovery of annual algal biomass to levels that occurred in Suisun Bay in the 1970s and early 1980s.

Further, the Tentative Permit overstates the evidence provided by field surveys in Suisun Bay. The Tentative Permit implies that Wilkerson et al. (2006)[12] provides evidence that "ammonia-induced inhibition of nitrate uptake prevents spring algal blooms from developing when conditions are otherwise favorable." (Tentative Permit at p. K-5.) However, no time series data are presented in Wilkerson et al. (2006) regarding several environmental parameters (such as stratification, benthic grazing by clams, zooplankton abundance, residence time, Delta outflow), which could control whether or not conditions are "favorable" for blooms. In the time series of Wilkerson et al. (2006), algal blooms occurred only twice out of five periods when ammonium concentrations fell below 4 µM. This amply illustrates that other factors frequently prevent

[11] 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., Cloern J.E., Cole B.E. (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.

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

blooms in Suisun Bay even when ammonium concentrations are below the "Dugdale" threshold. In fact, because drawdown of ammonium has been documented by Wilkerson et al. during the onset of blooms, 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*. 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 is mentioned in the Tentative Permit and which has not been made available in a public report, but which was presented at the Bay-Delta Science Conference September 27-29, 2010.[13]

4. The Evidence Identified in the Tentative Permit Does Not Support That Ammonia Causes a Decrease in Chlorophyll-a or Changes the Phytoplankton Composition Downstream from the SRWTP

Despite the momentum that ammonium-inhibition has gained in the debate about ammonia's potential role in the Delta ecosystem, many dire predictions based on the ammonium-inhibition theory (and other ammonia/algae hypotheses) have been contradicted by results from recent studies funded by the Department of Water Resources (DWR), CalFed, the Regional Board, and the State Water Contractors. Unsubstantiated predictions include (1) chlorophyll-a production would be lower and slower in river water below the discharge, compared to above the discharge, (2) the SRWTP discharge would trigger a change in the relative biomass of large (diatom) phytoplankton in the Sacramento River, (3) biomass of phytoplankton would not increase in the river in reaches where ammonium uptake exceeded nitrate uptake, and (4) ammonia concentrations would explain the occurrence of *Microcystis*. In addition, in Attachment K, the Tentative Permit does not place ammonia-related hypotheses in context with other well-regarded hypotheses for recent changes in the biomass or composition of phytoplankton in the upper estuary. Evidence that contradicts the predictions include the following:

- During 4-6-day experiments by Parker et al. (2010)[14], phytoplankton grew better in water collected at RM-44 below the SRWTP discharge than they did in water collected above the discharge, despite the fact that ammonium concentrations at RM-44 were well above the "Dugdale threshold" of 4 μM[15]. Although the detailed time courses for these "grow out" experiments (Nov. 2008, and March and May 2009), were not included in Parker et al. (2010) Final Report to the Regional Board, several were included in Parker's oral presentation at the Regional Board Ammonia Summit (slides 9-10 in Parker et al. 2009)[16].

[13] Marchi et al. (unpublished data presented on September 29, 2010).

[14] 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.

[15] 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 (Table 19-22 in Parker et al. (2010).

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

In all three months, phytoplankton growth was not delayed in water from RM-44 and there was more chlorophyll-a at the end of the experiments in water from RM-44 than in water collected above the discharge. These results led Parker et al. (2010) 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.)

- The SRWTP discharge does not explain the longitudinal decrease in phytoplankton biomass and primary production rates *which starts above the discharge* in the Sacramento River and extends downstream past the discharge. 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))[17] and Parker et al. (2010))[18] in 2008-2009. Both studies revealed that although chlorophyll-a consistently declined in the downstream direction from the I-80 above Sacramento to Rio Vista, no step decline was associated with the SRWTP discharge. In addition, the Parker et al. study indicated that the SRWTP discharge did not differentially affect small (<5 µm) versus larger (>5 µm) phytoplankton, the latter considered a proxy for diatoms.

