Proposal submission for the CALFED 2007 Supplemental PSP

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Title of Supplemental Proposal: Comparison of Nutrient Sources and Phytoplankton growth and species composition in two rivers: their roles in determining productivity and food web conditions in Suisun Bay and the Delta

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Original Proposal Title: Do Low Phytoplankton Growth Rates Signal the “Bad” Conditions in Suisun Bay Driving the Pelagic Organism Decline Phytoplankton communities in the San Francisco Estuary: monitoring and management using a submersible spectrofluoremeter

Year of Original Proposal Submission: 2006/2004

Joint CALFED Supplement to Agreement No. S-05-SC-054 and Agreement No. 1039

New Title: Comparison of nutrient sources and phytoplankton growth and species composition in two rivers: their roles in determining productivity and food web conditions in Suisun Bay and the Delta.

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1. Project Purpose

This project results from data collected this last year during two current CALFED and related IEP Pelagic Organism Decline (POD) studies that indicate a critical need to understand the contrasting nutrient and resultant phytoplankton communities and processes occurring in the San Joaquin (SJ) and Sacramento (Sac) Rivers. These rivers represent the dominant nutrient supply for the Suisun Bay and Delta food web and likely impact the "bottom-up" and habitat factors implicated in the POD.

Our research indicates that the form of nitrogen making up the DIN pool for phytoplankton determines biomass and possibly community structure. High levels of ammonium (NH_4) prevent phytoplankton nitrate (NO_3) uptake leading to low levels of production (Wilkerson et al 2006, Dugdale et al. 2007, Dugdale CALFED proposal and progress report). The most important source of NH_4 in the Delta and Suisun Bay is discharge from the Sacramento Regional Wastewater Treatment Plant (Sac WWTP) and the City of Stockton WWTP (Stockton WWTP, Fig. 1), although smaller WWTPs (e.g. Contra Costa, Fairfield, Tracy) and agricultural sources (Schemel and Hager, 1986, Hager and Schemel, 1992) are regionally and seasonally important (Jassby in press, Jassby and Van Nieuwenhuyse 2005, Mueller-Solger in prep.). The Sac WWTP discharges into the Sac River near Freeport and the Stockton WWTP discharges into the SJ River just upstream of the Port of Stockton (Fig. 1).

The Stockton WWTP recently began advanced secondary (2°) treatment. According to a new NPDES permit, this treatment will reduce NH_4 effluent concentrations from often more than 20 mg/L (1.5 mM) to never more than 2 mg/L (150 μM) by the summer of 2008, with a simultaneous increase in effluent NO_3 concentrations (Fig. 2). It is likely that this drastic shift in N output will have ecological consequences, especially at the phytoplankton level. **Assessment of these consequences should begin now, as they are occurring.**

The Sac WWTP has 2° treatment that releases mostly NH_4; this flux has been steadily increasing as the Sacramento metropolis grows (Jassby in press). In contrast to the Stockton WWTP, the Sac WWTP currently has no requirement to upgrade treatment, nor does it specify plans for increased NH_4 removal in its 2020 master plan (http://www.srcsd.com/srwtp-2020mp.html). This plan, however, was recently legally invalidated (Contra Costa Times, 12/11/07). Moreover, the 5-year operating license needs to be renewed by the Central Valley Regional Water Quality Control Board (CVRQWCB) in 2008 and the California State and Regional Water Boards have acknowledged the need for additional evaluation, and possibly regulation, of increasing NH_4 pollution in Bay-Delta waterways (SWRCB 2007-0079, R5-2007-0161). In these resolutions, the Water Boards committed to "execute a contract to conduct screening studies of potential inhibition of primary productivity […] associated with ambient ammonia concentrations in the Delta in consultation with the IEP's POD investigations, and implement appropriate regulatory controls to protect beneficial uses." **Thus, our proposed study is clearly timely**.

Although not finalized, we have some indication that we might receive a one-year contract by the CVRWQCB following the 12-2007 resolutions. They are asking us to focus on the Sac River near the WWTP. This initial "screening study" would be modeled after traditional toxicity tests. In contrast, the comprehensive study proposed here would make use of cutting edge experimental and monitoring techniques, build more closely on our previous and ongoing CALFED and IEP studies, and integrate with ongoing IEP, and other monitoring and the IEP POD investigations. Here, we focus on the contrasting nutrient regimes in the Sac and SJ Rivers. This approach will inform regulators and others immediately involved with discharge permits and setting water quality objectives, and lead to an improved understanding and management of factors driving phytoplankton and food web dynamics including the POD - in the upper SFE.

2008 will also be marked by water management decisions, including court directed ones related to Delta Smelt. Historically, these decisions have not considered the different nutrient regimes of the Sac and SJ Rivers (and consequent phytoplankton productivity) when re-routing water through the Delta. In spring especially, evaluation of the contribution of SJ (NO_3 rich, relatively NH_4 poor) vs. Sac (NH_4 rich) water to Suisun Bay, the central Delta and its effects on potential spring phytoplankton blooms and the pelagic food web is essential to understanding the conditions on primary productivity in the Delta and northern estuary. Similarly, water quality of export waters is also affected by nutrients and phytoplankton composition and production. These considerations are important with respect to the renewed discussion about routing Sac River water in a canal around the Delta toward the export pumps near Tracy, possibly in a "dual conveyance system" that also maintains some "through-Delta" pumping capabilities (e.g. Isenberg et al 2007). For example, the canal intake that was considered during the previous, voter-terminated discussions in the 1980s was located only a few miles downstream from the Sac WWTP discharge. We believe that decisions about canal intake location, amount and timing of water withdrawn, etc, would all benefit from the results of the study proposed here. Moreover, the new real-time phytoplankton monitoring technologies that are part of this project will likely be essential for rapidly obtaining the information needed to make these difficult decisions.

Our research to date, combined with analyses of the contribution of the two rivers to the upper SFE, has led to these following supplemental questions to our original proposals.

1) **How do differences in nutrient and phytoplankton community composition between the SJ and Sac Rivers influence conditions downstream in the Delta and Suisun Bay?**
2) **How do phytoplankton growth rates and community structure respond to the differences in nutrient concentrations in the SJ River vs. the Sac River, resulting from the differences in waste water treatment?**

In this study we propose to address these questions and compare different approaches to monitor the phytoplankton community. We also plan to address some of the comments received at local meetings (e.g. EET, IEP workshops) about preliminary results of our current CALFED, for example the mechanism of NH_4 inhibition in relation to light (see section 5).

2. Background and Conceptual Model (what's new)

Phytoplankton in the SFE. As discussed at length in our original proposals, phytoplankton production and community composition are crucial elements of the Bay-Delta ecosystem and need to be accurately monitored in an adaptive management framework. Here, we summarize a few relevant points; See original proposals for full discussion and references.

In the SFE, phytoplankton production supports much of the pelagic production that includes important fish species such as Delta Smelt and Striped Bass, even though phytoplankton productivity is low in the SFE relative to other estuaries. Not all phytoplankton species, however, are equally nutritious. Diatoms and cryptophytes are generally considered good food for estuarine and freshwater consumers, while green algae and especially cyanobacteria are deemed nutritionally inferior (e.g. Brett & Mueller-Navarra 1997). The observed shift in phytoplankton community composition from dominance by diatoms to increasing dominance by other, mostly smaller species including miscellaneous (green) phytoflagellates (Lehman 2000, 2004) and more recently cyanobacteria (*Microcystis aeruginosa*) (Lehman et al. 2005, 2007) is thus worrisome. Moreover, some phytoplankton species (e.g. *M. aeruginosa*) can impair water quality, while others can clog filters and canals that are vital to water project operations. Blooms of such harmful algae have become increasingly common in the SFE and a concern to water project operators, drinking water managers, and ecologists alike. Any ecosystem restoration, flow management, or agricultural and urban water management actions need to consider and monitor the consequences for the amount and quality of phytoplankton production in an adaptive management framework, or else risk jeopardizing the estuarine food web, including already at risk species, water project operations, and human health.

Accurate phytoplankton monitoring is essential for science-based phytoplankton management in an adaptive management framework. Unfortunately, estuarine phytoplankton monitoring is notoriously difficult due to the highly dynamic nature of the estuarine environment

and phytoplankton communities. Traditionally, discrete phytoplankton monitoring samples have been analyzed microscopically or chemically (pigment analysis) for species composition and biomass, but these methods are laborious, expensive, slow, often miss small cells, and allow for only low temporal and spatial resolution due to the need for discrete sample collection. In highly dynamic systems such as the SFE, these limitations of the traditional methods greatly impede advances in phytoplankton ecology and management and also prevent early detection, prediction, and effective management responses to harmful algal blooms.

New technologies such as the bbe FluoroProbe and the Cytosense flow cytometer (http://www.cytobuoy.com/ Dubelaar and Gerritsen, 2000) overcome at least some of these limitations. Testing and application of the bbe FluoroProbe is the subject of the original project by Mueller-Solger et al. Instrument details were given in the original proposal, and initial results are promising (Progress Report). The Cytosense flow cytometer produces scanned profiles of forward scatter, sideward scatter and one or more wavelengths of fluorescence for each particle (size range of 2 to 800 µm) in a water sample A mixture of particles generates clusters, each representing a group with different morphology/optical properties that can be related to "fingerprints" from known algal cultures. The CytoSense is routinely used in the Dugdale lab (e.g. Lew, 2007; Takabayashi et al. 2006) to count different sized phytoplankton, diatom chains and to "recognize" cryptophytes and cyanobacteria due to their accessory pigments. Originally developed for cyanobacterial filaments, it should be effective in enumerating *Microcystis.* We plan to compare the FluoroProbe, the CytoSense and microscopic enumeration using the Utermöhl settling technique in a dynamic range of planktonic communities to be sampled in the proposed study. Finally, we also intend to compare and integrate the results from the proposed study with the continuously collected phytoplankton fluorescence data at the EMP continuous sites (Fig. 1) and during the monthly EMP cruises.

