Romberg Tiburon Center, SFSU

IEP Study Concepts 2012

Assessment of Wastewater Effluent Effects on Phytoplankton Carbon and Nitrogen Assimilation in the Sacramento – San Joaquin Delta

Alex Parker
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IEP 2012 STUDY CONCEPTS PROPOSAL

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I. Name of the Principal Investigator with affiliation.

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II. Name of Co-Investigator(s), with affiliation(s) and role in proposed project.

Richard C. Dugdale, PhD, RTC, SFSU – Estuarine/River surveys
Frances P. Wilkerson, PhD, RTC, SFSU – Nutrient bioassays

III. Project title. Assessment of Wastewater Effluent Effects on Phytoplankton Carbon and Nitrogen Assimilation in the Sacramento – San Joaquin Delta

IV. Total budget. $227,293 (includes $82,995 in SFCWA match). IEP portion: $144,298

The proposed project has two tasks. SFSU has secured a commitment of funding from the State & Federal Contracts Water Agency (SFCWA) for Task# 1 contingent upon funding provided by the IEP for Task# 2. Attached, please find the letter of support from SFCWA confirming their commitment to the project.

V. Study duration:

One year, beginning January 1, 2013. If these proposal hypotheses are validated and funds are available, a second year of funding will be requested (with separate detailed budget) to follow-up on year 1 results with the specific goal of identifying the class of chemical toxicant within the effluent stream. Future work would be carried out with an additional PI with this specific expertise. The total project duration should not exceed three years of study.

VI. Priority research topic and questions addressed (please refer back to the Call for Study Concepts) and overall relevance.

Topic 2: Physical and Chemical Effects on Fish Populations
Topic 3: Food Web Effects on Fish Populations (Bottom-up Effects)

IEP-Q 2C: What is the distribution of acute and chronic toxicity?
IEP-Q 2D: What is the effect of contaminants on fish food items in the estuarine low salinity zone?
IEP-Q 3A: To what extent are native fish limited by food supply?

VII. Overall project purpose.

The proposed project seeks to address the question: ***Does wastewater treatment plant (WTP) effluent inhibit phytoplankton C (primary production), NO_3, and NH_4 uptake and contribute to diminished food resources for POD species?*** Results will build on previous studies by this laboratory

that has guided permitting of WWTPs by RWQCB Regions II and V, as well as being of national interest. The results may lead to the development of an alternative to *Selenastrum* in the three species bioassay effluent toxicity test (EPA-821-R-02-013). The project will form the basis of an MSc thesis. Funds will be leveraged through match by SFCWA and competitive student fellowships.

VII. Project background and conceptual model.

Wastewater effluent from the Sacramento Regional Wastewater Treatment Plant (SRWTP) has been shown to inhibit phytoplankton carbon (C), ammonium (NH_4) and nitrate (NO_3) (collectively referred to as N) uptake rates in field and laboratory studies carried out in the Sacramento – San Joaquin Delta (Parker et al 2010; 2012a). Based on a combination of transect surveys and multi-day enclosure experiments, the inhibition has been attributed specifically to the elevated NH_4 found in effluent treated to the secondary-level which is known to inhibit phytoplankton NO_3 uptake (e.g. Wilkerson et al 2006; Dugdale et al 2007; Parker et al. 2012a) as well as C uptake (Parker et al. 2012a) throughout the SFE including at stations within the low salinity zone (LSZ). This phenomenon has been referred to within the SFE research community as the "NH_4 hypothesis". Results in support of the "NH_4 hypothesis" have been reported for secondary-level wastewater effluent plumes in coastal southern California (MacIsaac et al. 1979), a Canadian River (Waiser et al. 2011) and in the Delaware Estuary (Yoshiyama and Sharp 2006). Within the Sacramento River, Parker et al (2012c) found that in addition to C and NO_3 suppression, there appeared to be an NH_4 inhibition effect on phytoplankton NH_4 uptake at NH_4 concentrations >20μmol L^{-1} (Fig 1B). This result had not been observed in other SFE studies related to the "NH_4 hypothesis" (but see Yoshiyama and Sharp 2006 for discussion in the Delaware River) but suggested that either NH_4 or some unidentified effluent contaminant (UEC) within the effluent was acting to reduce phytoplankton NH_4 uptake.

As noted by Jassby (2008) and Brooks et al (2012), wastewater treatment plants (WTPs), and not agriculture fertilizer loadings, are the major source of dissolved inorganic nitrogen (DIN) to the SFE Delta and LSZ. Of the WTPs in the Delta roughly 70% are Secondary (NH_4-rich effluent) and 30% are Advanced-secondary (NO_3-rich) (Table 1; Brooks et al. 2012). In addition to large loadings of DIN, especially NH_4, WTPs are sources of contaminants such as herbicides and insecticides, and medications including antibiotics (Loraine and Pettigrove 2006; Brooks et al 2012). While some contaminants, are eliminated during treatment (e.g. pyrethroids are removed during Advanced-secondary treatment) other contaminants (e.g. antibiotics) pass through all treatment processes unchanged. Gradients in DIN species (i.e. NH_4 and NO_3) may provide a tracer of Secondary and Advanced-secondary wastewater and the additional contaminant loadings that are associated with their effluent within the rivers, Delta and the LSZ.

Parker et al (2010) conducted a preliminary laboratory experiment in which additions of a composite SRWTP effluent sample (~2mM NH_4) were made approximating a range of river dilutions of SRWTP effluent to natural phytoplankton collected immediately upstream of the SRWTP outfall (Table 1). A parallel incubation experiment was conducted with additions of laboratory-grade NH_4Cl in order to distinguish whether C and N inhibition effects could be tied directly to NH_4 or whether the inhibition was due to some other constituent in the effluent. The authors found that NO_3 was inhibited in both SRWTP effluent and NH_4Cl addition experiments by >80% at relatively low NH_4 concentrations (~1μM), implying that NH_4 was responsible for NO_3 inhibition (Table 2). However, while additions of SRWTP effluent also resulted in reduced phytoplankton carbon and NH_4 uptake (25 to ~33%; Table 2), NH_4Cl additions did not result in a similar carbon and NH_4 uptake reductions (Table 2; Fig. 2). These

results are equivocal in that rates measurements from several NH_4Cl bottles were lost during analysis, however the results do suggest that a UEC, rather than NH_4, may be causing inhibition of phytoplankton carbon and NH_4 uptake. This single effluent dilution series experiment must be repeated to evaluate the reproducibility of results as well as the magnitude and frequency of occurrence across effluent types.