- The Tentative Permit acknowledges 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 <u>a</u> 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*

---

[17] 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.

[18] 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.

the discharge point, and may not be contributing to the decline downstream of the discharge point. (Tentative Permit, p. K-6.)

- Longitudinal transects by the Parker/Dugdale team during their 2008-2009 Sacramento River project included rate measurements (uptake of carbon, ammonia and nitrate) at 21 stations from I-80 above Sacramento downstream into Suisun Bay (see Attachment 4 in Engle Testimony (2010)[19]. These rate measurements, which were not included in the Parker et al. (2010) report to the Regional Board[20], show that primary production rates were not explained by relative rates of ammonium- versus nitrate uptake.

    - No step-change in phytoplankton biomass or carbon fixation rates was associated with either (1) the location of the SRWTP discharge, or (2) the shift from primarily nitrate uptake by phytoplankton to primarily ammonia uptake below the discharge. Carbon fixation rates decreased *starting upstream* of the SRWTP, despite the fact that nitrate dominated N uptake in that reach of the river.

    - Significant *increases* in phytoplankton concentration (chlorophyll-a) and carbon fixation occurred between Rio Vista and Suisun Bay, although inorganic nitrogen uptake was dominated by ammonium in that reach.

- Attachment K implies that *Microcystis* is a "less desirable form" of algae that may be associated with ammonia from the SRWTP. However, available research from the Delta —which is not referenced in the Tentative Permit—argues against a simplistic association between *Microcystis* and nutrient form or concentration. Studies conducted by Lehman et al. (2008, 2010)[21] and Mioni (2010)[22] in the Delta 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. An association between water temperature and *Microcystis* blooms in the Delta is supported by the upward trend in spring-summer mean water temperature

---

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

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

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

[22] 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.

in the freshwater Delta between 1996-2005 (Jassby (2008))[23] 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 blooms (Pearl et al. (2009), Pearl & Huisman (2008), Fernald et al. (2007)).[24]

- The Tentative Permit omits information that *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. 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 (Pearl et al. (2006)).[25] The role of flow and residence time in regulating estuarine microfloral 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).)[26]

---

[23] 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.

[24] 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.

Paerl, 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.

[25] 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.

[26] 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.

The idea that flows influence diatom abundance is not new in the Delta. Lehman (1996, 2000)[27] associated a multi-decadal decrease in the proportional biomass of diatoms in the Delta and Suisun Bay to climatic influences on river flow. The 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))[28].

- Top-down effects on phytoplankton composition - caused by selective grazing by clams and zooplankton - are not acknowledged in the Tentative Permit, but are likely to influence the species composition of phytoplankton in the SFE, and may contribute to the occurrence of *Microcystis*. Clam grazing selectively removes larger particles from the water column (Werner & Hollibaugh (1993))[29]; clams may consume a larger fraction of diatoms than smaller plankton taxa such as flagellates. Kimmerer (2005)[30] attributed a step decrease in annual silica uptake after 1986 to efficient removal of diatoms by *Corbula 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))[31]; 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*

---

[27] 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.

[28] 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.

[29] 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.

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

[31] 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

> *Vanderploeg et al. (2001) reported for zebra mussels (Dreissena polymorpha) in the Great Lakes.* (Meyer et al. (2009).)[32]

In addition to 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)).[33]

- The Tentative Permit echoes a hypothesis advanced in (Glibert 2010) that changes in N:P or ammonia:nitrate ratios are responsible for the observed shift in the Delta phytoplankton community from an assemblage historically dominated by diatoms to one that is now dominated by flagellates and blue-green algae. Unfortunately, Glibert's conclusions are not based on direct experimental evidence of differential phytoplankton responses to nutrient ratios in the San Francisco Estuary (SFE). Instead, Glibert arrives at her conclusions using an improperly applied statistical transformation (cumulative sums of variability, or CUSUM) to produce artificial correlations between nutrient parameters and biological parameters (phytoplankton, zooplankton, fish abundance). Glibert's approach was analytically and conceptually flawed, as detailed in Engle & Suverkropp (2010):[34]