N sources and flows. As in most estuaries, water in the SFE is a complex mix from different freshwater sources and the ocean. What distinguishes the SFE is its highly altered and exceedingly complicated – and contended – water flows: seasonally and tidally variable freshwater and tidal ocean inflows, large water export pumps in the southern Delta, many local agricultural and urban inputs and diversions, and various flow barriers and gates all contribute to a highly dynamic mixing zone that extends throughout most of the Delta and Suisun Bay.

While much of the water routing strategies employed in the SFE focus simply on water quantity, water quality is also a very important factor. Traditionally the Sac River has been viewed as having "better" water than the SJ River because of its lower salinity and contaminants loads. Recently, SJ River water has been viewed as a potentially important food source for food-limited fishes and other organisms in the SFE (IEP 2008). The research proposed here adds another facet to this discussion by focusing on the SJ River as a "good N" source (NO_3) and the Sac River as a "bad N" source (NH_4). If our conceptual model (see below) about the importance of these different N species holds true in the Delta, the traditional notion of what constitutes "water quality" in the Delta will have to be revised and water management (including flow management and wastewater treatment) may have to change accordingly.

In recent decades, NH_4 levels at IEP long-term monitoring stations in the Sac and SJ Rivers closest to the Sac and Stockton WWTPs have been higher and experienced more substantial increases than NH_4 levels at all other monitoring stations in the Delta and Suisun Bay, and have also increased the most (Fig. 3). This is mostly due to the large and increasing NH_4 output from the WWTPs (Fig. 2 & Fig. 4). In contrast, NO_3 concentrations have been highest and rising in the SJ upstream of the Stockton WWTP (mostly from agricultural inputs, Dubrovsky et al 1998), but relatively low and constant at the stations near the two WWTPs (Fig 2). Silica trends may reflect decreased diatom production in Suisun Bay and increased diatom production in the SJ River upstream of the Stockton WWTP (Fig. 3).

N output from the Stockton WWTP has been highly seasonal with high effluent NH_4 levels and loads in winter and high NO_3 levels and loads in early summer due to NH_4 nitrification in wastewater treatment ponds during the warmer summer months. This seasonality is also reflected in river N loads downstream of the Stockton WWTP (Fig. 5). Since October 2006, this pattern

has changed, however, due to the implementation of more advanced 2° treatment with year-round nitrification by the Stockton WWTP. As is already noticeable (Fig. 2 & Fig. 4), this improved treatment greatly increases NO_3 output and decreases NH_4 output.

In contrast to the Stockton WWTP, the Sac WWTP effluent contains much more NH_4 than NO_3 with little seasonality and no recent changes in operations. Due to its larger size, NH_4 loads from the Sac WWTP are on average about seven times greater than the pre-2007 Stockton NH_4 loads and about 20 times greater starting in the end of 2006. While average NO_3 loads from the two plants were comparable before 2007, Stockton WWTP NO_3 loads are now much higher. In winter, NO_3 loads in the Sac River have historically been much higher than in the lower SJ because of NO_3 "flushing" from the large Sac watershed area in agricultural use. During the rest of the year, however, NO_3 loads in the Sac River are much lower and comparable to and sometimes exceeded by NO_3 loads in the lower SJ River, in spite of much smaller flows in the latter. NO_3 loads in the SJ River may more commonly exceed those in the Sac River in the future due to the operational changes at the Stockton WWTP.

In a recent modeling study, Monsen et al (2007) showed how operation of the Delta Cross Channel on the Sac River, the Head of Old River Barrier (HORB) on the SJ River, and export reductions at the SWP and CVP pumps in the southern Delta could all have a large influence on the relative amounts of Sac and SJ source water and thus water quality in the central Delta and Suisun Bay (see Fig. 1 for locations). These and other water project operations and seasonal effects result in large variations in relative amounts of Sac and lower SJ water at different Delta and Suisun Bay locations (Fig. 6). Overall, much more water enters the Delta through the Sac than through the SJ River, and much of the SJ River water is diverted through the CVP & SWP export pumps throughout much of the year, resulting in overall much lower contributions of SJ water to the central Delta and Suisun Bay (Fig. 6). However, the SJ River represents an important source in spring when inflows are high and especially in April-May when the DCC is closed and after the HORB is put in place and exports are reduced to protect migrating salmon. This is also an ecologically critical period when phytoplankton spring blooms fuel rapid invertebrate growth providing food resources for larval and juvenile fish rearing in the Delta and Suisun Bay. Our previous research has indicated that historically occurring phytoplankton spring blooms in Suisun Bay are now suppressed by high NH_4 levels. Here we propose to extend this research upstream and specifically focus on the role of the different N sources from the Sac and SJ Rivers in spring bloom formation in Suisun Bay and the central Delta in April-May before and after the salmon protection measures are put in place. In addition, this study will also target the summer low flow period when Sac River flows are expected to dominate in the Delta and Suisun Bay and the high Sac River NH_4 content may directly benefit *Microcystis*.

***Importance of NH_4 and NO_3 for phytoplankton in the SFE*.** Prior work in Central and San Pablo Bays showed that phytoplankton blooms occur when NH_4 concentrations are low (~4 µM or less) with suitable irradiance conditions (Fig. 7). Occasionally these blooms occur in Suisun Bay, but are less common, due to the increasing gradient of NH_4 going up estuary towards the Sacramento and Stockton WWTPs. This effect is a result of the well known condition whereby NH_4 inhibits phytoplankton NO_3 uptake preventing access in SFE to the larger pool of N (Fig. 8). Preliminary results from our current CALFED project strongly suggest that NH_4 inhibition of NO_3 does occur in the freshwater reaches of the estuary. The degree to which the occurrence of NH_4 inhibition of NO_3 uptake in the rivers influences nutrient delivery and NO_3:NH_4 ratios in the Delta and Suisun Bay is lacking. Without better spatial sampling along riverine transects (not budgeted in our current project) this cannot be addressed.

Another important aspect of high NH_4 concentration is the competitive advantage for NH_4 adapted species (e.g. *Microcystis*), and disadvantage for sensitive species such as diatoms. Evidence for this in the upper SFE includes initial FluoroProbe results which show a marked switch in dominance from diatoms to other algae along a transect toward the Stockton WWTP in late fall when NH_4 output increased (Progress report; Fig. 8 original proposal) and by others demonstrating long-term shifts from diatoms to other algae in the upper SFE (see above), and, more recently, striking differences in the algal assemblage and productivity in the SJ River

upstream (high net primary productivity, diatoms) and downstream (lower net primary productivity, flagellates) of the Stockton WWTP (Lehman 2007). *Microcystis* blooms have become increasingly common and severe in the central Delta. *Microcystis* is a "nitrogen specialist" that thrives on high levels of NH_4, while also benefiting from high levels of NO_3 (Rückert, and Giani 2004). It is also able to efficiently acquire and store inorganic phosphorus (P) (Marinho and Feliciano 2007). P output from the Sacramento WWTP declined dramatically in the mid 1990s, likely contributing to the demise of high-P adapted species (Van Nieuwenhuyse 2007) in areas dominated by Sac River water. Together, the N and P characteristics of Delta waters which during the summer months are to a large degree attributable to operations of the Sac WWTP may help explain the increasing occurrence of *Microcystis* blooms in the Delta. Testing this, and differentiating between the importance of N versus P for bloom formation will be addressed by the study proposed here.

Conceptual Model (Fig. 9). This conceptual model shows the major DIN pools and pathways (in relative terms as shown by their size) from the two river sources to Suisun Bay. The hypothetical scenario describes two extreme ecosystems with different phytoplankton community structure. One extreme is hypothesized when the DIN pool contains NH_4 at levels that inhibit diatom access to the NO_3 pool and the community will shift towards non-diatoms and low carbon productivity. This right side of the conceptual model illustrates our present understanding of the causes of low primary production in Suisun Bay, i.e. high ambient NH_4 concentrations result in inhibition of NO_3 uptake and access to the largest source of DIN for the phytoplankton (Wilkerson et al. 2006; Dugdale et al. 2007; Parker et al. (in rev). In Fig. 9, the major source of high NH_4 to Suisun Bay is through the Sac River and the Sac WTTP.

The alternative (the left side) occurs when the water from the SJ River is the dominant source, NH_4 levels are reduced (maybe below inhibitory levels) and NO_3 is higher due to advanced secondary waste water treatment. Diatoms should dominate both within the river and in the water exported to Suisun Bay, leading to higher and more nutritious phytoplankton biomass and a rich carbon productivity foodweb. The river phytoplankton may be conceived as filters of the source nutrients (from the WWTP's) making it important to know the characteristics of the phytoplankton, whether they are more likely to use NH_4 or NO_3. Another process that may be important is nitrification in the rivers in which NH_4 is oxidized by bacteria to NO_3, helping to reduce NH_4 concentrations, but with a cost in oxygen depletion (Hager and Schemel 1992, Jassby and Van Nieuwenhuyse 2005). Nitrification is likely to be more important in summer when temperatures are elevated and reaction rates increased. Finally, other nutrients (e.g. P) and environmental conditions (flow, light, temperature) also play a role in modulating the phytoplankton N response and will be considered in the study proposed here.

3. Summary of Progress (what's learned)

The two projects have been collecting data for the last year (Tables 1, 2 Progress Reports) and these results have been presented to a variety of stakeholders, agencies and scientists (Table 2). Some of these results have led to our revised conceptual model (Fig. 9). Relevant to this proposal, our previous results have shown that the FluoroProbe is particularly effective in distinguishing the diatoms, green algae and cyanobacteria within the phytoplankton community, but more testing is needed to assure meaningful results. This proposed study offers a unique opportunity to combine additional instrument tests with a highly relevant, "real-life" application.