Recent transect surveys of the San Joaquin River (Fig. 3, Kress et al, *in press*) showed reduced chlorophyll-a (Fig 3A,B), lower phytoplankton abundance (Fig 3C,D), and lower carbon and N uptake rates (data unpublished) near the Stockton Wastewater Treatment Plant (SWTP). SWTP is an Advanced-secondary treatment facility in which effluent-N is predominantly in the form of NO_3, with little NH_4 (Kress et al. *in press*); The reduced phytoplankton biomass observed in the San Joaquin River study also suggests the possibility of a UEC other than NH_4.

The conceptual model describing the potential for an unidentified contaminant influencing phytoplankton C, NH_4 and NO_3 uptake in the SFE Delta ecosystem, as well as its relationship to the existing "NH_4 hypothesis" is illustrated in Figure 4. In this model dissolved inorganic nitrogen in the Sacramento and San Joaquin rivers, SFE Delta and the LSZ is assumed to come from WTPs (Jassby 2008; Brooks et al. 2012). As a result, gradients in NO_3 or NH_4 are established moving away from Advanced-secondary or Secondary WTPs, respectively. Additionally, both types of WTPs are sources of a UEC that reduces phytoplankton C and NH_4 uptake. The result is that phytoplankton experience spatial gradients in NH_4 (high to low concentrations moving from near-field to far-field from secondary WTPs), NO_3 (high to low concentrations moving from near-field to far-field from Advanced-secondary WTPs) and the EUC (high to low concentrations moving from near-field to far-field from either type of WTP) (Fig 4). Previous work on the "NH_4 hypothesis", which did not reveal an inhibition effect of NH_4 on phytoplankton NH_4 uptake, was carried out at locations within the estuary far enough from the near-field of WTPs to avoid high concentrations of the unidentified contaminant. The response of phytoplankton C, NH_4 and NO_3 uptake will be a function of where along these concentration gradients they are found, In position "A" (Fig 4A) phytoplankton experience high NO_3, low NH_4 but high UEC. This results in low phytoplankton NH_4 uptake (NH_4 substrate limitation), and reduced C and NO_3 uptake (due to the WTP contaminant) and low biomass. This is hypothesized to drive the pattern observed in the San Joaquin River (Fig 3; Kress et al. *in press*). In position "B" (Fig. 4B) the phytoplankton experience elevated NO_3, low NH_4 and low UEC; they display elevated NO_3 uptake (as there is no NH_4 inhibition on NO_3 uptake) and elevated C uptake (e.g. Wilkerson et al. 2006; Dugdale et al. 2007; Parker et al. 2012c) and high biomass. This scenario best describes the condition in the Upper Sacramento River or San Pablo Bay (Parker et al. 2012a, 2012c). In position "C" (Fig. 4C), phytoplankton see moderate NH_4, low to moderate NO_3 and UEC. The result is that there is little to no NO_3 uptake due to NH_4 inhibition (Wilkerson et al. 2006; Dugdale et al 2007), and lower C and NH_4 uptake and lower biomass (Dugdale et al. 2007; Parker et al. 2012c). This represents the conditions at station USGS 6 in Suisun Bay as reported by Wilkerson et al. (2006). Finally, in position "D" (Fig. 4D) phytoplankton experience low NO_3, high NH_4 and high UEC. The response of the phytoplankton is no NO_3 uptake (NH_4 inhibition of NO_3 uptake) and greatly reduced C and NH_4 uptake due to inhibition by the UEC. This scenario is representative of the WWTP and Central Sacramento River (Parker et al. 2012a) or station USGS 7 in Suisun Bay (adjacent to the Central Contra Costa Sanitation District Secondary discharge), as reported in Parker et al (2012a). At other locations in the estuary the effect will be a function of the effluent source concentration as well as NH_4 transformation rates (e.g. nitrification or phytoplankton N assimilation) and degradation of the UEC.

While the SFE research community is still refining our understanding of the factors that lead to low primary production and phytoplankton biomass in the LSZ (Kimmerer et al. 2012) (e.g. light-limitation (Cole and Cloern, 1984), clam grazing (Alpine and Cloern, 1992), and nutrient impacts (Dugdale et al. 2007; Glibert et al. 2011)), it is clear the food resources are low, and likely contributing to the pelagic organism decline (Sommer et al. 2007). Recent attention to the potential for nutrient impacts on low phytoplankton biomass has yet to directly consider the potential that other contaminants (UECs) that associated with WTPs, for which elevated N may act as a "tracer" may also be important. This one year proposal seeks to make a preliminary assessment of this potential.

IX. Estimated number of all FESA and ESA-listed fishes that would be captured by the field component of your study. None

X. Project description:

This project is divided into two interrelated and interdependent tasks. **Task #1** is to complete four surveys of the near-field environment at secondary and advanced-secondary WTPs in the Sacramento and San Joaquin rivers, Delta and Suisun Bay. If available, we will include storm water and oil refinery effluent for evaluation. This task will provide a basis for evaluating experimental results obtained in Task #2 to the environment. **Task #2** is to perform a series of short duration nutrient addition bioassays, using NH_4Cl, KNO_3, a composite sample of Secondary wastewater effluent (containing NH_4) and a composite sample of Advanced-secondary wastewater effluent (containing NO_3) to investigate the impact on phytoplankton primary production (carbon uptake), NO_3 uptake and NH_4 uptake. Task #2 is contingent on Task #1 for sample collections of phytoplankton and to ground truth the experimental results.

The guiding **hypothesis** for the project is that an unidentified effluent contaminant (UEC), associated with both Secondary and Advanced-secondary wastewater effluent reduces phytoplankton C, NO_3 and NH_4 uptake and can be attributed to at least some of the reduced phytoplankton rates and standing stocks observed in the Sacramento River (Parker et al, 2012c), San Joaquin River (Kress et al. *in press*) and Suisun Bay (Dugdale et al. 2012). It is not the aim of this one year proposal to identify the UEC. A follow-up study, developed with the specific aim of identifying an UEC would need to be developed with the appropriate expertise. However, using a variety of effluent sources (Secondary and Advanced-secondary treatment) may provide some indication of the class of compound acting as a contaminant (Brooks et al. 2012). Parker will identify an additional PI with expertise in contaminant identification (e.g. TIEs) and develop a follow-up proposal if an effluent effect, independent of an NH_4 effect, is established during the project proposed here.