> The correlation approach used by Glibert (using CUSUM values instead of measured values) violated assumptions for linear regression, and can produce spurious relationships between variables that are unsupported by the underlying data. Although she analyzed chemical and plankton data from only one station in the freshwater Delta (Sacramento River at Hood), and two stations in Suisun Bay, Glibert generalizes her results to the whole of the upper San Francisco Estuary SFE. Although they are not well articulated in the article, a number of problematic ecological assumptions are required to infer cause and effect from her correlation analysis. Key analyses that are necessary to support her conceptual model are missing from the publication. Many well-known alternative hypotheses for the observed changes in plankton composition and fish abundance in the SFE (and in estuaries, generally)—which would have been testable using her CUSUM methodology—were omitted from the analysis and from discussion in the article. Finally, owing to the

---

[32] 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.

[33] 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.

[34] 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.

> 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) could be shown as highly correlated with pelagic fish abundance using CUSUM correlations. As an example included in Section 1 of this memo, it is shown 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. (Engle & Suverkropp (2010).)

After referencing Glibert's (2010)[35] hypothesis regarding bottom-up effects on algal composition, the Tentative Permit acknowledges "*whether this [shift in algal communities] is the result of changes in nutrient concentrations and/or ratios is not known*." (Attachment K at p. K-7.) Attachment K additionally acknowledges that additional studies are in fact necessary to determine if nutrient control would actually "*cause the community to revert back to diatom-based system*."

5. The Tentative Permit Does Not Present Evidence That a Shift in Phytoplankton Composition in the Estuary Represents a Degradation of Food Resources at the Bottom of the Food Web

The Tentative Permit references a shift in phytoplankton composition that has been observed in the upper SFE (the brackish and freshwater Delta), characterized by a decline in the relative abundance of diatoms and an increase in other taxa including flagellates, green algae, and cyanobacteria. A required assumption for linking ammonium inhibition to the POD is that these changes in phytoplankton composition signal a deterioration in the quality of food for estuarine mesozooplankton, and calanoid copepods in particular, that may have repercussions for pelagic fish which eat them. For example, the Tentative Permit parrots a common claim that large diatoms are better food for SFE zooplankton than other classes or sizes of phytoplankton. However, there is no direct evidence in the Tentative Permit to support this supposition. In fact, with the exception of the recent occurrence of the toxic alga *Microcystis*, there is currently little basis for the assumption that the observed shift in phytoplankton composition is a negative development for the key copepods which are prey for POD fishes, or for other zooplankton in the estuary. At least six lines of evidence challenge the simplistic diatom -> copepod -> fish "paradigm" that is used to justify much of the attention regarding ammonia and the SFE food web:

1. Feeding experiments conducted in the SFE 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)[36], Bouley & Kimmmer (2006), Gifford et al.

---

[35] Glibert, Patricia M. Long-term Changes in Nutrient Loading and Stoichiometry and Their Relationship with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Bay Estuary, California.

[36] Bollens, Gretchn C. Rollwagen, Penry, Deborah L. Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA).

(2007))[37]. In other words, these copepods do not rely on diatoms—or even on phytoplankton—as a direct food source, and frequently discriminate against phytoplankton altogether (even during diatom blooms) depending on season and location in the estuary.

2. The reproductive implications of food choices is virtually unstudied for the copepods of the SFE. For example, a recent review of almost 400 research articles revealed that only three published studies measured egg production or hatching success for SFE-pertinent copepod species fed mixtures of diatoms and non-diatoms (Engle Slides (2010))[38]. In other words, there is essentially no direct evidence that changes in phytoplankton composition in the estuary have population-level consequences for copepods.

3. Non-diatom classes of phytoplankton include species which are considered highly nutritious for zooplankton. Examples are the cryptophytes (which include *Cryptomonas* and *Rhodomonas* spp.) and *Scenedesmus* spp. (a green alage), which are both used to rear zooplankton in laboratories.

