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April 21, 2010

Dr. Alex Parker
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Via e-mail to: aeparker@sfsu.edu

Re: Comments on the Draft Final Report, Effect of Ammonium and Wastewater Effluent on Riverine Phytoplankton in the Sacramento River, CA. Parker et al. March 17th 2010

Dear Dr. Parker:

The Sacramento Regional County Sanitation District (SRCSD) is providing comments on the "Effect of Ammonium and Wastewater Effluent on Riverine Phytoplankton in the Sacramento River, CA, Draft Final Report", Parker et al. March 17th 2010. We appreciate the opportunity to comment on this current research in which we were a participating party. We hope you find these comments helpful and useful in revising the Draft Final report.

SRCSD is concerned about the Pelagic Organism Decline (POD) in the Delta, and is working in many different areas to understand the underlying causes of the POD. To date there has not been any definitive research indicating that treated wastewater discharged from SRCSD's treatment plant has had an adverse effect on the Delta. It is important to clarify that this study does not demonstrate any scientific linkage of ammonium in the Sacramento River to the POD, and it may be appropriate to state this in the final report.

SRCSD's primary concern is that it seems premature to conclude from this study that SRCSD's effluent affects primary production rates or ammonium uptake. As detailed in the general and specific comments below, the conclusions about effluent effects are 1) based on a single bench-top test; 2) not consistent with environmental observations or results from grow-out experiments; and, 3) the concentrations where effects may occur are not necessarily indicative of ammonia levels that typically occur in the Sacramento River.

The report clearly indicates that environmental factor(s), unrelated to SRCSD's discharge, apparently affect phytoplankton biomass and carbon uptake throughout an extended reach of the Sacramento River, from I80 to Rio Vista. However, the recommended future research still presumes that SRCSD's discharge is a key environmental factor influencing phytoplankton dynamics in the Sacramento River, even though some of these affects are observed upstream of SRCSDs discharge. As a result, we recommend future research consider nutrient concentrations upstream of SRCSD as potentially limiting the growth of algae in the Sacramento River. Grow out experiments and detailed measurements during river surveys in this study, and analogous survey work by the Central Valley Regional Water Quality Control Board (CVRWQB), do not support the hypothesis that SRWTP's effluent is negatively impacting phytoplankton growth rates or biomass in the North Delta.

The District's comments and recommendations focus on the following key issue:

- Balanced comparison of laboratory data and ambient data

Following are general comments, with specific comments on sections of the report following the general comments.

General Comments

The report focuses on the Sacramento Regional Wastewater Treatment Plant (SRWTP) discharge at the expense of other factors which operate throughout the entire reach of the river surveyed. In Section 6.1 the authors acknowledge that the decline in chlorophyll-a in the Sacramento River (when it is observed) occurs along the full extent of the north-south transect from I80-Rio Vista. This result is consistent with chlorophyll-a data from multiple transects between Tower Bridge in Sacramento and Isleton collected in 2009 by CVRWQCB (Foe et al. 2009, Ammonia Summit presentation)[1]. Surprisingly, two of the hypotheses prompted by this observation (hypotheses 1 and 3, listed in Section 6.1) rely on proposed effluent-related processes that would begin below the SRWTP discharge, as opposed to well upstream at I80. Given that longitudinal transects between I80 and Rio Vista do not show step changes at RM44 in chlorophyll-a, particle size distribution, fluorescence, or carbon uptake - why aren't the investigators focusing on environmental factors which apply to the entire reach surveyed? After all, the report states that phytoplankton populations at RM-44 (below the discharge) are in a physiologically *unimpaired* condition (page 7). At this point, the continuing focus on SRWTP and NH_4 -vs-NO_3 uptake appears to be stimulated by the results of one bench-top experiment using a single day's worth of effluent, but not justified by ambient data collected over several seasons or by the results of several "clean NH_4Cl" experiments to date.