Given low food resources for POD species, and the recent attention being given to nutrient reduction strategies at WTPs, it is critical to ensure that simple point source reductions will lead to improved growth conditions for phytoplankton in the SFE Delta. Detailed proposed hypotheses, methodological approach and budget for each task are provided below.

TASK 1:

1. Task Title: Near-field surveys of phytoplankton standing stock and physiology at Secondary and Advanced-secondary WTPs.

2. Investigator(s) responsible for carrying out the task

Parker – Task lead
Dugdale – Task lead

3. Specific questions the task seeks to address

Task Questions:

Q1a. Over what spatial and temporal scales does WTP effluent influence the nutrient field within river and Delta habitats as well as Suisun Bay?

Q1b. How do phytoplankton C, NH_4 and NO_3 assimilation rates respond to WTP nutrient loads *in situ*?

Q1c. Are there differences in the above phytoplankton responses under Secondary and Advanced-secondary treatment nutrient loads *in situ*?

Task Hypotheses:

H1a. Distinct gradients in nutrient concentrations will be observable in association with WTP outfalls; high NH_4 will be associated with Secondary treatment WTPs and high NO_3 will be associated with Advanced-secondary WTPs. Nutrient gradients will be modulated seasonally due to freshwater flow and dilution of the WTP load.

H1b. Phytoplankton C, NH_4 and NO_3 uptake rates will all be depressed at stations adjacent to WTP outfalls despite elevated nutrients.

H1c. Phytoplankton C uptake response will be similar (i.e. depressed production) around Secondary and Advanced-secondary WTP outfalls. Phytoplankton NO_3 uptake will be completely inhibited downstream of Secondary WTP outfalls. Phytoplankton NH_4 uptake will be low around Advanced-secondary WTP due to low substrate (NH_4) concentrations.

4. Approach and Methods:

The **objectives** of Task #1 are: 1) to provide the environmental context for which the experimental results obtained through task #2 will be interpreted. 2) to obtain natural phytoplankton communities and Delta water from the environment immediately upstream and downstream of the WTPs for use in task #2 laboratory experiments. Previous surveys like those proposed have been fruitful, showing depressed phytoplankton biomass and rates of phytoplankton C, NH_4 and NO_3 uptake in regions associated with WTP outfalls (Parker et al. 2012c; Kress 2012; Dugdale et al 2012).

We will characterize nutrients and phytoplankton in the river, Delta and Suisun Bay regions of the SFE adjacent to the selected WTPs outfalls (Table 3). A total of four surveys with 10 stations each will be completed during the one year project Station selection will be made under advisement with staff at Regional Water Quality Control Boards Regions II and V, the Bay Area Clean Water Association (BACWA) and the Central Valley Clean Water Association (CVCWA). Two surveys will be completed during spring (a period of relatively high freshwater flow) at Secondary and Advanced-secondary WTPs and two surveys will be completed during the summer (a period with relatively low flow) at Secondary and Advanced-secondary WTPs. Transects will bracket the WTP outfall by sampling upstream and downstream of the point of discharge; in Suisun Bay, this will be achieved by sampling on a single tide and making a longitudinal transit of the discharge point (see Dugdale et al. 2012). At each station, a Seabird Electronics SBE-32 rosette mounted with three 6-L Niskin bottles and fitted with a Seabird SBE-19 plus CTD will allow continuous vertical profiles of salinity, temperature and *in vivo* fluorescence (Wetlabs fluorometer) from the water's surface to approximately 0.5 m off of the bottom. Light attenuation will be assessed using a LiCor PAR sensor mounted on the rosette. Both surface and near bottom samples for nutrients will be collected to ensure that we assess heterogeneity in discharge.

Inorganic nutrient concentrations (NO_3, NO_2, silicate ($Si(OH)_4$), PO_4) will be determined using a Bran and Luebbe AutoAnalyzer II (Whitledge et al. 1981 for NO_3, NO_2 and PO_4; Bran and Luebbe, 1999 for $Si(OH)_4$). NH_4 will be determined by the phenol-hypochlorite colorimetric method of Solorzano (1969) and urea will be measured according to Revilla et al. (2005). Both analyses will be performed using a Hewlett Packard diode array spectrophotometer and 10-cm path length cell. Dissolved inorganic carbon (DIC) concentrations will be measured according to Friederich et al. (2002) using a Monterey Bay Research Institute-clone DIC analyzer with acid-sparging and a LiCor nondispersive infra-red detector (Model 6252).

Extracted chlorophyll-a and phaeophytin will be determined on GF/F filters (for all cells >0.7-µm) and 5-µm pore-size Nucleopore filters (>5.0-µm cell sized phytoplankton) fluorometrically on acetone extracts according to Arar and Collins (1992) using a Turner Designs Model 10 AU fluorometer calibrated with commercially available chlorophyll-a (Sigma Chemical). A limited number of samples will be collected for phytoplankton enumeration and identification by a subcontractor (Alemdar Consulting). Carbon and nitrogen uptake will be estimated using dual-labeled stable isotope tracer techniques (Legendre and Gosselin, 1996; Parker et al 2012c) on surface water samples. Incubations will be conducted over 24-hr under 50% of surface PAR with particulate collection onto 25 mm GF/F filters. Phytoplankton ^{13}C and ^{15}N enrichment will be measured on a Europa 20/20 gas chromatograph – mass spectrometer and transport rates (ρ, µmol L^{-1} d^{-1}) and specific uptake (V, in d^{-1}) will be calculated according to Dugdale and Wilkerson (1986) and Legendre and Gosselin (1996).

5. Interaction with existing monitoring surveys or other studies

This task will complement previous surveys conducted by this laboratory supported by the State Water Contractors, CALFED and the Delta Science Program, and the Interagency Ecological Program. If funded, transects will by synchronized with the proposed work of Dugdale et al (IEP 2012 Concepts – Suisun Sampling). Whenever possible we use stations established by ongoing monitoring programs (USGS Polaris, Department of Water Resources and IEP sampling) to support those efforts. This project will compliment the proposed work of Senn, Kendall and Guerinn (IEP 2012 Concepts – Synthesis and Modeling) to synthesize loads and transformations of N in the Delta**.**

6. Feasibility:

High. Our laboratory has conducted more than 100 surveys within the Sacramento and San Joaquin rivers and the SFE Delta and Suisun Bay since 2005. Our methodology has been optimized for working in the study area. We have produced several publications as a result of these surveys (Wilkerson et al. 2006, Dugdale et al. 2007, 2012, Parker et al., 2012a, 2012b, 2012c, Kress et al. 2012).