---

[37] Bouley, P. & Kimmerer, W. J. (2006) Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. *Marine Ecology-Progress Series,* **324,** 219-228.

Gifford, S. M., Rollwagen-Bollens, G. & Bollens, S. M. (2007) Mesozooplankton omnivory in the upper San Francisco estuary. *Marine Ecology-Progress Series,* **348,** 33-46.

[38] 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.

4. The Tentative Permit does not acknowledge that a large body of literature exists indicating that direct feeding on diatoms can cause reproductive failure in copepods (see Ianora & Miralto (2010), and references therein)[39]. This potential harmful effect of diatoms on copepods, first described in the early 1990s, prompted a re-evaluation of the classic paradigm that "diatoms-beget-copepods-beget-fish" that continues today. There are at least 24 recent experiments indicating harmful effects of diatom grazing for copepod species pertinent to the SFE (i.e., SFE species and their cofamilials; Engle Slides (2010)[40]).

5. Chlorophyll-a levels below 10 µg/L are frequently cited as evidence that zooplankton in the Delta are food limited (Muller-Solger et al. (2002))[41]. However, this threshold is based on growth experiments conducted with a single cladoceran zooplankton species (*Daphnia magna*) and it is unclear whether this threshold is appropriately applied to copepods in this system.

6. 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))[42]

---

[39] Ianora, A. & Miralto (2010) A. Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology,* **19,** 493-511.

[40] 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.

[41] 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.

[42] 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.

6. The Copepod Toxicity Tests Referenced in the Tentative Permit Are an Improper Basis for Requiring Full Nitrification

The Tentative Permit relies on an oral presentation by Teh et al. (2009)[43], given at the Regional Board's Ammonia Summit, regarding acute toxicity tests with the copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* to allege that the U.S. EPA acute criterion for ammonia may not be protective of these invertebrates. However, none of the LC50s reported in Teh et al. (2009) for either copepod species exceeded the U.S. EPA acute criterion for ammonia.[44] Furthermore, the data referenced in the 2009 oral presentation for *P. forbesi* have never appeared in a draft or final report, and consequently have not been subject to stakeholder or peer review.

Attachment K implies that an "ACR analysis" included by Teh in his 2009 oral presentation provides an indication of potential ambient chronic toxicity for copepods. (Tentative Permit at p. K-2.) In Teh's ACR approach, the LC50s from his lowest test pH (7.2) were divided by a mean ACR from U.S. EPA (1999) to yield a hypothetical chronic criterion for the 2 copepod species. However, as explained in Engle Memorandum (2010)[45], use of the lowest test pH (which was not representative of ambient pH in the brackish or freshwater Delta) biased the analysis. When the LC50s from exposures at *environmentally relevant* test pH (7.6)[46] are used in an analogous ACR analysis, the resulting hypothetical chronic criteria for the two copepod species was exceeded by only 4 out of 2487 measurements of un-ionized ammonia from the upper SFE during the last decade (Engle Memorandum (2010)).[47]

Allegations based on Teh et al. (2009) that Sacramento River water below the discharge contains ammonium concentrations that can cause mortality to either *E. affinis and P. forbesi* rely on test results using misrepresentative pH. Regarding the experiments described by Teh et al. (2009), the Tentative Permit mentions that ten percent mortality occurred to both *E. affinis* and *P. forbesi* at ambient concentrations present in the river below the SRWTP. By doing so, the Tentative Permit disregards qualification of these particular results in the Regional Board staff's Summary of the Ammonia Summit. In this summary, Foe (2009)[48] acknowledged 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:

---

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

[44] In Teh et al. (2009) LC50s (as total ammonia) for *E. affinis* ranged 7.56-10.97 mg N/L and for *P. forbesi* ranged 5.87-7.68 mg N/L [ranges reflect tests at different pH]; the USEPA acute criterion is 11.4 mg N/L at the representative pH 7.6.