The potential for treated wastewater to affect phytoplankton growth was evaluated in bench-top experiments that - based on ambient data - may not accurately reflect in situ processes. This uncertainty regarding laboratory test results is not well addressed in the report. For example, if phytoplankton growth is impaired in river water containing SRWTP effluent the longitudinal transects should have revealed a step change in chlorophyll-a below the discharge site (rather than steady declines or no decline starting well above the discharge site)- but they did not. Similarly, if

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

diatoms (presumably measured by the chlorophyll-a concentrations in >5 um fraction) are impaired by SRWTP effluent there should be a step change in this parameter below the discharge in transect data-- but there is not. In 3 of 4 survey months, there was no discernable longitudinal pattern in carbon uptake from I-80 to Rio Vista (Nov 08, March 09, May 09), and in the sole transect showing a longitudinal trend (Apr 09) the decrease was monotonic throughout the study reach -- with no step change below the SRWTP discharge site.

As an example of the puzzling continued emphasis on NH_4 and SRWTP, why is it proposed in section 6.4:

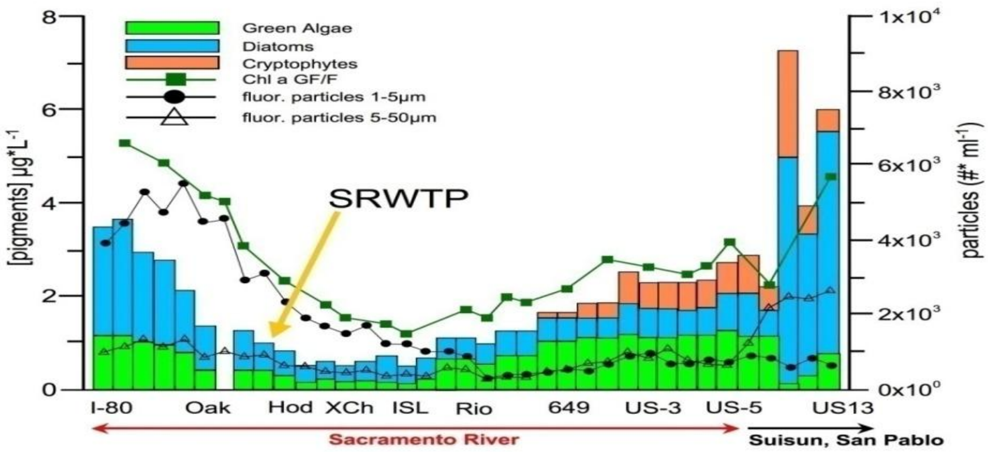
"Experiments need to be made to sequentially address the questions 1) is NH4 the only factor suppressing primary production in the river downstream of SRWTP (RM-44)..."
when none of the results provided in this report indicate that NH_4 suppresses primary production in the Sacramento River - at all? (see grow outs results in Tables 19-22, clean NH_4 addition results in Table 24, clean NH_4 addition results in Table 26, and clean NH4 addition results in Table 27).

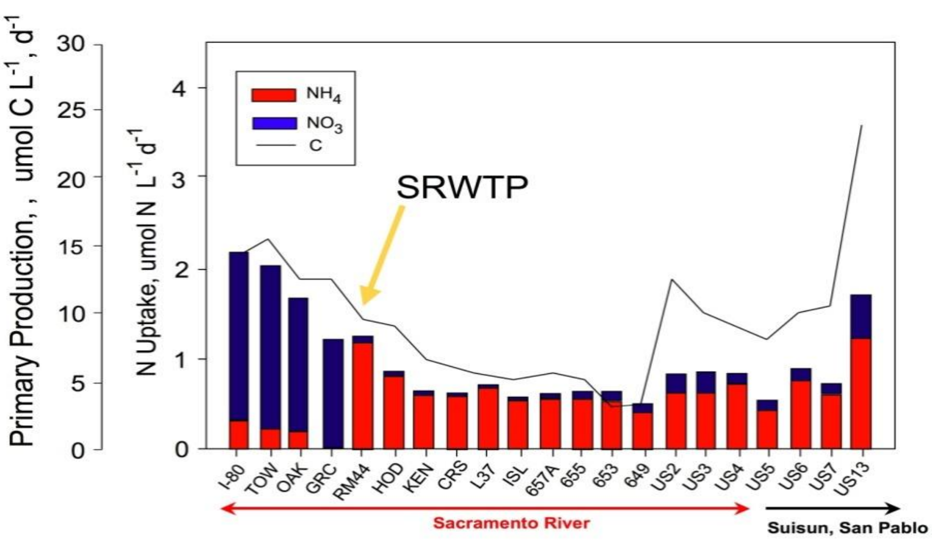
Much of the discussion of study results is crafted to support a perceived dichotomy of upstream- versus downstream-of-SRWTP effects, but longitudinal trends for many parameters extended throughout the study reach. Including the location of the SRWTP discharge on the figures in the report would allow readers to gauge whether or not spatial trends are affected by the location of SRWTP's discharge.