7. Deliverables (e.g. report(s), journal publication(s), IEP newsletter article(s), presentation(s)

Final report, one presentation at IEP, EET or other regional conference. Results will be incorporated into an MSc thesis completed at SFSU.

8. Detailed budget: $53,893 + $10,779 IDC (IDC set at 20% by SFCWA).

$35,393 is salary and benefits support for the PIs research technician and student stipend. $10,000 is requested for ship time (*RV Questuary*) and an additional $1,000 for transportation costs (vehicle and fuel charges). $4,000 is requested for disposable supplies and $1,500 is requested for phytoplankton counts with an outside consultant (see "Approach and Methods"). $2,000 is requested for travel to a regional or national meeting (one lab representative).

9. If you are proposing a multi-year study, please give a task schedule. Not applicable – If additional years of study are requested (see "Study Duration") those would be requested with a new scope of work and an additional PI.

10. If applicable, number of FESA and ESA-listed species specimens required by task. Indicate life stage (larval, juvenile and adult) and hatchery or wild origin. Not applicable

TASK 2:

1. Task Title: Parallel "clean"-NH_4, "clean"-NO_3, Secondary (NH_4) effluent and Advanced-secondary (NO_3) effluent nutrient bioassays.

2. Investigator(s) responsible for carrying out the task

Parker – Task lead
Wilkerson – Task lead

3. Specific questions the task seeks to address

Task Questions:

Q2a: Does NH_4 concentration influence rates of phytoplankton C, NH_4 and NO_3 uptake in natural autotrophic assemblages in the SFE and Delta?

Q2b: Does NO_3 concentration influence rates of phytoplankton C, NH_4 and NO_3 uptake in natural autotrophic assemblages in the SFE and Delta?

Q2c. Does secondary (NH_4) effluent concentration influence rates of NH_4 and NO_3 uptake in natural autotrophic assemblages in the SFE and Delta?

Q2d: Does Advanced-secondary effluent concentration influence rates of phytoplankton NH_4 and NO_3 uptake in natural autotrophic assemblages in the SFE and Delta?

Task Hypotheses:

H2a. NH_4 concentrations will: 1) increase C uptake at low N (0-10 μmol L^0) before reaching saturating C uptake. 2) increase NH_4 uptake at low N (0-10 μmol L^0) before reaching saturating uptake. 3) inhibit NO_3 uptake at low concentration (1 μmol L^{+1}).

H2b. NO_3 concentrations will: 1) increase C uptake at low N (0-10 μmol L^{-1}) before reaching saturating C uptake that is higher than achieved at comparable NH_4 concentration. 2) not influence NH_4 uptake at any N concentration. 3) increase NO_3 uptake at low concentration (1-10 μmol L^{+1}) and saturate at higher uptake compared to comparable NH_4 uptake rates,

H2c. Secondary (NH_4) effluent concentrations will: 1) increase C uptake at low N (0-10 μmol L^{-1}), reach saturating C uptake at NH_4 of 10-20 μmol L^{+1}, before inhibiting C uptake at effluent NH_4 > 20 μmol L^{+1}. 2) increase NH_4 uptake at low N (0-10 μmol L^{-1}), reach saturating NH_4 uptake at 10-20 μmol L^{+1} before inhibiting NH_4 uptake at effluent NH_4 > 20 μmol L^{+1}. 3) inhibit NO_3 uptake at low concentration (1 μmol L^{+1}).

H2d: Advanced-secondary (NO_3) effluent concentrations will: 1) increase C uptake at low N (0-10 μmol L^{-1}), reach saturating C uptake at NO_3 of 10-20 μmol L^{+1} and inhibit C uptake at effluent NO_3 > 20 μmol L^{+1}. 2) not influence NH_4 uptake at NO_3 0-20 μmol L^{-1} but inhibit NH_4 uptake at NO_3 >20 μmol L^{+1} 3) increase NO_3 uptake at low N (0-10 μmol L^{-1}), reach saturating NO_3 uptake at NO_3 of 10-20 μmol L^{+1}) before inhibiting NO_3 uptake at effluent NO_3 > 20 μmol L^{+1}.

4. Approach and Methods:

The **objective** of Task #2 is to determine experimentally whether NH_4 or an UEC is responsible for inhibition of NH_4 uptake, NO_3 uptake and carbon uptake by phytoplankton. We will conduct a series of laboratory nutrient bioassays similar to those described in Parker et al (2010). We will use natural phytoplankton collected as part of Task #1, immediately upstream of wastewater inputs (Table 3). Parallel additions of Secondary (high NH_4), Advanced-secondary (low NH_4, high NO_3), and laboratory grade NH_4Cl and KNO_3 will be made at a range of N concentrations (0.1 to 100 μM) to approximate a range of river dilutions (Table 2). The specific WTP effluents that will be tested will be determined under consultation with the RWQCB Regions II and V as well as BACWA and CVCWA.

This experimental protocol follows closely that used in experiments on Los Angeles effluents (MacIsaac et al. 1979). A flow-weighted 24 hour composite of dechlorinated final effluent from Secondary and Advanced-secondary WTPs will be analyzed for inorganic nutrient concentrations prior to experimental additions. Four sets of 20 280-ml incubation bottles will be filled with ambient station

water. To one set of incubation bottles serial additions of secondary effluent will be added To a second set of incubation bottles serial additions of NH_4Cl will be made to parallel the effluent additions (0 to 100 µmol N L^{-1}). The third and forth sets of bottles will receive Advanced-secondary (NO_3) effluent, and KNO_3 additions at parallel concentrations (0 to 100 µmol L^{-1}) isotopic tracers of NO_3, NH_4 and HCO_3 will be made to trace C, NH_4 and NO_3 uptake. Incubations will be conducted for 6-hr at ambient water temperatures and under natural light attenuated to 50% of surface PAR. A limited number of samples will be collected for phytoplankton enumeration and identification by a subcontract (Alemdar Consulting). Detailed methodology is provided under Task #1 description. Resulting transport rates (ρ) and biomass-specific uptake rates (V) (see Dugdale and Wilkerson 1986) will allow for characterization of quantitative relationships between N substrates and carbon, NH_4 and NO_3 uptake (e.g. Michaelis-Menten kinetics) and/or inhibition.

5. Interaction with existing monitoring surveys or other studies:

We will work with the Central Valley Clean Water Association and the Bay Area Clean Water Agencies to acquire wastewater effluent. Additionally, we will inform the Regional Water Quality Control Boards, Regions II and V with our progress and findings (see letters of support from N. Feger (Region II) and C. Foe (Region V).