[45] Engle, D. (2010) Memorandum: Comments Regarding the Regional Board Staff Analysis of the 2009 Ammonia Summit. 20 pp. January 13, 2010.

[46] Based on IEP, USGS, and DWR monitoring data for the period 2000-2010, the median and mean pH for the brackish delta are 7.6 and 7.7, respectively, and the median and mean pH for the freshwater Delta were both 7.6 (Engle 2010).

[47] Engle, D. (2010) Memorandum: Comments Regarding the Regional Board Staff Analysis of the 2009 Ammonia Summit. 20 pp. January 13, 2010.

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

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

When environmentally representative pH is considered, test results using *E. affinis* do not indicate a potential for acute toxicity in the Sacramento River or the Delta. Acute tests with *E. affinis* referenced in the Teh et al. (2009) oral presentation were described as Appendix A in a progress report for the UC Davis POD project (Reece et al. (2009))[49] and again as chapter IV.3 in Werner et al. (2010)[50]. The LC10[51] for *E. affinis* obtained at the most environmentally relevant test pH used (pH 7.6) was 5.0 mg N/L total ammonia. This concentration (5.0 mg N/L) is about five times higher than the maximum concentrations observed in the Sacramento River from RM-44 and points downstream. This LC10 is higher than the 99.91-% percentile of ammonia concentrations occurring 350 feet below the SRWTP diffuser[52]. *In other words, ambient concentrations of total ammonia in the Sacramento River essentially never exceed the lowest acute thresholds (LC10) thus far reported for E. affinis for representative pH conditions*. The lack of reasonable potential for acute toxicity for *E. affinis* for the rest of the Delta is reflected by long-term monitoring data; in terms of *un-ionized* ammonia, the LC10 for representative pH 7.6 (0.08 mg N/L un-ionized ammonia) is well above the 99th percentile for freshwater concentrations of un-ionized ammonia in the freshwater Delta for 2000-2010 (0.014 mg N/L un-ionized ammonia, Engle Testimony (2010))[53].

The Tentative Permit relies on another oral presentation (Teh et al. (2010)) to infer that ambient concentrations of total ammonia in the Sacramento River potentially cause chronic toxicity to *P. forbesi*. (Tentative Permit at p. K-2.) The oral presentation described the results of preliminary chronic tests (30-day full life cycle tests) using *P. forbesi*, conducted during the summer 2010. In the Tentative Permit, the lowest test concentration from this experiment (0.36 mg N/L total ammonia) is included in the rationale for denying a 60 foot acute and 350 foot chronic mixing zone for ammonia. The exaggerated significance on this experimental outcome is inappropriate for several reasons:

---

[49] Reece, C., D. Markiewicz, L. Deanovic, R. Connon, S. Beggel, M. Stillway, and I. Werner. 2009. *Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delt*a. UC Davis Aquatic Toxicology Laboratory, Progress Report, 29 September 2009.

[50] Werner, I., et al. Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010. (Final Report (July 24, 2010).)

[51] LC10 is the concentration at which it is estimated there is 10% mortality.

[52] 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.

[53] 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.

- The test result concentration (0.36 mg/L) does not represent an EC20 for the species. EC20s are the thresholds used by the U.S. EPA (2009) for derivation of the chronic ammonia criterion.

- The concentration referenced in Attachment K (0.36 mg/L total ammonia) is from weeks-old laboratory work that has not been presented in written form for stakeholder or peer review.

- The tests were conducted with a novel test organism (a copepod species), for which there are no established protocols and no comparable test results from other laboratories.

- There were irregularities, for which the investigators have no explanation, in the test results (the fact that an inverse relationship was observed between toxicity and test pH, which is opposite from the expected responses for organisms included in the U.S. EPA ammonia database).