Our "Bad Suisun" Project uses grow-out experiments to evaluate if the phytoplankton in Suisun Bay have impaired productivity capacity compared to those downstream in Central Bay (control location) and upstream in the Sac and SJ Rivers. Our initial view was that Suisun Bay was a unique "bad" spot where phytoplankton consistently showed reduced productivity even under optimal light conditions. This lower productivity is due in part to the high levels of ambient NH_4 that limits their access to the larger NO_3 pool for growth. An alternative view is that Suisun Bay represents part of a productivity continuum with lower anticipated phytoplankton growth also occurring upstream in the Sac and SJ Rivers and Delta. Data

collected in 2007 during four different times (Table 1) showed that Suisun Bay phytoplankton can sometimes grow well and draw down NH_4 and use NO_3 like phytoplankton in Central Bay.

Fig. 10a shows a typical response of phytoplankton to optimal light conditions in a grow-out experiment using Central Bay water. First NH_4 is drawn down and then NO_3 is taken up rapidly after Day 2 and the chlorophyll biomass increases to ~ 30 $\mu g\ l^{-1}$ by Day 4. All the NO_3 is drawn down to zero and nutrient limitation sets in. Prior to our CALFED studies, grow-outs with Suisun Bay water showed little chlorophyll buildup, and all the physiological processes were delayed in time with NH_4 uptake occurring slowly and some NO_3 drawdown starting after 4 days (cf. 2 days in Central Bay). This "bad" scenario was observed in BS2 (Fig. 10c). However in BS1, ambient NH_4 in Suisun Bay was low (4 µM) and the grow-out acted like Central Bay and showed NO_3 (36 µM) drawdown to zero, once NH_4 was below inhibitory levels (Fig. 10b). Chlorophyll accumulation reached 50 $\mu g\ l^{-1}$ by Day 5.

However, the grow-outs carried out with water from the Sacramento and SJ Rivers in June (BS3, Figs 10 d, e) showed almost no NO_3 drawdown and a small amount of NH_4 drawdown with little buildup of chlorophyll. It is important to note that our "SJ" sampling took place in the lower SJ in an area that often receives a large quantity of NH_4-rich Sac River water during the summer due to export pumping, open Delta Cross Channel gates, low SJ River inflows, etc. Based on these results, we now believe that when it occurs, the "bad" Suisun condition is actually part of a continuum from the rivers downstream to the seaward embayments.

Our CALFED project includes a modeling component that uses a nutrient-phytoplankton model originally developed for coastal upwelling systems (Dugdale et al., 1992) with an NH_4 inhibition term. It simulated the Central Bay grow-outs very well reproducing the high NO_3 uptake rates and rapid exhaustion of NO_3 once relatively high NH_4 concentrations were reduced to low levels. The initiating factor in both upwelling and enclosures is the sudden availability of light. Experimentally we have shown light to be sufficient at 5-15% of surface irradiance.

Some preliminary modeling efforts were made to relate river nutrient processes to the export of nutrients to Suisun Bay and the other downstream embayments. Using mean NH_4 uptake rate measured in Suisun Bay (Wilkerson et al., 2006) and ambient NH_4 concentrations just below the Sac WWTP, calculations show river phytoplankton were unable to reduce the NH_4 concentration to 4 µM, the critical value for the initiation of blooms in SFE (Dugdale et al. 2007), under average flow conditions during the transit from Sac to Suisun Bay. If conditions were to change in the Sac River such that NH_4 concentrations were reduced to below an inhibitory level, the model shows that the phytoplankton would access the available NO_3 and spring bloom conditions could develop. However with increasing NH_4 concentrations, a critical level is reached when both phytoplankton biomass and growth rate collapse to low values. These model results are consistent with our conceptual model (Fig. 9). Our plan is to continue development of the nutrient/phytoplankton model with new data on river nutrient and phytoplankton processes with the object of understanding the role of the river phytoplankton in modifying the river water nutrients entering Suisun Bay and the Delta. These results indicate more knowledge is needed about conditions in the river and how Suisun Bay responds to changes in the relative contribution of nutrients and phytoplankton of Sac vs. SJ water.

4. Approach and Scope of New Work

The scope of proposed work will consist of four sampling campaigns in early April 2008, 2009; early May, 2008 and August 2008) to characterize the Sac, SJ and Old Rivers and stations in Suisun Bay, along with grow-out experiments (Table 3). During the early spring we anticipate the largest influence of SJ River water in Suisun Bay and Delta; in August, a large influence of Sac River water, with essentially no contribution from the SJ River (Fig.6). Transects within Old River and Central Delta are proposed because this area represents a mixture of Sac and SJ water with little SF Bay influence and it is an important habitat for some POD fishes. August is also when *Microcystis* blooms are likely to occur, and the capability for "observing" this with the Fluoroprobe and CytoSense instruments can be tested.

These campaigns will be made using the R/V Questuary, capable of completing one river transect per day. We plan to center our land efforts (i.e. grow-outs) at the SF NERR Rush Ranch

facility near Suisun City where there are simple lab and dormitory facilities available. Sampling along transects will include continuous underway measurements of physical and some chemical and biological parameters with more detailed characterization at 24 discrete station locations (Fig. 1). These stations include previously designated DWR stations to provide the capability for comparison with the long-term DWR water quality dataset. Underway sampling will include measurements of temperature, salinity, turbidity, fluorescence, as well as continuous sampling using flow cytometry and the FluoroProbe. Discrete sampling at stations will include vertical profiles of temperature and salinity, and light penetration using a CTD fitted with a PAR sensor as well as by secchi disk. Water will be sampled at the surface and near the bottom for determination of inorganic nutrients (NO_3, NO_2, NH_4, PO_4, $Si(OH)_4$), dissolved inorganic carbon (DIC), and size fractionated chlorophyll. These methods are described in detail in the original Dugdale et al proposal. Primary production and phytoplankton NO_3 and NH_4 uptake will be assessed under saturating light (50% of PAR) and light limited (10% of PAR) conditions using $^{15}N/^{13}C$ tracer methods in light attenuated, flow-through incubators. Comparison of NH_4 gradients along the river with $^{15}NH_4$ uptake rates will provide an indication of nitrification. Samples will be collected for phytoplankton identification by microscopy, flow cytometry and the FluoroProbe at each of the 24 discrete stations. Microscopic enumerations will be conducted by the taxonomist who is also analyzing the routinely collected IEP EMP samples to ensure cross-study coordination and data comparability. We will augment our original experimental grow-out approach (outlined in the Dugdale et al proposal) adding grow-outs at locations downstream of the Sac and Stockton WWTPs. If NH_4 depletion rates in grow-outs are greater than measured $^{15}NH_4$ uptake rates this will suggest the presence of nitrification.

5. Relevance to CALFED Science Program

This proposal meets objectives of the CALFED-Bay-Delta Program; to study and improve ecosystem quality and water quality. It will generate knowledge to help guide decisions and evaluate actions critical to CALFED, including, e.g., major aspects of the DRERIP models currently under development by CALFED. It addresses the role of lower trophic levels in the POD: the phytoplankton community structure and productivity in relation to their nutrient supply. Mueller-Solger has been working on nutrient trends, NH_3 toxicity, and food web issues as a PI in the IEP POD study, is a member of the POD Management Team and is affiliated with the IEP EMP. These ties assure coordination with these projects and relevance to the POD. The proposal is related to the "Foodweb" Project (CALFED, Kimmerer), which includes Dugdale, Wilkerson and Parker, is directed towards top down controls and carbon supply to the LSZ. Our project is complementary, focusing at the phytoplankton community level and upstream supplies of nutrients from contrasting rivers.

This project also addresses comments received while presenting our preliminary results and original "bad" Suisun" conceptual model to a diverse group of stake holders with interests about the POD and water quality in the Delta. Specifically

1) *NH_4 inhibition in Suisun Bay has been extrapolated to the Delta but has not been tested, nor quantified in comparison with light limitation and grazing.* Our preliminary data suggests the inhibition effect occur~~s~~ in the rivers. We have acknowledged that light limitation represents the major "bottom-up" control on primary production. However, historical chlorophyll peaks have been observed in the Delta and Suisun Bay, initiated by improved light; it is during these periods that phytoplankton growth may be modulated by ambient NH_4. This proposal will investigate the NH_4 inhibition phenomenon at low light and will focus on this along the two rivers.

2) *The NH_4 inhibition effect has not been linked to WWTP in a quantitative way.* We hope to do experiments with Sac WWTP effluent, but we have no contract with CVRWQB.

3) *The linkage between changes in primary productivity and the POD has not been clearly established, and the presumed food web impacts related toNH_4concentrations have not been demonstrated.* The collaboration of these two CALFED projects will ensure that phytoplankton community structure will be measured in parallel with productivity and nutrient conditions.

Table 1. Progress to Date: Field Work

CALFED Agreement No.S-05-SC-054 (Phytoplankton communities in the SFE: monitoring and management using a submersible spectrofluorometer – see progress report for the (mostly bureaucratic) obstacles encountered by this project resulting in a long delay in implementation).

Date	Experiment Name	Description
11/8/2007	Laboratory testing	Laboratory testing with 8 species of green, blue-green and cryptophyte algae (see Mueller-Solger progress report for further details)
11/26/2007	Field deployment	Ran FluoroProbe during DWR "Dissolved Oxygen" monitoring cruise. Measurements taken at 14 stations.

CALFED Agreement 1039 (Do low phytoplankton growth rates signal the “bad” habitat conditions in Suisun Bay driving the pelagic organism decline

Date	Experiment Name	Encl #1	Encl #2	Encl #3	Encl #4
4/3/07	Bad Suisun 1	Rio Vista 38º 08.9' N 121º 41.3' W	Sta 649 38º 3.7N 121º 48.0'W	"5 psu" 38º 05.5N 122º 00W	
4/23/07	Bad Suisun 2	"0.5 psu" 38º 03.7N 121º 48.6W	"2 psu" 38º 02.8N 121º 55 W	"5 psu" 38º 05N 121º 59.7W	"SW" 37º 53.8’ N, 122º 25.5’ W
6/26/07	Bad Suisun 3	Rio Vista 38º 08.9' N 121º 41.3' W	"San" 38º 02.3N 121º 29.3W	"5 psu" 38º 04.4N 121º 58.4W	"SW" 37º 53.8’ N, 122º 25.5’ W
7/16/07	Bad Suisun 4	Rio Vista 38º 08.9' N 121º 41.3' W	"San" 38º 02.3N 121º 29.3W	"5 psu" 38º 02.6N 121º 55.3W	"SW" 37º 53.8’ N, 122º 25.5’ W

Encl= grow-out experiment, with Encl 1 filled with water from Sac River, Encl 2 filled with water from lower SJ River, Encl 3 filled with water from Suisun and Encl 4 filled with water from Central bay (RTC seawall).