6. Feasibility: High. This laboratory has conducted a preliminary experiment (Parker et al. 2010) similar to the experiments proposed here. Additionally, F. Wilkerson and R. Dugdale have been involved in issues related to WTPs nutrient loads both locally and abroad; R. Dugdale was a co-author in the experiments conducted in MacIsaac et al. (1979). Parker has contacted BACWA and CVCWA as well as Kurt Ohlinger (Sacramento Regional WTP) to initiate discussion about effluent requests. These requests will be brought to the respective Boards shortly (September 5, in the case of CVCWA) for their consideration. Michael Connor (East Bay Dischargers Authority – See letter of support) has agreed to work with us to select WTP effluents and has agreed to supply secondary-treated effluent to the project.

7. Deliverables (e.g. report(s), journal publication(s), IEP newsletter article(s), presentation(s)

Final report, one presentation at IEP or other regional conference. Results will be incorporated into an MSc thesis completed at SFSU. One manuscript will be submitted for consideration in a peer-reviewed journal.

8. Detailed budget: $97,908 + $46,390 IDC. $77,908 is requested for salary and benefits for the PIs, research technician and graduate student stipend. $12,000 is requested for graduate student tuition. $3,000 is requested for travel to present findings at a regional (2 representatives) or national (1 representative) conference. $4,000 is requested for disposable supplies. $1,000 is requested for phytoplankton identification by an outside consultant.

9. If you are proposing a multi-year study, please give a task schedule: Not applicable – If additional years of study are requested (see "Study Duration") those would be requested with a new scope of work and an additional PI.

10. If applicable, number of FESA and ESA-listed species specimens required by task. Indicate life stage (larval, juvenile and adult) and hatchery or wild origin. Not applicable

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Table 1: Additions of either wastewater effluent or NH_4Cl were made to a series of incubation bottles containing resident phytoplankton collected at Garcia Bend, CA, immediately upstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP) (ambient DIN <2µmol L^{-1}). Additions were made to simulate a range of effluent dilutions in the Sacramento River (from 14:1 (2010 SRWTP permit requirement) to 5000:1. (From Parker et al 2010).

Dilution	NH_4 (µmol L^{-1})
14 : 1	142.86
20 : 1	100.00
32: 1	62.50
64 : 1	31.25
100 : 1	20.00
200 : 1	10.00
400 : 1	5.00
800 : 1	2.50
1600: 1	1.25
5000 : 1	0.40

Table 2: Summary of effluent and NH_4Cl effects on primary production (C uptake), phytoplankton NH_4 uptake and phytoplankton NO_3 uptake, as reported by Parker et al. 2010.

	Process	Effective Concentration	Effect	SRWTP Dilution Equivalent
Effluent	Primary Production	(>8 μmol L^{-1})[2]	22-36% decrease[2]	333 : 1
	NH_4 Uptake	(>4 μmol L^{-1})[2]	29% decrease[2]	333 : 1
	NO_3 Uptake	(≥ 1 μmol L^{-1})[2]	>80% decrease[2]	2000: 1
NH_4	Primary Production	Unclear[1]	Unclear[1]	None
	NH_4 Uptake	(>1 μmol L^{-1})[1] Unclear[2]	34%[1] Unclear[2]	None
	NO_3 Uptake	(≥ 1 μmol L^{-1})[1] (≥ 1 μmol L^{-1})[2]	>80% decrease[1] >80% decrease[2]	2000 : 1
Current SRWTP Dilution				14 : 1

Table 3: Representative wastewater treatment facilities that supply nutrients via effluent loading to the San Francisco Estuary Delta and Suisun Bay. Loading estimates from NPDES permits (except *Michael Conner, personal communication).

Facility	**Treatment level**	**Loading (mgd)**
Sacramento Regional Wastewater Treatment Plant	Secondary	181
Stockton Wastewater Treatment Plant	Advanced-secondary	32
Central Contra Costa Sanitation District	Secondary	54
Delta Diablo	Secondary	13
Fairfield - Suisun	Advanced-secondary	17
East Bay Dischargers Authority	Secondary	66*

Figure 1: Biomass-specific (A) NO_3 and (B) NH_4 uptake versus ammonium concentrations in the Sacramento River. Linear relationship shown in panel (B) for five stations immediately downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP) during two transits made in March (closed circles) and April (open circles) 2009 (from Parker et al. 2012c).

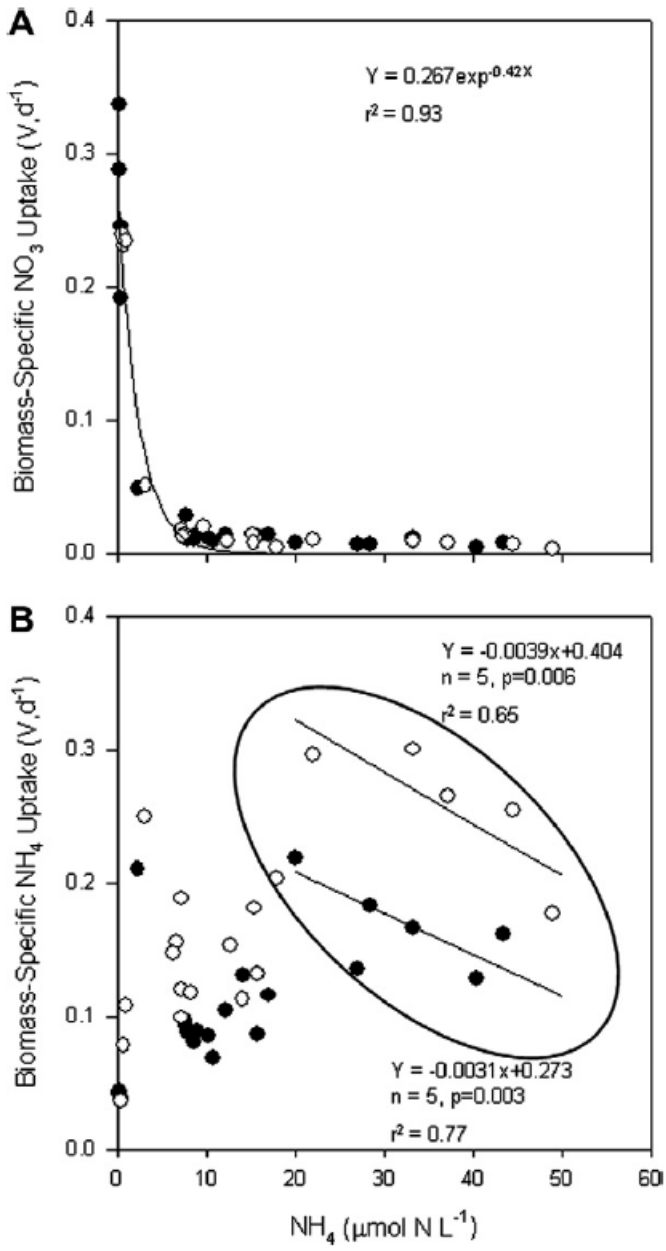


Figure 2: Laboratory experiment completed in April 2010 to investigate NH_4 and wastewater effluent effects on phytoplankton carbon, NO_3 and NH_4 uptake (shown here is impact on biomass specific NH_4 uptake, VNH_4). Phytoplankton were collected at Garcia Bend, on the Sacramento River upstream of the SRWTP (From Parker et al 2010).