Omitted from discussion in the report are other results by the SFSU team (Parker et al. 2009[2], see figures excerpted below) and CVRWQCB (see slide 8 in Foe et al. 2009 Ammonia Summit presentation) which show that significant increases in phytoplankton abundance and carbon fixation can occur between Rio Vista and Suisun Bay, even when inorganic nitrogen uptake is dominated by ammonium, and even when chlorophyll-a and primary production trend downward between I80 and Isleton. These results are particularly noteworthy given greater importance of the confluence zone as habitat for delta smelt compared to upstream reaches of the Sacramento River proper.

Acknowledgement of these results would support de-emphasis of SRWTP as an explanatory factor for phytoplankton biomass trends in the Sacramento River and the confluence zone, and encourage a more holistic examination of other factors which are known to influence riverine phytoplankton.

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





Figures excerpted from Parker et al. 2009, arrows delineating the Sacramento River and Suisun/San Pablo Bays were added.

Specific Comments

The following comments are referenced to specific sections of the draft report.

Executive Summary

The following passage from the report's introduction omits Jassby's (2008) characterization of recent phytoplankton trends in the Delta:

"Compared to most temperate estuarine systems, primary production and rates of phytoplankton nitrogen uptake are low in Suisun Bay and in the Sacramento River as far upstream as Rio Vista. This has also been documented as a long-term decline in phytoplankton abundance in the San Francisco Bay Estuary (SFE) (Jassby 2008). Jassby et al. (2002) estimated that annual primary productivity in the Delta decreased by 43% between 1975 and 1995." (Parker et al. Draft Report)

This passage leaves out Jassby's (2008) additional finding in the same research article that *"The decline in Delta GPP appears to have halted in 1991–1995"* (p. 16) and that annual productivity in the Delta increased between 1995-2005:

"Regional phytoplankton biomass trends during 1996–2005, however, are positive in the Delta and neutral in Suisun Bay, the two major sub-regions of the upper estuary. The trend in Delta primary productivity is also positive." [Jassby (2008) abstract]

The executive summary concludes that SRWTP discharge has a negative effect on primary production and phytoplankton NH_4 uptake. This conclusion is based on selected laboratory results (the effluent dilution series) but is not supported by ambient data or grow-out experiments. For example, the second paragraph states that phytoplankton NH_4 uptake increased downstream of the wastewater discharge, presumably due to higher NH_4 from SRWTP. This contradicts the above conclusion. The third paragraph states that "Grow-out experiments conducted at RM-44 produced more chlorophyll-a than experimental grow-outs conducted at GRC", an outcome which also contradicts the overall study conclusion.

1.0 Study Background

The study is described as an initial investigation regarding whether the NH_4 and NO3 uptake interactions observed in Suisun Bay also occur in the fresh water Sacramento River. Nowhere in the report is it clearly stated whether the same interactions are occurring or not.

2.0 Study Components/Experimental Design

Clarifying who funded the various studies under this contract would be helpful in addition to clarifying what is meant by the statement that the eight tasks were developed for funding convenience rather than how the experiments were conducted. Perhaps attaching the agreement to the final report would help the reader understand what is meant by the above statement.

4.0 Results

4.1River Characterization

It seems relevant to include some summary statistics for ambient ammonium concentrations in the five (5) sampling events conducted by this study, such as the 95^{th} percentile upper confidence limit of the mean (a conservative estimate of the maximum likely concentration to be encountered in the river). The maximum ammonium concentrations in the Sacramento River observed during each event also provide the reader with a sense of environmental relevance to gauge bench-top experiments that were conducted using concentrations up to 100 uM NH_4.

4.2 River Grow-Out Experiments

Chlorophyll-a accumulations in samples of Sacramento River water from RM44 (below the SRWTP discharge) exceeded those in GRC samples in 3 out of 4 experiments, and were very close (it is not clear if there is a statistically significant difference) to GRC chlorophyll accumulations in the one experiment where they were not exceeded. These results should be used to qualify the results of laboratory experiments where they were contradictory.