Table 2 Progress to Date: Presentations

Project	Authors	Title	Conference, Year
CALFED 1039	Dugdale, R.C., Parker, A.E., Mueller-Solger, A., Wilkerson, F., Marchi, A., Hogue V.E.	Origins of high nutrients and low productivity conditions in Suisun Bay, and the Sacramento and San Joaquin Rivers.	Interagency Ecological Program Science Meeting, Pacific Grove, CA. 2008
	Dugdale, R. C., Wilkerson, F., Parker, A. E., Hogue, V., Marchi, A., Kleckner, A., Fuller, J.	The declining ecosystem health of the northern San Francisco Bay-Delta may be linked to poor phytoplankton productivity.	Biennial State of the Estuary Conference, Oakland, CA 2007
	Dugdale, R.C., Sharp, J.H., Wilkerson, F., Parker, A.E.	DIN is an inadequate descriptor for evaluating nitrogen based effects on estuarine ecosystems.	Estuarine Research Federation Meeting Providence, RI. 2007
	Wilkerson, F., Hogue V, Parker AE, Dugdale RC, Marchi A, Kleckner A, Fuller J.	Anomalously low phytoplankton productivity in northern San Francisco Estuary.	Estuarine Research Federation Meeting Providence, RI. 2007
	Parker, A. E., Dugdale, R.C. , Wilkerson, F.	Bad Suisun: Exploring the role of anthropogenic NH_4 in determining primary production in the northern San Francisco Estuary.	Contaminants Workgroup meeting, June 15, 2007 UC Davis
	Dugdale, R., Wilkerson, F., Parker, AE, Marchi, A., Hogue, V., Lew, K	Do Low phytoplankton growth rates signal the “bad” habitat conditions in Suisun Bay driving the pelagic organism decline.,	Interagency Ecological Program Science Meeting, Pacific Grove, CA. 2007
CALFED S-05-SC-054	Mueller-Solger, A.	Using a FluoroProbe to examine phytoplankton fluorescence by taxonomic group.	Estuarine Ecology Team Meeting, Dec 4, 2007 Romberg Tiburon Center

Table 3. Scope of New Work: Approximate number of additional field samples from four proposed campaigns

	Sacramento River	**San Joaquin River**	**Old River**	**Suisun Bay**
Temperature/salinity	32 discrete + continuous	32 discrete + continuous	16 discrete + continuous	16 discrete + continuous
Nutrients (NO_3, NH_4, PO_4, Si)	32	32	16	16
Chl-*a* (5µm>X, X<5µm)	32	32	16	16
FluoroProbe	32 discrete + continuous	32 discrete + continuous	16 discrete + continuous	16 discrete + continuous
CytoSense	32 discrete + continuous	32 discrete + continuous	16 discrete + continuous	16 discrete + continuous
Microscope counts	64	64	32	32
Secchi / PAR	32	32	16	16
$^{13}C/^{15}NH_4$ uptake	64	64	32	32
$^{13}C/^{15}NO_3$ uptake	64	64	32	32
Dissolved inorganic carbon	32	32	16	16
Enclosure experiments	4, in addition to original proposal	4, in addition to original proposal	4	4

Additional microscopy samples for FluoroProbe and CytoSense verification will be required.

Fig. 1

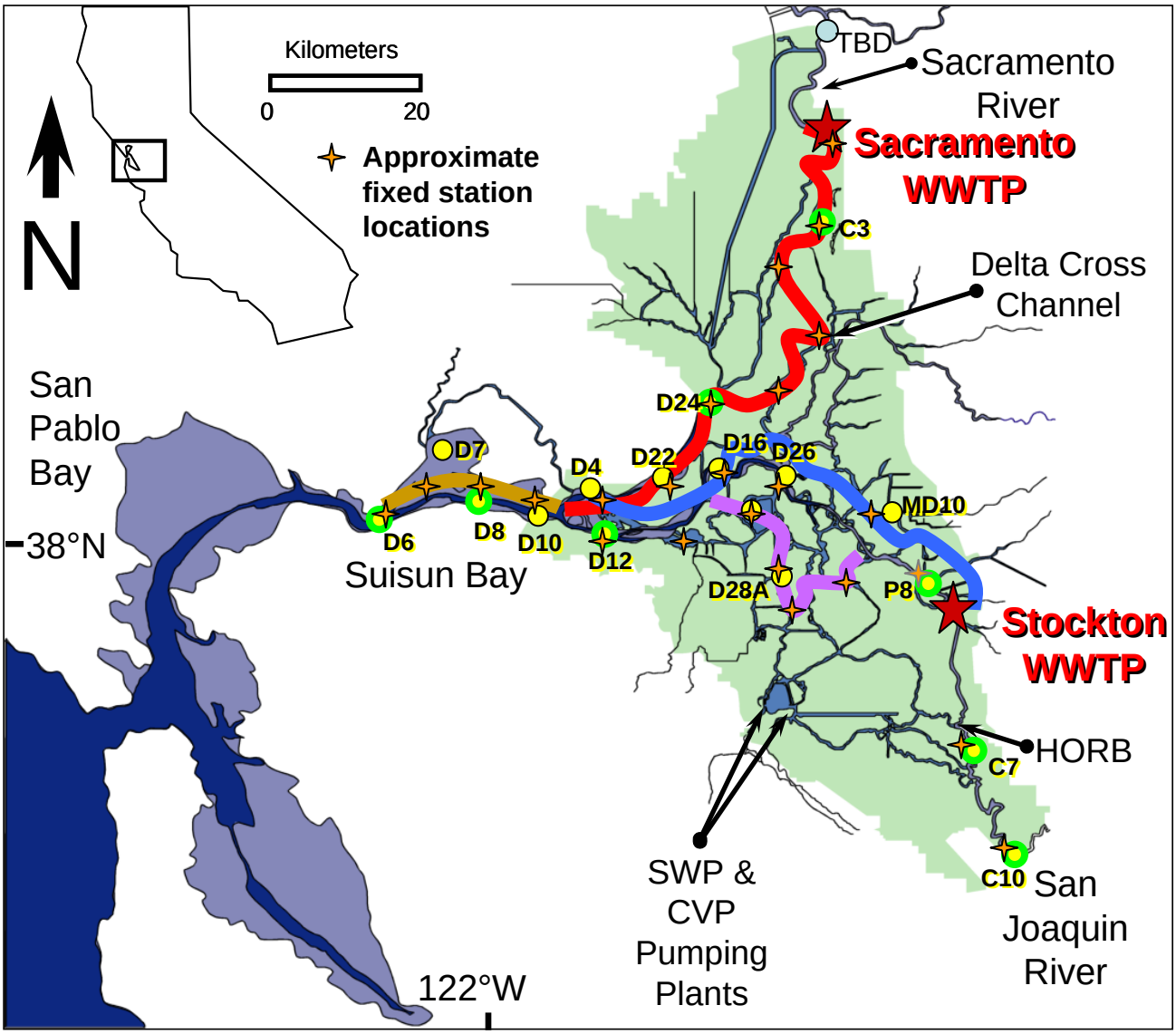


Fig. 1: Major waterways of the San Francisco Estuary. The freshwater Delta area is shown in green. Also shown are: locations of the Sacramento and Stockton WWTPs and important water project operation structures (Delta Cross Channel, Head of Old River Barrier (HORB), and SWP and CVP pumping plants); locations of long-term IEP water quality monitoring sites (solid yellow circles) with stations that have continuously recording fluorometers indicated by green circles; locations of four transect segments proposed for this study (brown: Suisun Bay segment, red: Sac River segment, blue: SJ River segment, purple: Old River/Central Delta segment) as well as approximate fixed station locations along the study transects (orange stars). Discrete sampling will also take place at the EMP stations C7 and C10 upstream of the Stockton WWTP and upstream of the Sacramento WWTP (location(s) to be determined).