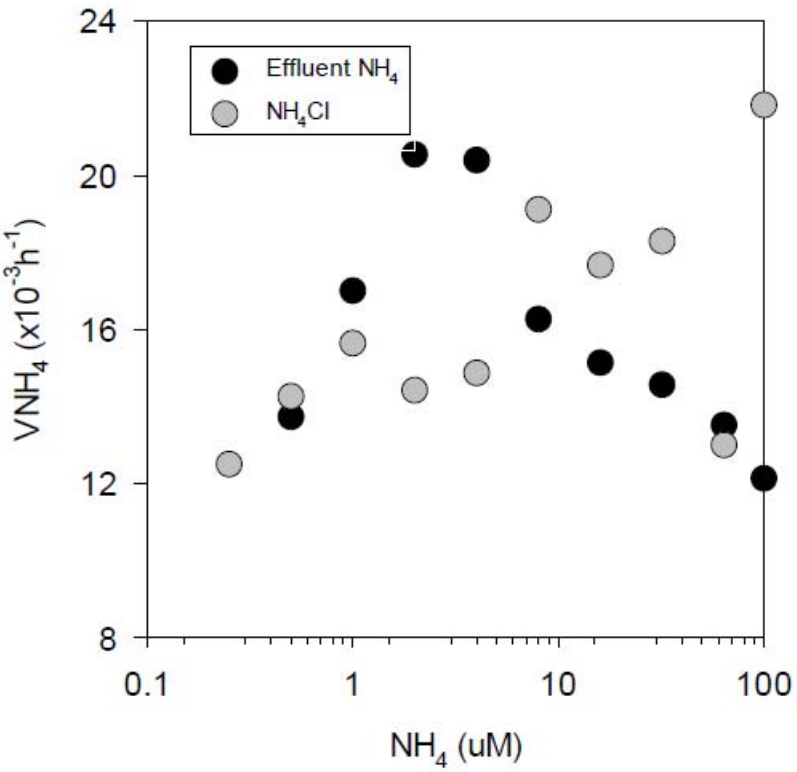


Figure 3: (A, C) Chlorophyll-a concentrations and (B,D) fluorescent particle abundance in the San Joaquin River during April (A,B) and August (C,D) 2010. Shaded area refers to cells >5-µm diameter, open area are cells <5- µm diameter. Lower chlorophyll-a was observed during each survey in the region downstream of the Stockton Wastewater Treatment Plant (SWWTP). STB represents a station in the Stockton Turning Basin that consistently showed elevated chlorophyll-a (from Kress et al., in press).