Grow-out results are provided only as initial and final concentrations in Tables 19-22. Plots should be provided showing the daily measurements made during the grow-out experiments (such as were shown in earlier project updates and Parker's Ammonia Summit talk). The time series would allow the reader to evaluate the shape of the response (timing of chlorophyll-a increases in relation to nutrient drawdown, variability within treatments between initial and final endpoints, etc.). Some pertinent information from the grow-outs has been lost in the move from figures to tables, such as the observation that phytoplankton growth rates can collapse after nitrate is depleted in GRC water, within the same time frame that NH4 in RM44 water can continue to support phytoplankton growth. Potential benefits of nitrogen additions from SRWTP treated wastewater should be noted.

Note that 96-hour exposures are equal to 4-days, not 5-days as indicated in the table titles (Tables 19 through 22).

4.3 River Water Aging Experiment

The time frame proposed for nitrification in the Executive Summary (7-14 days) is based on a time series of inorganic nutrient concentrations measured in river water obtained on one date, July 21, 2008, and held for eight weeks in carboys. Little should be made of the results of this experiment. Whether or not the composition of the microbial population sampled on this single day is reflective of the Sacramento River generally -- and across seasons -- is unknown. Also, how well do microbial processes in water held in carboys for 8 weeks characterize nitrogen transformations *in situ*?

4.4 NH4Cl Addition Experiment

Clearly this experiment found little to no effect on primary production from NH_4 additions to GRC water. While the addition of NH_4 does seem to restrict NO3 uptake (due to inhibition or preference) this experiment reinforces the finding that NH_4 does not seem to affect algae in the Sacramento River.

4.5 Effluent Addition Experiments

<u>Minor Editorial Comments/Clarifications</u>

"NH_4" and "NO_3" in the column headings of the 1st-4th column in Table 27 refer to the trace addition of $^{15}NH_4$ versus $^{15}NO_3$. Without this additional information, Table 27 is confusing, so perhaps this distinction can be made in the table caption. The units for columns 5-8 should be µmol N, rather than µmol C.

Based on information from the author, the control from the $^{15}NO_3$ addition series is the basis for the statement on page 24: "The difference in ^{13}C-primary production estimates between "GRC+0" effluent-NH_4 and "GRC+100" effluent-NH_4 addition represented a 22-27% decline in primary production." Because the $^{15}NH_4$ series lacked a control measurement for primary production (n.d. in Table 27), this detail should be explained in the text.

On page 24, it is not clear which data fit the Michaelis Menton kinetic uptake relationship at low, but not high, concentrations. Does this refer to both NH_4Cl and effluent-NH_4 treatments, as described earlier in the paragraph? What is meant by low and high?

Data Interpretation and Environmental Relevance of Test Concentrations

It does not seem appropriate to conclude that there are environmental effects from ammonia when clear effects were only observed in lab experiments at concentrations exceeding those that are environmentally relevant. For balance, the authors should address contradictory findings between the lab tests and the ambient data, and to re-focus the data interpretation on effects in the environmentally relevant range rather than at the extreme end of concentrations tested.

Ambient ammonium concentrations collected at locations downstream of SRWTP discharge for this study, at the time of these effluent addition experiments were 32 µM. This concentration exceeds the likely average ammonia concentration in the Sacramento River downstream of SRWTP discharge, which is 25.3 µM, based on the 95th percentile upper confidence limit of the mean (a conservative estimate of the maximum likely concentration to be encountered in the river). Tested concentrations of effluent-ammonium ranged from 0.4 µM to 100 µM, bracketing environmentally relevant concentrations. Tested concentrations above those environmentally relevant (25.3 µM) are not important points of discussion for possible real world effects – yet the effects observed at 100 µM are emphasized throughout the report.

Table 27 shows only slight differences in ^{13}C uptake rates at environmentally relevant concentrations when ammonium is supplied in effluent versus in NH_4Cl. Also, the ^{14}C-based measurements in the effluent addition experiment provide little support for the conclusion that an effluent concentration yielding >8 µM NH_4 in dilution water represents a primary production impairment threshold. ^{14}C-based primary production rates in 2 of the 4 concentrations above 8 µM effluent-NH_4 (+16 µM and +64 µM) were higher than the control. Yet, one of the author's overarching conclusions from the study is that SRWTP effluent has a negative effect on primary productivity in the Sacramento River. The conclusion seems an overstatement, especially given that carbon uptake rates in longitudinal surveys (Figure 6) are inconsistent with the authors' interpretation of the bench-top experiment.