Fig. 2

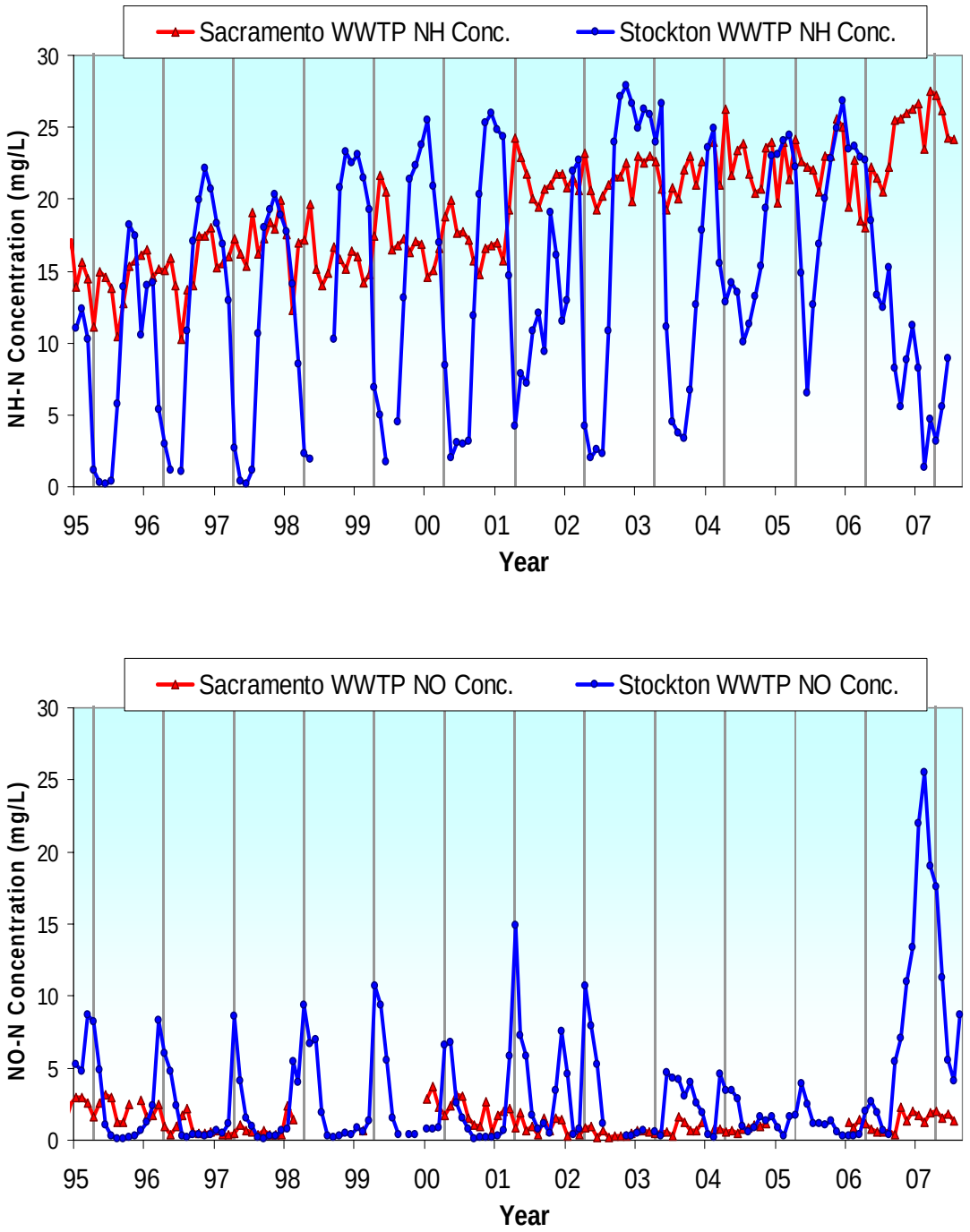


Fig. 2: Top panel: 1995-2007 times series of average monthly total ammonia nitrogen (NH-N) concentrations in the effluent from the Sacramento (red line with triangles) and Stockton WWTPs (blue line with circles). NH-N consists mostly of ammonium nitrogen (NH4+), but also contains a small amount of unionized ammonia (NH3)). Vertical bars indicate April, a focus month in this study. Data from Steve Nebozuk, Sacramento Regional County Sanitation District-1, personal communication to Anke Mueller-Solger. Bottom panel: Same as top panel, but for nitrate plus nitrite nitrogen (NO-N).

Fig. 3

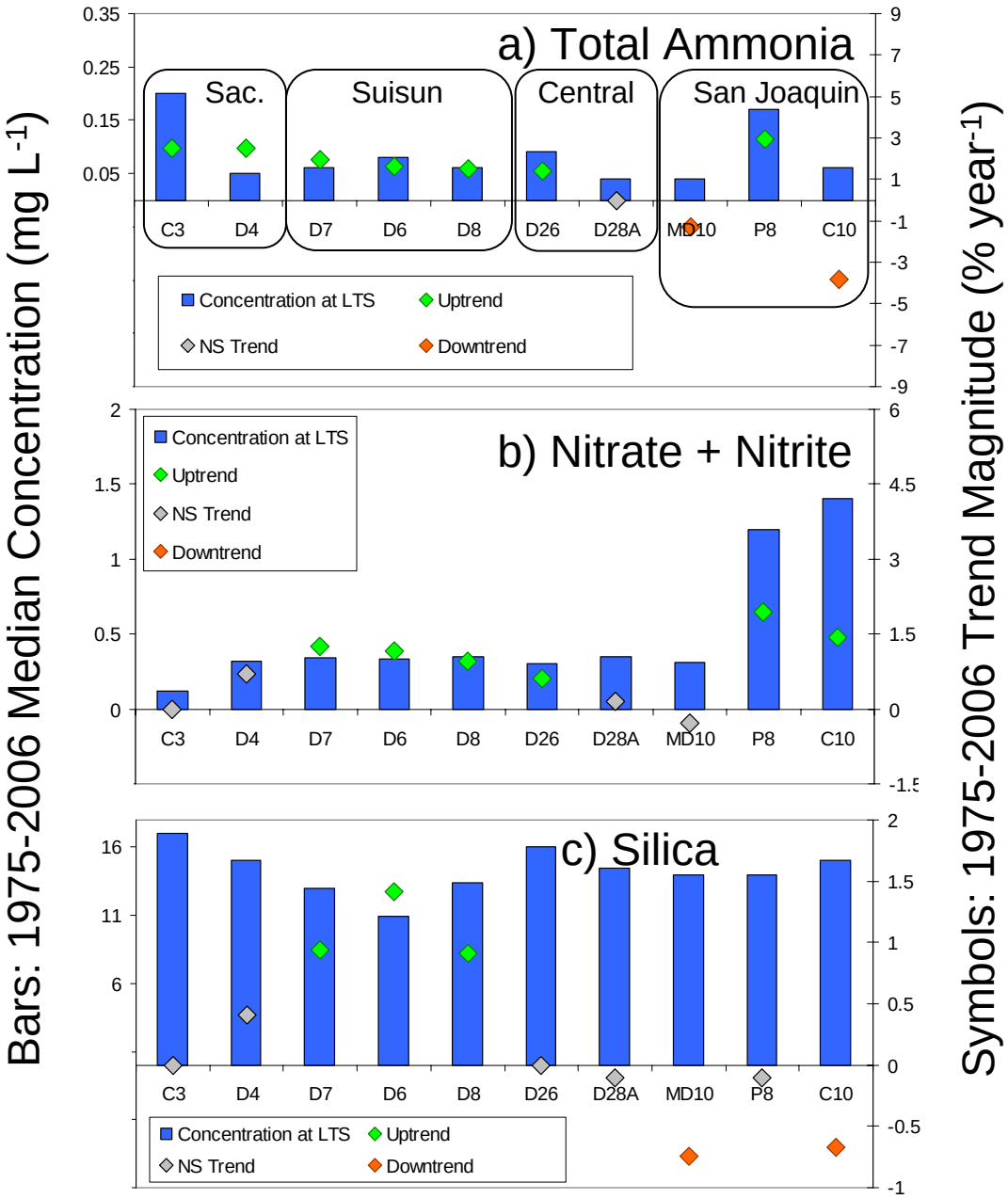
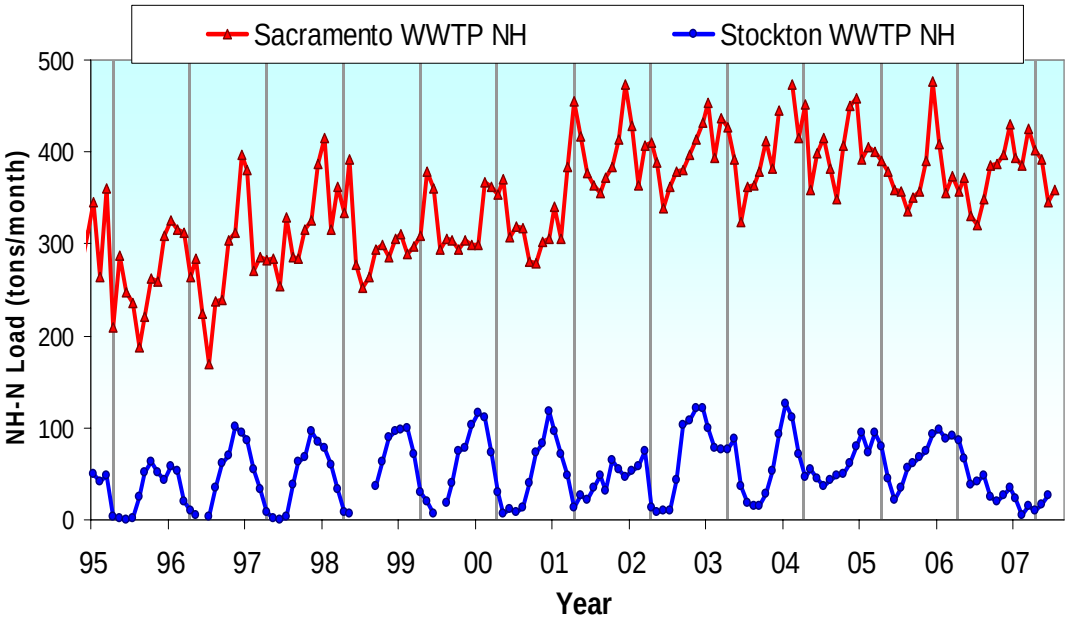


Fig. 3: Median magnitudes (columns) and annual trends (symbols) for concentrations of a) ammonia, b) nitrate + nitrite, c) silica at long-term IEP EMP monitoring stations throughout the Delta and Suisun Bay from 1975 to 2006. Trends were estimated and tested with USGS "Estrend" for S-plus using seasonal Kendall tests with months as seasons and no flow adjustment (since actual concentrations were of interest here). For station locations see Fig. 1. Data and metadata are available at http://www.baydelta.water.ca.gov/emp/