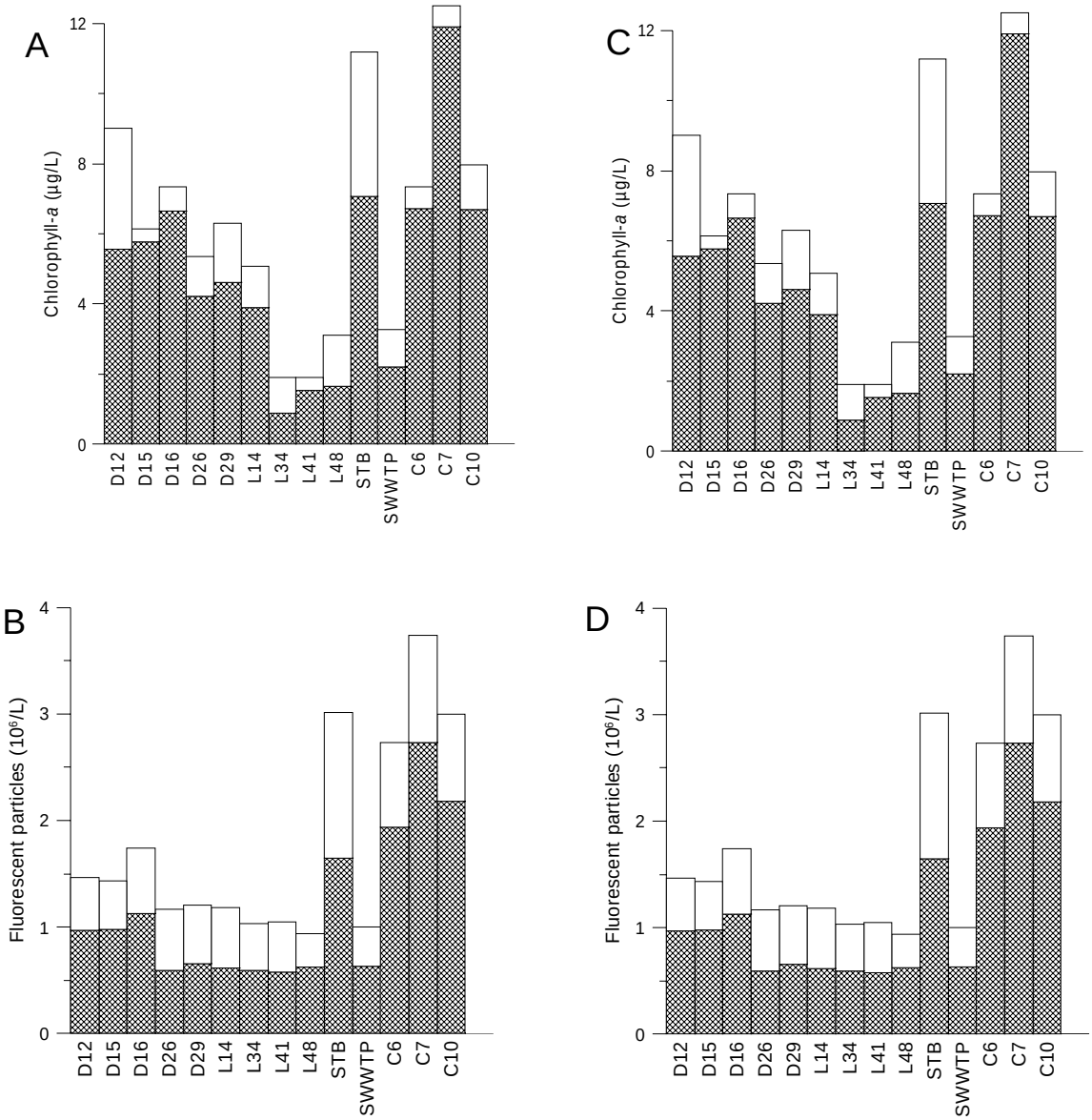
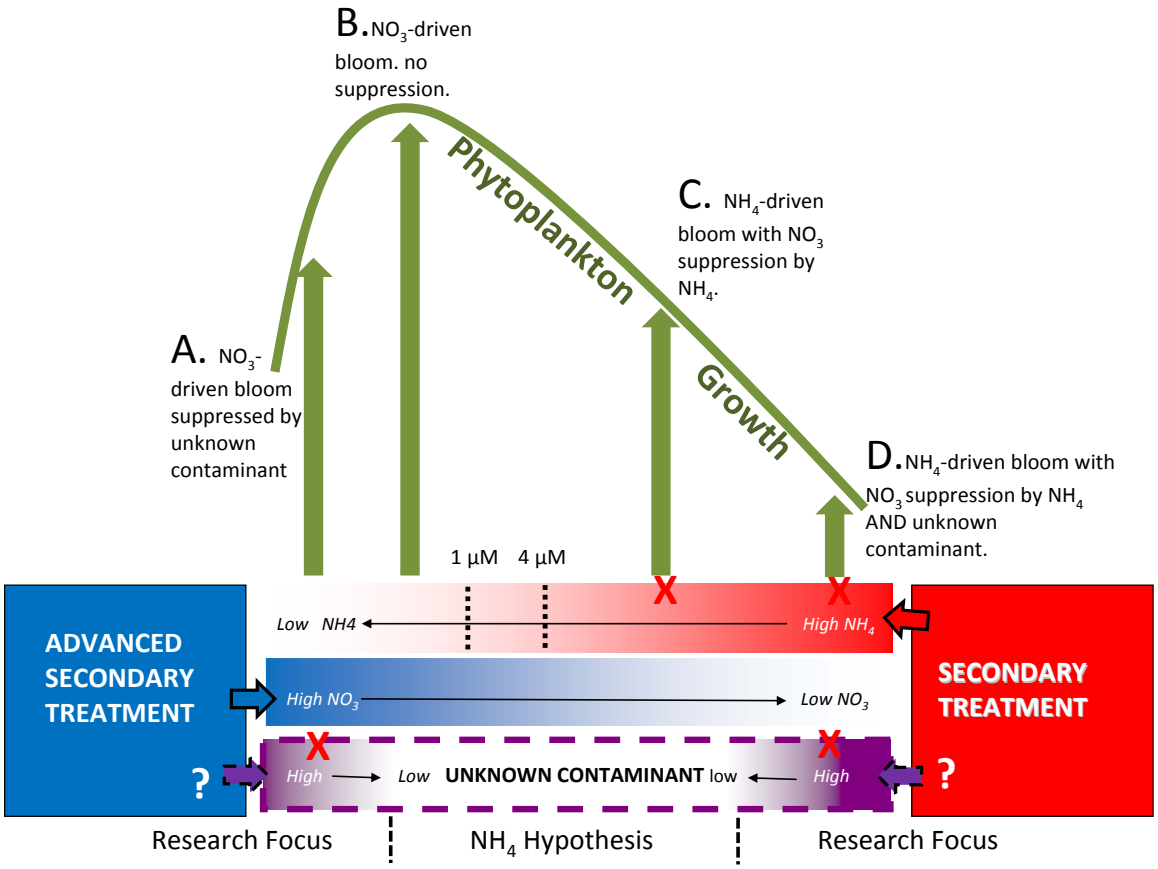


Figure 4: Conceptual model of potential wastewater effluent effects on primary production in the Sacramento and San Joaquin rivers, the San Francisco Delta and San Francisco Estuary. Advanced-secondary WTP releases high NO_3 (blue bar) while secondary WTP release high NH_4 (red bar). We hypothesize that both Secondary and Advanced-secondary WWTP release an unidentified effluent contaminant (UEC) (purple bar). Phytoplankton growth responses: A. High NO_3 and low NH_4 but UEC results in suppressed carbon, NO_3 and NH_4 uptake (San Joaquin River downstream of Stockton WTP). B. High NO_3, low NH_4 and low UEC results in high carbon and NO_3 uptake (e.g. San Pablo Bay) C. Elevated NH_4 but low EUC results in suppression of NO_3 uptake and reduced carbon uptake (Suisun Bay). D. High NH_4 and high UEC results in suppression of NO_3 uptake, reduced NH_4 uptake and low carbon uptake (e.g. Sacramento River downstream of SRWTP). Red crosses indicate points where inhibition of phytoplankton growth (as measured by carbon, NO_3, or NH_4 uptake) occurs.



State & Federal Contractors Water Agency

1121 L Street, Suite 806, Sacramento, CA 95814

August 16, 2012

Alexander E. Parker, PhD
Romberg Tiburon Center
San Francisco State University
3150 Paradise Drive
Tiburon, CA 94920 USA

Dear Dr. Parker,

The State and Federal Contractors Water Agency (SFCWA) fully supports (pending our external scientific review) the research project aimed at evaluating the effect of Secondary and Advanced-secondary wastewater effluent on phytoplankton standing stocks and rates processes in the San Francisco Estuary Delta. SFCWA will provide financial support for Task # 1 In the amount of $53,893 plus up to 20% IDC. This will provide for the field component of the study. SFCWA support is contingent on Task 2 being funded by the IEP or other source.

Also, please note that on August 19, 2010 the State and Federal Contractors Water Agency Board of Directors adopted a policy regarding payment of overhead costs. The policy states that SFCWA will pay overhead amounts up to 20%.

We respectfully request that you lower your overhead rate so that we may continue working with and be in compliance with the SFCWA Board approved policy.

If you have any questions regarding this letter of SFCWA's participation in this project I can be reached at 530 219-9295.