At environmentally relevant concentrations, it is not clear that effluent or NH4 causes any differences in NH4 uptake rates by algae. Maximum NH_4 exceeded 25 µM in only one of 5 river transects – and at those concentrations effluent-ammonium treatments yielded higher specific NH_4 uptake rates greater than the ammonium-only additions (Figure 8). The second highest concentrations of NH_4Cl and effluent-ammonium (~75 µM; shown in Figure 8) produced similar specific NH_4 uptake rates. These data do not support the overall conclusion that effluent-NH_4 and "clean NH_4" produce different ammonium uptake rates. Why were these data points excluded from Figure 9? Given the variability of tests results within the range of environmentally relevant concentrations (i.e., <70 µM) it is inappropriate to make sweeping conclusions based on this single bench-top experiment.

Use of Effluent Concentrations or Dilution Constants as Impairment Thresholds for Unidentified Constituents

The report improperly implies that test results using a single day's worth of effluent should be extrapolated to diluted effluent in the Sacramento River, generally and implies that effluent percentages - or effluent ammonium levels -- associated with particular treatments in one experiment are candidate impairment thresholds. First, the SRWTP permit limit of 14:1 (7.1% effluent) does not represent effluent concentrations that occur in the river as a result of actual plant operations. Based on 7-day running averages for Sacramento River flow at Freeport from 1998-2009, the 99.5th percentile value for percent effluent in the river (based on current discharge 141 mgd) is 2.8% (or 36:1)[3]. More importantly, it is very speculative to assume that (a) an unidentified constituent from a single 24-hr composite sample of effluent occurs frequently in effluent, (b) that the concentration of an unknown constituent would be similar in other effluent samples, (c) that the relative concentrations of ammonia and the unknown constituent are constant in effluent or (d) that the constituent behaves similarly to ammonium in the river once discharged with respect to fate and transport (is it conservative?, does it remain in suspension?, does it become modified during transport?, etc.)

5.0 Discussion

5.2 SRWTP Effect on Phytoplankton N Uptake Rates

As previously mentioned, it would be very helpful for the authors to provide some explanation of the contradictory findings in the ambient data (i.e., uptake rates of C and N in ambient samples; Figure 6) compared to bench-top experiments showing reduced primary production.

Concerns raised over lab results showing negative impacts on primary production and NH_4 uptake by SRWTP effluent-NH_4 >8 µM are not supported by the field data. Perhaps this discrepancy is related to the fact that although NH_4 uptake appears to decrease with higher effluent-NH_4 concentrations in one experiment, the NH_4 uptake rates at 8 µM effluent-NH_4 are still greater than the NH_4 uptake rates at NH_4Cl concentrations up to 100 µM NH_4 (Table 27).

5.3 SRWTP Effect on Primary Production and Phytoplankton Blooms

This section focuses on statements that experimental results showed effluent-NH_4 reduced primary production rates. This was not necessarily true. Table 27 shows primary production in effluent-NH_4 treatments greater than in NH_4Cl treatments at many concentrations tested (i.e., at least up to 16 µM). Concentrations without any adverse effects account for the majority of ambient concentrations below the SRWTP effluent when river transect data were collected on five occasions for this study. Please include a consideration of environmentally relevant concentrations in this discussion rather than focusing on bench-top experiments that do not mimic reality.

It is not clear how 4-hr experiments are sufficient to indicate that phytoplankton are not N-limited in the Sacramento River above the SRWTP discharge. This discussion should consider that it takes days to weeks for water to travel from Sacramento to Suisun Bay and 4-hours is not the relevant timeframe for determining if N is limiting for phytoplankton in transit.

The results of the effluent-addition experiment should be regarded as highly preliminary. This is especially true considering (1) that ambient data reveal no step change in phytoplankton-related parameters downstream of the discharge, and (2) effluent generally has a stimulatory effect in algae toxicity tests that are conducted quarterly by SRCSD. Please also consider including contradictory data in the discussion, for example that many of the tested concentrations in bench-top experiments with 13C showed that primary production was positively affected by effluent-NH4 (Table 27).

On page 28, a hypothesis is developed based on phaeophytin concentrations in river transects that senescence of phytoplankton outweighed new production below GRC:

[3] Mitch Mysliwiec, Larry Walker Associates, unpublished data.