Fig. 4



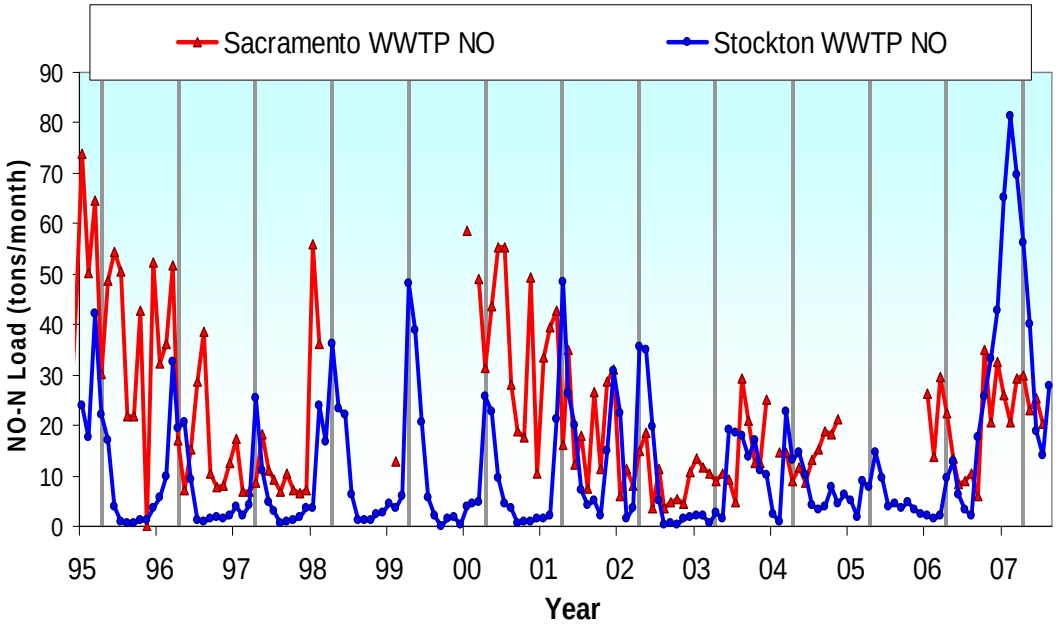


Fig. 4: Same as Fig. 2, but showing effluent NH-N and NO-N loads rather than concentrations. Vertical bars indicate April.

Fig. 5

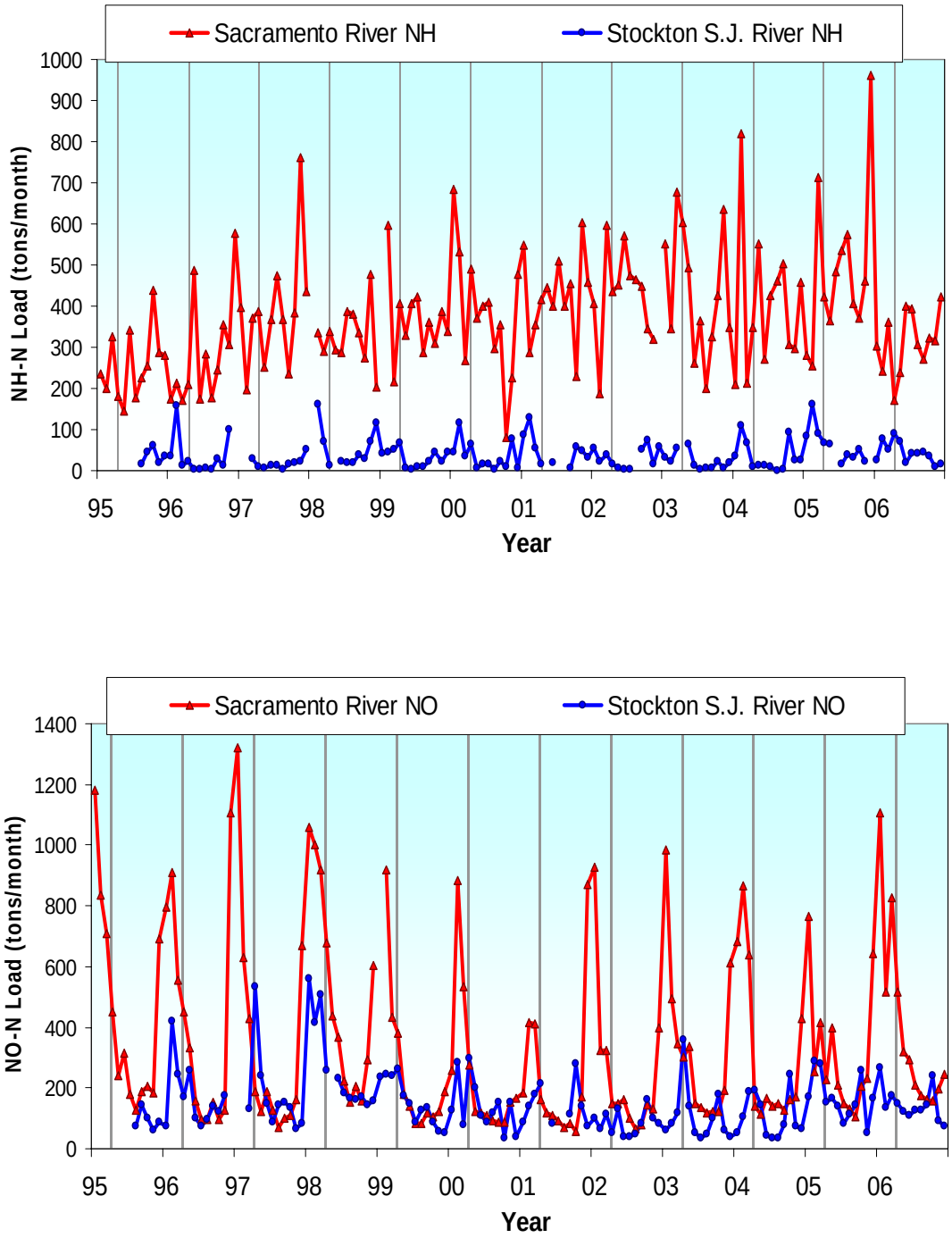


Fig. 5: Top panel: 1995-2006 times series of average monthly total ammonia nitrogen (NH-N) loads in Sacramento (red line with triangles) and San Joaquin River (blue line with circles) water near the Sacramento and Stockton WWTPs. Data: IEP EMP, stations C3/C3A and P8, see Fig. 1 for locations. Vertical bars indicate April. Bottom panel: Same as top panel, but for nitrate plus nitrite nitrogen (NO-N).

Fig. 6

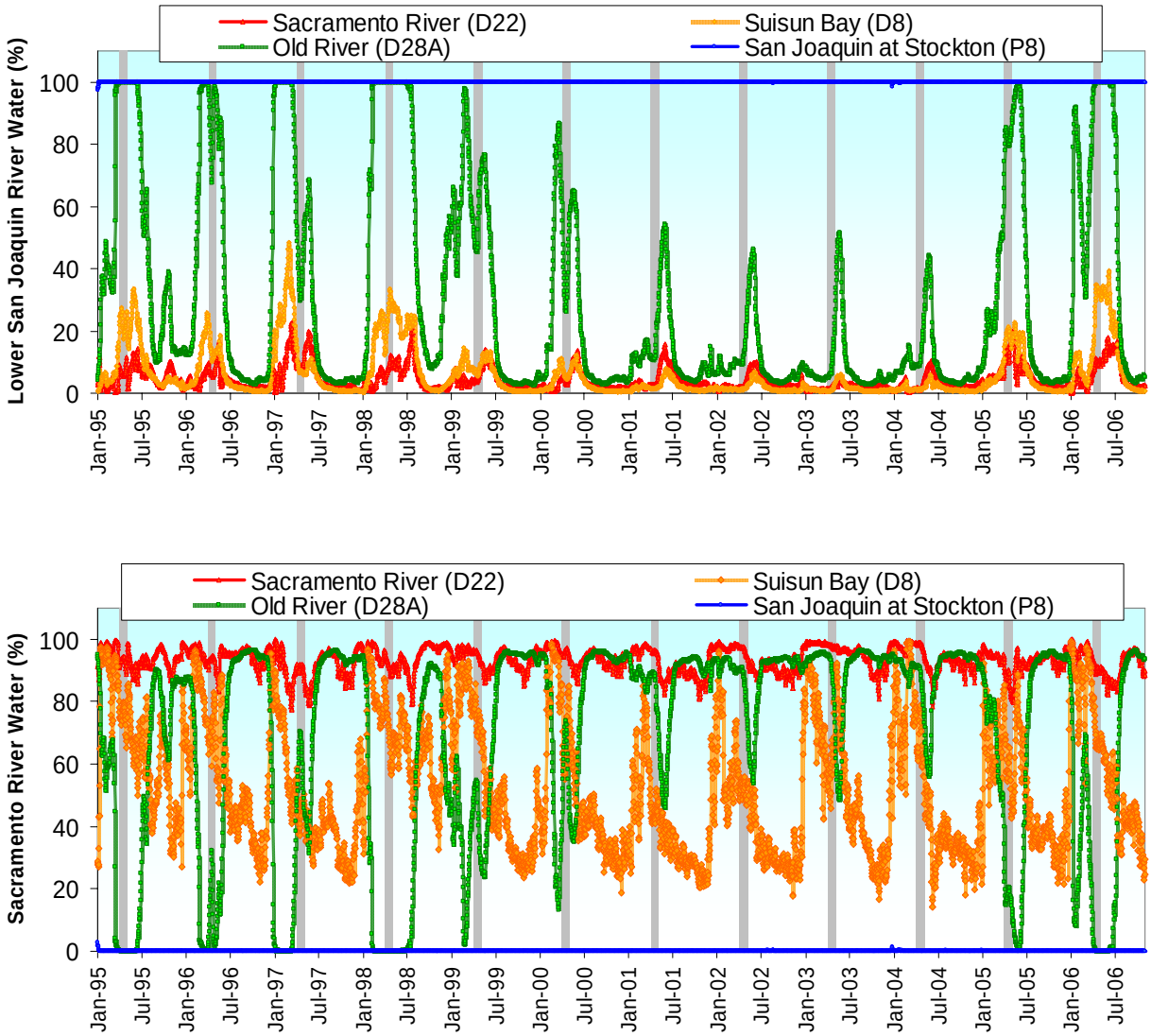


Fig. 6: 1995-2006 times series of source water contributions to the total water volume at four EMP monitoring stations located on the four study transect segments, see Fig. 1 for station locations and transects. Top panel: contribution of Lower San Joaquin River water defined as San Joaquin plus Cosumnes plus Mokelumne River water plus Delta agricultural drain water. Together, these four water sources make up 100% of the water present near the Stockton WWTP. Lower panel: Contribution of Sacramento River Water at the same fours stations. Data: Extracted from historical volumetric "fingerprints" at these stations generated with the Delta Simulation Model 2 (DSM2, http://modeling.water.ca.gov/delta/models/dsm2/index.html). These data were provided to Anke Mueller-Solger by Bob Suits, DWR.