Finally, by treating a test concentration from the preliminary work with *P. forbesi* as if it were an established, chronic endpoint for the species, the Tentative Permit contradicts its own evaluation of the state of knowledge regarding copepod sensitivity to ammonia:

> *Toxicity impacts from ammonia to more sensitive aquatic life, such as copepods, are continuing to be evaluated and* ***current findings need to be confirmed before the information can be used to determine that beneficial uses are impacted****.* (Tentative Permit Options at p. 6, emphasis added.)

III. Requirement for Denitrification-PAGES 50-53

The Tentative Permit proposes to require full "denitrification." The estimated cost of full denitrification is $780 million. The evidence does not support the requirement. Further, there is no basis to conclude that "full denitrification" would result in compliance with the effluent limitation that is proposed.

A. Scientific Evidence Has Not Been Presented in the Tentative Permit to Justify the Proposed Denitrification Requirements on the Basis of Protecting Aquatic Life Uses in the Delta

The "RPA Results" section of the Tentative Permit (p. F-71) refers to the Primary MCL for nitrate and concludes that reasonable potential exists to cause or contribute to exceedance in the future. However, the "WQBELs" section does not discuss a WQBEL based on the adopted water quality standard. Accordingly, no properly-derived WQBEL is being proposed.

No information has been presented or referenced regarding the positive or negative impact of reducing nitrate in a nitrified SRWTP effluent. The stated rationale for reducing nitrate from SRWTP is to keep the nitrogen to phosphorus (N:P) ratio from changing. (Tentative Permit at p. F-71 [*"There are theories that changing the ratio of nitrogen to phosphorus can change the* *ecology of a waterbody, so removal of nitrogen from the effluent would keep the nitrogen to phosphorus ratio from changing."*].)

In fact, the converse is true. Denitrification of SRWTP effluent would reduce existing N:P ratios in the Sacramento River and Suisun Bay, with unknown consequences. Deviations in atomic TN:TP ratios in water samples from the classic "Redfield Ratio" of 16 (named for the oceanographer who determined in 1934 that the mean atomic N:P ratio of marine phytoplankton is 16 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, but phosphorus limitation is generally not expected unless TN:TP ratios exceed 50 (Guilford & Hecky (2000))[54]. Boynton et al. (2008)[55] show that TN:TP ratios for 34 coastal, estuarine, and lagoon ecosystems trend somewhat above 16. Monthly samples for 3 IEP Suisun Bay monitoring stations for 2002-2007 provides a mean atomic TN:TP ratio of about 17 (16.7; Engle *unpublished* data[56]). This ratio is very close to the classic "Redfield Ratio." Lower ratios would be considered by some investigators as potential indicators of relative nitrogen deficiency in the water column. There are some indications that nutrient limitation shifts between P limitation and N limitation between spring and summer in temperate estuaries (Conley 2000)[57]. Regardless, 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.

At this time, assertions that current N:P ratios in the SFE have driven observed changes in phytoplankton composition are pure speculation. The Tentative Permit implies that Parker et al. (2010) and Glibert (2010) provide some kind of meaningful evidence that would support the hypothesis that current ammonia:nitrate or N:P ratios in the SFE provide a competitive disadvantage to diatoms and a competitive advantage to blue-green algae and flagellates. (Tentative Permit at p. K-6.) However, neither citation refers to direct evidence that nutrient ratios explain changes in phytoplankton composition in the SFE. Setting aside the fact that Glibert's statistical approach was invalid (Engle & Suverkropp (2010)), Glibert omitted correlation analyses between nutrient *ratios* (TN:TP, NO_3:NH_4, or DIN:DIP) and phytoplankton indices (chlorophyll-a or individual taxonomic groups) from her research article. In fact, Glibert's transformed data for NO_3:NH_4 were not compared to *any* of the biological data (phytoplankton, copepods, clams, or fish) in her article. They were only compared to trends in Delta outflow. *As a consequence, the publication did not even make the case (even accepting its flawed statistical approach) that nutrient ratios and phytoplankton composition are statistically related to each other*. In addition, the Glibert article reviews no direct experimental evidence from the SFE or other systems that supports her conclusions regarding nutrient ratios and estuarine phytoplankton composition. The Tentative Permit includes an incomplete footnoted

---

54 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.