"An alternative hypothesis is that chlorophyll-a was produced mainly at upstream locations with little additional in situ production of chlorophyll-a moving south of GRC. The increase in absolute phaeophytin and the relative increase in phaeophytin compared to chlorophyll-a may reflect the ongoing senescence of active chlorophyll-a with little new production of chlorophyll-a. "

Phaeophytin data from only 2 of the 4 river transects (July 08, April 09) supports this particular hypothesis. The failure of the other two transects (March 09, May 09) to support the hypothesis is attributed by the authors (potentially) to flow (i.e., higher river flows in March 09, May 09 diluted the phaeophytin signal). Wouldn't higher river flows dilute both chlorophyll-a and phaeophytin proportionally if the phaeophytin was derived from chlorophyll-a in cells in suspension?

5.4 Phytoplankton Species Composition Changes

The persistent suggestion that the Dugdale (2007) hypothesis – that diatoms are favored by nitrate-driven systems - explains trends in Sacramento River algae is not well supported by the results of this study. Repeated transect data clearly indicate that an abrupt switch from a nitrate- to an ammonium-dominated nitrogen pool below the SRWTP is not associated with a step change in concentrations of large algae

6.0 Recommendations for Future Study

6.1 Determination of the Underlying Causes for Declining Chlorophyll-a Concentrations From the City of Sacramento to Rio Vista, CA

An acknowledgement of the existing data refuting these hypotheses should be included in this discussion alongside statements describing the data that may support them.

Example 1) the fact that patterns in chlorophyll-a in the >5 um algae fraction in river transects (which includes presumably "nutritious" algae such as diatoms) were not influenced by the location of the SRWTP discharge should be acknowledged here.

Example 2) Hypothesis 1 is biased and not warranted by existing data. The hypothesis suggests that there is no algal growth downstream of SRWTP discharge because algae (1) do not take up nitrate in the presence of ammonium, and (2) ammonium uptake is also impaired (the single bench-top study that potentially demonstrates this is referenced). However, results regarding NH_4 uptake at environmentally relevant concentrations are ambiguous (as previously discussed) and data from grow outs certainly demonstrate that chlorophyll-a accumulates in river water collected downstream of SRWTP while utilizing ammonium.

Given that spatial trends in phytoplankton metrics between I80 and Rio Vista do not predict growth or biomass of phytoplankton between Rio Vista and Suisun Bay -- future transect work should extend through the confluence zone, and not be truncated at Rio Vista.

6.4 Determination of Underlying Causes of SRTWP Effluent Toxicity on Primary Production and Phytoplankton Nitrogen Uptake

It seems premature to conclude from this study that SRWTP effluent affects algae uptake of ammonium. As mentioned elsewhere herein, the authors' conclusion about ammonium uptake is 1) based on a single bench-top test; 2) not consistent with environmental observations or results from grow-outs; and, 3) the concentrations where effects may occur are not necessarily indicative of NH_4 levels "maintained within the Sacramento River." Maximum NH_4 exceeded 25 µM in only one of 5 river transects – and at more representative concentrations effluent-ammonium treatments had NH_4 uptake rates higher than those in NH_4-only additions (Figure 8).

6.X Questions not posed
One additional hypothesis that could be added to the list of possible future studies is: *Nutrient concentrations upstream of SRWTP discharge are potentially limiting to the growth of algae in the Sacramento River.* Potential nutrient limitation, is suggested by the grow out studies wherein ambient nitrate in GRC was depleted after several days – a time scale shorter than transit time for river water through the Delta. Results of a study designed to address this hypothesis would be useful to resource managers balancing water quality concerns.

We hope you find these comments and recommendations helpful for finalizing the report, and that you address our comments in revisions to this draft report before it is forwarded to the POD-CWT for discussion. SRCSD is ready to continue participating in these studies in search of solutions to the POD. Please contact me if you have any questions at 916-876-6030.

Sincerely,

Linda Dorn
Environmental Program Manager

cc: Chris Foe, Central Valley Regional Water Quality Control Board
Stephanie Fong, Central Valley Regional Water Quality Control Board
Terrie Mitchell, Manager Legislative and Regulatory Affairs, SRCSD
Stan Dean, Director of Policy and Planning, SRCSD