Fig. 7

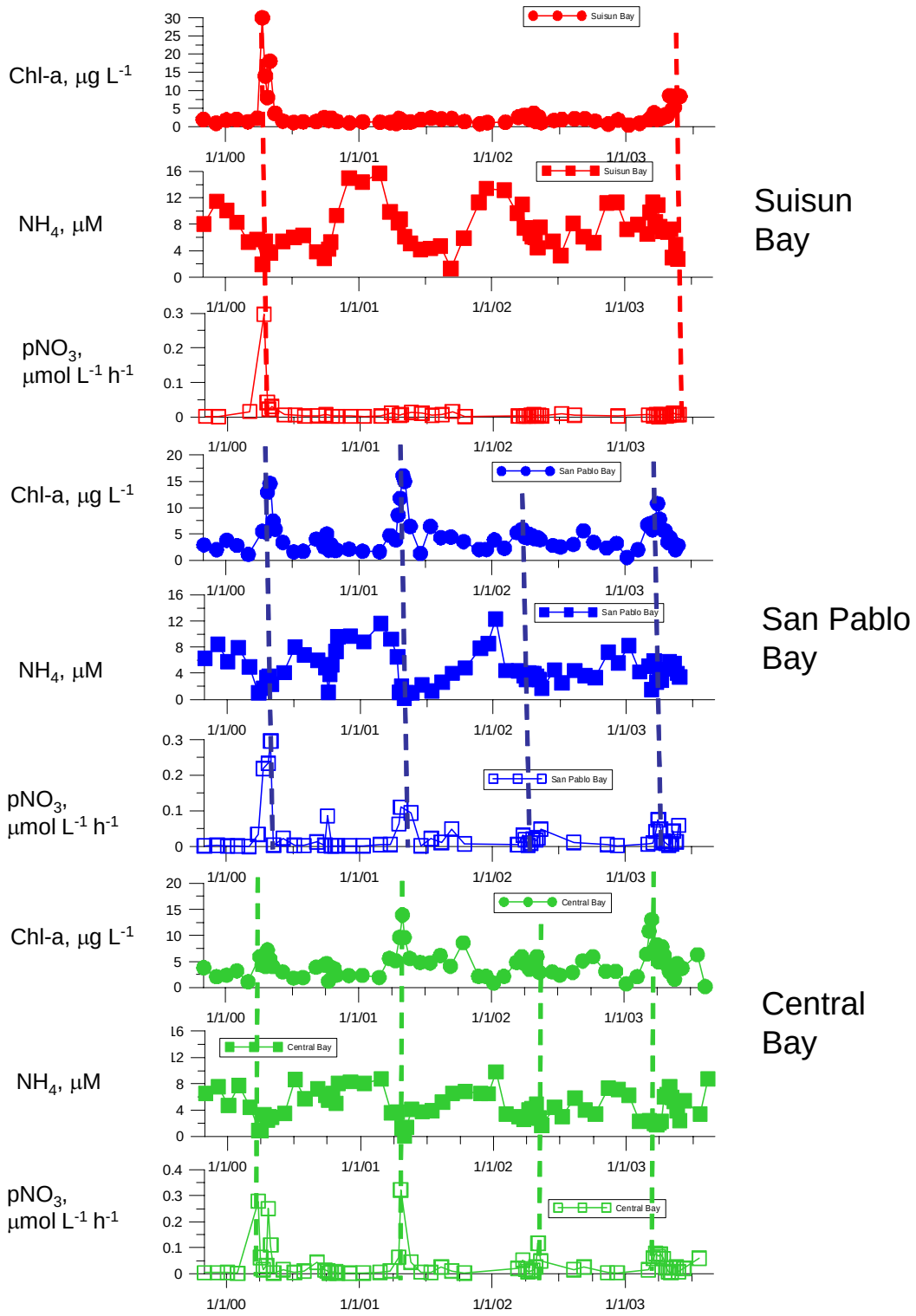


Fig. 7. Time series from Nov. 1999-Aug. 2003 for Suisun (red), San Pablo (blue) and Central Bays (green), dotted lines show timing of annual spring phytoplankton blooms, when chlorophyll is high, NH_4 low and NO_3 uptake high.

Fig. 8

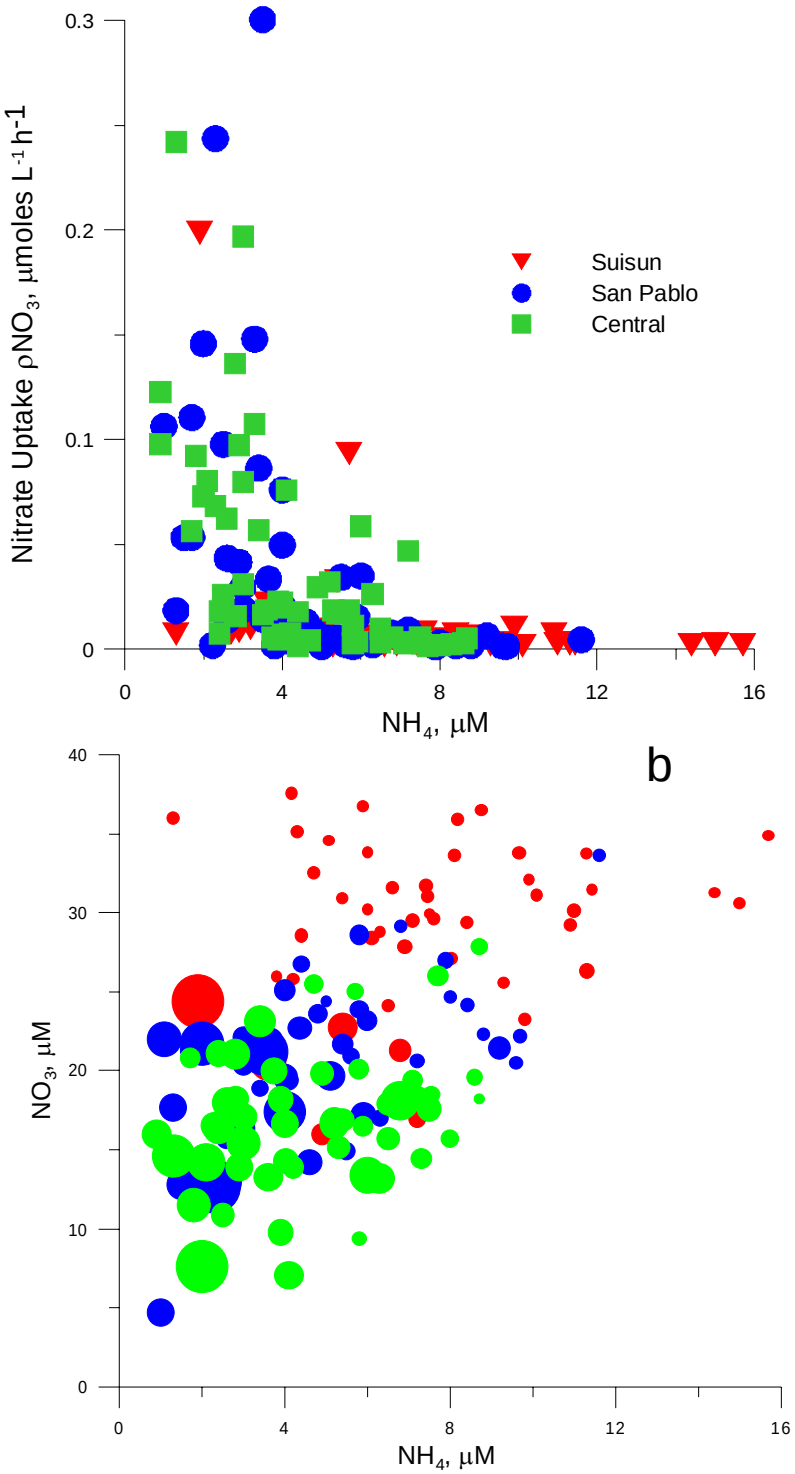


Fig. 8. upper) Nitrate uptake versus NH_4 concentration, for Suisun (red), San Pablo(blue) and Central (green) Bays shows high uptake values and low NH4 concentrations. Lower) More chlorophyll (larger bubbles) results with low NH_4 and high NO_3. Note, Suisun values (red) are all mostly small chlorophyll concentrations and occur at high NH_4 values.