Sincerely,

Valerie M Connor

Valerie Connor, PhD.
Science Manager

EBDA

EAST BAY DISCHARGERS AUTHORITY
2651 Grant Avenue
San Lorenzo, CA 94580-1841
(510) 278-5910
FAX (510) 278-6547

A Joint Powers Public Agency

August 20, 2012

Alexander E. Parker
Romberg Tiburon Center
San Francisco State University
3150 Paradise Drive
Tiburon, CA 94920

Subject: Assessment of Wastewater Effluent Effects on Phytoplankton Carbon and Nitrogen Assimilation in the Sacramento – San Joaquin Delta

Dear Dr. Parker:

I write in support of your IEP study concept proposal "Assessment of Wastewater Effluent Effects on Phytoplankton Carbon and Nitrogen Assimilation in the Sacramento – San Joaquin Delta". I am happy to see that you propose to follow up on your preliminary work, presented at the Bay Delta Conference in 2008, suggesting that some unidentified contaminant, rather than NH4, may be causing inhibition of phytoplankton C and NH4 uptake. I hope that you will consider testing effluent from multiple POTWs, including those with secondary and advanced secondary levels of treatment. I would be happy to provide technical guidance on POTW selection and would be willing to work with you by supplying effluent from East Bay Dischargers Authority.

Your proposed research will help to address unanswered questions related to the potential importance of NH4 and other nutrient reductions by POTWs and the anticipated benefits of such actions.

Sincerely,

Dr. Michael S. Connor
General Manager

CALIFORNIA
Water Boards

EDMUND G. BROWN JR.
GOVERNOR

MATTHEW RODRIQUEZ
SECRETARY FOR
ENVIRONMENTAL PROTECTION

Central Valley Regional Water Quality Control Board

23 August 2012

Kelly Sousa
Anke Mueller-Solger
IEP Management Team

I strongly recommend funding the proposal entitled, "*Assessment of Wastewater Effluent Effects on Phytoplankton Carbon and Nitrogen Assimilation in the Sacramento-San Joaquin Delta*" by Alex Parker. I make this recommendation for two reasons. First, one hypothesis for the pelagic organism collapse in the Delta is the current low primary production rate. I am convinced that increasing primary production will result in a more robust and stable aquatic community. Dr. Parker demonstrated in a double dilution series experiment funded by the Central Valley Regional Board that there was an unknown contaminant, besides ammonia, present in effluent from the Sacramento Regional Wastewater Treatment Plant (SRWTP). The unknown contaminant was of sufficient strength to produce 25-percent suppression in primary production at 0.3-percent effluent mixed into 99.7-percent upstream Sacramento River water. The result is troubling because the sum of all sewage treatment plant discharges in the Delta is 1-2 percent of the total water volume. The unknown contaminant appears to be of sufficient strength, if chemically stable, to cause delta-wide impacts. Other pelagic primary production work conducted downstream in the Sacramento River at Rio Vista is consistent with the presence of an unknown toxicant. The potential presence of an unidentified contaminant needs to be followed up on to (1) determine its magnitude, duration and frequency of occurrence and, if sufficiently toxic, (2) its identity. The Regional Board cannot develop a regulatory program without the identification of the unknown contaminant.

The second reason I support the proposal is because I believe developing a replacement for the *Selenastrum capricornutum* portion of the USEPA three species bioassay procedure may be of national significance. The USEPA recommends that the three species bioassay be used to screen effluents and receiving waters for toxicity. The Central Valley Regional Board has successfully used the invertebrate portion of the bioassay procedure to identify and regulate toxic pesticide applications on rice, orchards and alfalfa, the fish bioassay for ammonia from sewage treatment plants, and the invertebrate and fish bioassays for metal toxicity from mines. The *Selenastrum* portion of the three species bioassay has been less useful. In part this is because *Selenastrum* is a green alga and the more common and ecologically important species in the Delta are diatoms. Diatoms and green alga are phylogenetically very different and may not respond to the same toxicants. The *Selenastrum* test did not detect toxicity from the unknown contaminant in either the Sacramento River or in SRWTP effluent. Modifying the three species bioassay to use the ambient upstream algal community instead of amending a filtered sample with *Selenastrum* may be a more ecologically relevant screening tool. Funding this proposal would be an initial test of that concept.

In conclusion, I recommend fully funding the proposal by Dr. Alex Parker. Please feel free to call me if you have any questions (916-661-2259).

Sincerely,

Christopher Foe, PhD
Staff Scientist

CALIFORNIA
Water Boards

EDMUND G. BROWN JR.
GOVERNOR

MATTHEW RODRIQUEZ
SECRETARY FOR
ENVIRONMENTAL PROTECTION

San Francisco Bay Regional Water Quality Control Board

August 23, 2012

Anke Mueller-Solger
Kelly Souza
Greg Erikson
Interagency Ecological Program

Subject: Letter of Support for Proposal entitled "Assessment of Wastewater Effluent Effects on Phytoplankton Carbon and Nitrogen Assimilation in the Sacramento-San Joaquin Delta"

Dear IEP 2013 Workplan Co-Chairs:

We are writing to support funding for the subject proposal as a part of the Interagency Ecological Program's (IEP's) 2013 Workplan. This project, proposed by the Romberg Tiburon Center (RTC) would address whether wastewater treatment plant (WWTP) effluent may be impacting phytoplankton nutrient uptake rates, with resultant effects on the food web, potentially including the observed pelagic organism decline. Specifically, this project would contribute to our understanding of effluent-phytoplankton dynamics, further our understanding of how the Bay may respond to changes in WWTP effluent composition and, potentially provide better insight into conditions that signify nutrient impairment in the Bay.

The goals of the study are to identify the influence of WWTP effluent on nutrients and phytoplankton nutrient assimilation rates. The study proposal suggests that if nutrients are not a dominant factor driving phytoplankton growth dynamics, then a UEC, or unidentified effluent contaminant, may be the driver and that this is the guiding hypothesis for the project. Our understanding is that this hypothesis is based on studies conducted previously by the RTC for the Central Valley Water Board and that they too support this continued line of research.

We support this proposal because, as a regulatory agency responsible for permitting wastewater discharges to the Bay, we need a clear understanding of the impact wastewater discharge has on primary productivity. In addition, we recognize that the consequence of managing nutrients or other wastewater constituents includes the possibility that a significant capital investment will be required to address treatment in order to achieve the environmental benefits.

We recommend funding this proposal. Please contact me, at nfeger@waterboards.ca.gov if you have any further questions.

Sincerely,

Naomi Feger
Planning Division Chief