55 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.

56 A print-out of the data used for this calculation is provided in the District permit response package.

57 Conley, D.J. (2000) Biogeochemical nutrient cycles and nutrient management strategies. Hydrobiologia 410:87-96.

citation for Parker et al. (2010) that refers to an abstract for an oral talk given by A. Parker on Sept. 28, 2010, at the recent Bay-Delta Science Conference (K. Harder, CVRWQCB, pers. comm.). (Tentative Permit at p. K-6.) However, neither the abstract for the talk nor the oral presentation at the conference provided any information regarding relative growth rates of different phytoplankton taxa presented with different nutrient ratios. In fact, only bulk parameters which apply to the aggregate phytoplankton community (e.g., chlorophyll-a) were described in this presentation. These citations notwithstanding, the Tentative Permit admits to the lack of information for the SFE on this topic. (See Tentative Permit at pp. K-6 - K-7 ["*Dr. Peggy Lehman and T. Brown have documented that the algal community in the Delta has changed from a diatom to a flagellate/blue-green algal dominated community consistent with the predictions of Dugdale et al. and Glibert. Whether this is the result of changes in nutrient concentrations and/or ratio is not known.*"]; see also Tentative Permit Options at p. 7 ["*adverse impacts from changed nitrogen:phosphorus ratios in the Delta have not been demonstrated. The overall impact of . . . nitrogen on the Delta is not understood.*"].) Finally, as discussed in section II.A.4-5, the Tentative Permit does not acknowledge other physical and biological factors which can shift phytoplankton composition in estuaries, nor does it provide evidence that the shift in phytoplankton composition is harming populations of copepods that are prey for POD fishes, or that it has any other significant trophic effects.

Potential negative ramifications of lower N:P ratios, or removing nitrate from a nitrified effluent, do not appear to have been considered in development of the Tentative Permit. 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 seed populations are present (Piehler et al. (2002))[58]; both taxa are present in the upper SFE[59]. A stated concern in the Tentative Permit regarding ammonia levels in SRWTP effluent is that it inhibits uptake of nitrate by phytoplankton in Suisun Bay, resulting in possible unquantified effects on diatom blooms, and (highly speculative) effects at higher trophic levels. Significant concern exists regarding the low productivity of the Delta (Baxter et al. (2007))[60], and currently only a small fraction of in-Delta freshwater phytoplankton production escapes loss processes such as burial, in-Delta grazing, and direct export in water diversions, and is transported into the brackish Delta (confluence zone and Suisun Bay) where the early life stages of POD fishes rear (Jassby et al. (2002))[61]. Because there is evidence from Parker et al. (2010) that Sacramento River phytoplankton are nitrogen-limited upstream from the SRWTP (see section II.A.4), it is reasonable to question whether primary productivity in the Sacramento River would increase following implementation of a full nitrification-denitrification requirement.

---

[58] 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.

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

[60] 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.

[61] Jassby, A.D., Cloern, J.E., Cole, B.E. (2002) Annual primary production: patterns and mechanisms of change in a nutrient-rich tidal estuary. Limnol Oceanogr 47:698-712.

Given (1) the absence of direct evidence in the Tentative Permit that lowering N:P ratios would provide any benefits to the food web, (2) the lack of consideration of possible negative impacts of lowering N:P ratios, (3) the currently low subsidy of phytoplankton biomass provided to the brackish Delta from the freshwater Delta, (4) the possibility that a nitrified discharge would alleviate observed N-limitation for Sacramento River phytoplankton entering the freshwater Delta, and (5) that a tacit goal of the Tentative Permit is to increase access of diatoms to nitrate downstream from the SRWTP, the requirement for denitrification seems somewhat contradictory and has not been justified by ecosystem-related arguments in the Tentative Permit.