Fig. 9

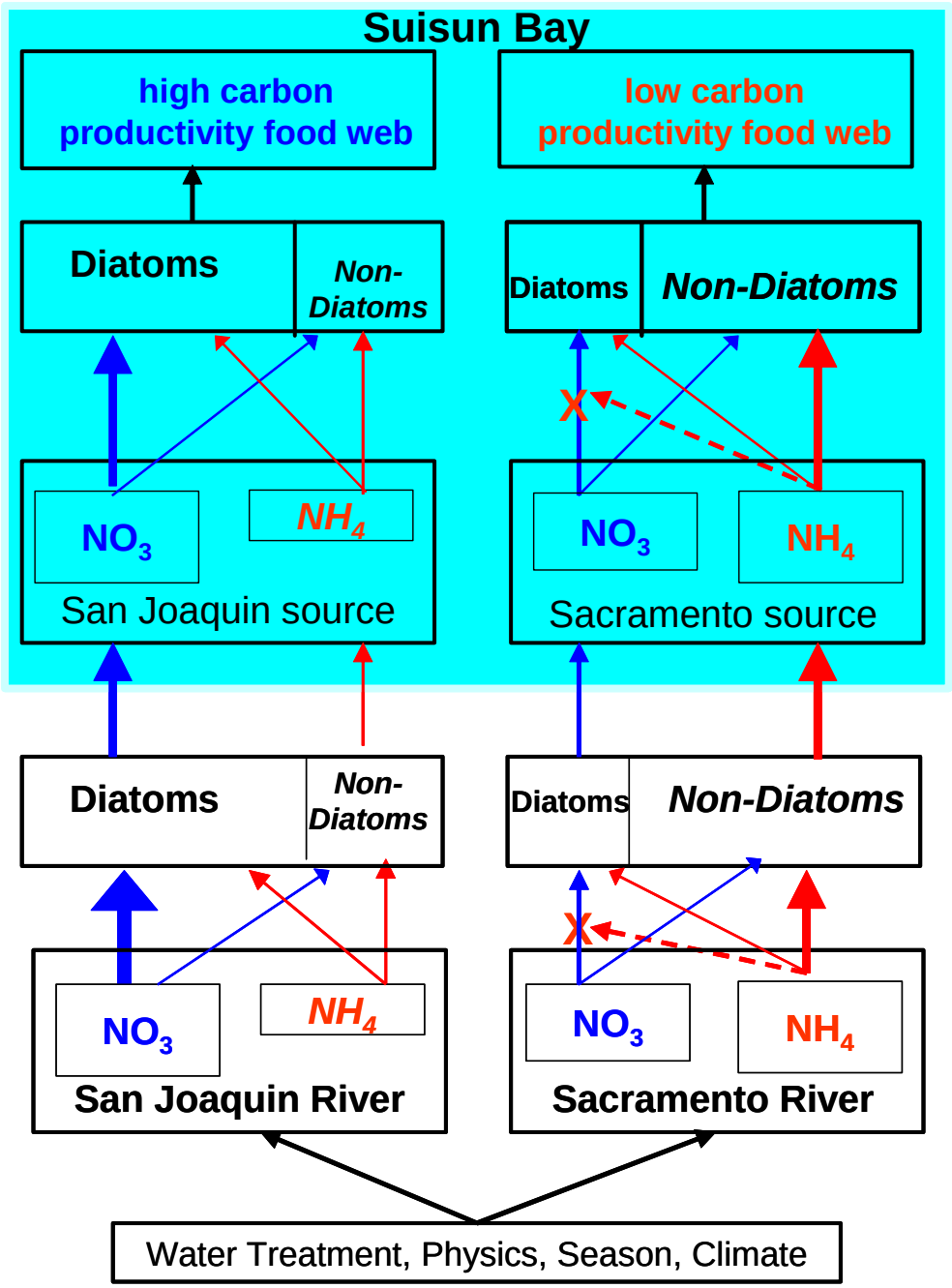


Fig. 9 Conceptual Model. Blue lines show the pathway for NO_3 uptake and transport down-river. Thickness of both NO3 and NH4 lines are proportional to concentration or transport of the particular nutrient. Red lines show NH_4 pathways with a dotted line when it is inhibiting NO3 uptake (shown with a X). All phytoplankton are considered capable to take up NH_4 and NO3 although diatoms are considered to be better competitors for NO_3 and non-diatoms for for NH_4.

Fig. 10

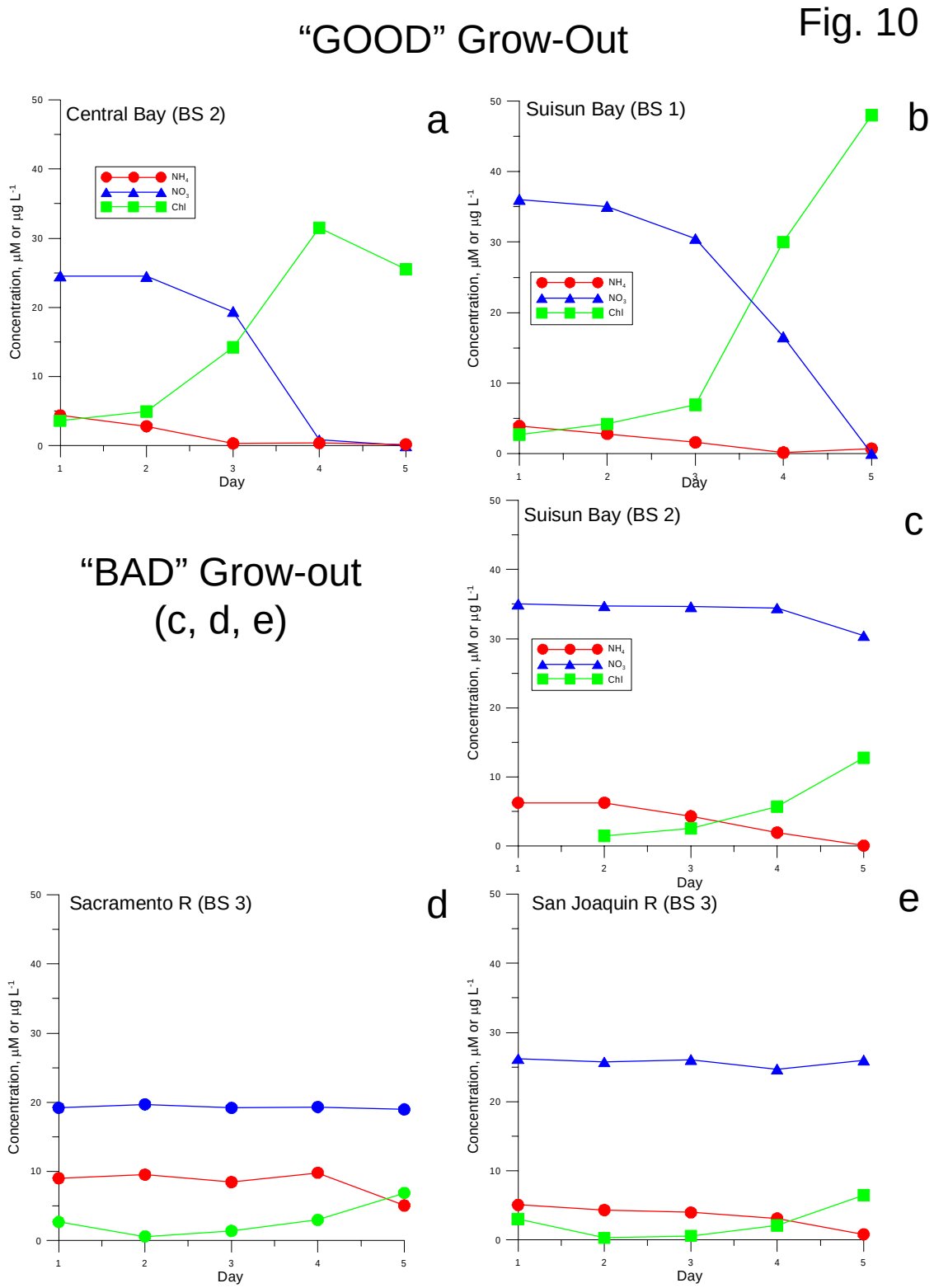


Fig. 10 Enclosure experiments (grow-outs) form our current CALFED project (Bad Suisun) using water from Central and Suisun Bays and from Rio Vista (Sac River) and 8-mile Slough (lower SJ River) showing "good" response (a,b) when NO_3 drawdown occurs and chlorophyll accumulates and "bad" response when ambient NH_4 is elevated and remains elevated, NO_3 is not drawn down and chlorophyll does not increase (c,,d,e). Reason(s) for the low NH_4 uptake rates will be investigated.

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TITLE:	**Bad Suisun Supplement**				
SPONSOR:	**CalFED**				
DURATION:	**2 years, 1 Apr 2008 to 31 Mar 2010**				
			Total	**Total**	**Total**
			Requested	**Requested**	**Requested**
			Yr 01	**Yr 02**	
PERSONNEL					
Principal Investigator, Frances Wilkerson					
$91,706	Academic Year Salary				
$7,642	Per Month				
6%	RRT		5,502	2,889	8,391
3%					
43%	Fringe Benefits		2,366	1,242	3,608
Principal Investigator, Richard Dugdale					
$124,614	Calendar Year Salary				
$10,385	Per Month				
5%	RRT		6,231	3,925	10,156
3%					
11.5%	Fringe Benefits		717	451	1,168
Post-doc, Alex Parker					
$47,250	Calendar Year Salary				
3938	Per Month				
5			19,688	16,538	36,225
4					
49%	Fringe Benefits		9,647	8,103	17,750
Research Technician, V. Hogue					
$50,016	Calendar Year Salary				
$4,168	Per Month				
3.0	months yr1		12,504	4,376	16,880
1.0	months yr 2				
47%	Fringe Benefits		5,877	2,057	7,934
Research Technician, A. Marchi					
$52,076	Calendar Year Salary				
$4,340					
3.0	Months (100% Time/Effort)		13,019	9,113	22,132
2.0	months yr 2				
40%	Fringe Benefits		5,208	3,645	8,853
Graduate Student Research Assistant, To Be Determined (1)					
$15.00					
720	Per Hour				
480	Hours (AY: 20 hrs/wk x 32 wks)		10,800	11,340	22,140
1.5%	Hours (Summer: 40 hrs/wk x 12 wks)		7,200	7,560	14,760
	Fringe Benefits		270	284	554
Total Salaries			74,944	55,741	130,685
Total Benefits			24,084	15,783	39,867
Total Salaries and Benefits			**99,027**	**71,524**	**170,551**
PERMANENT EQUIPMENT					
Subtotal Permanent Equipment			**0**	**0**	**0**
TRAVEL					
Domestic Travel - Annual National Meeting (2 Project Representatives)					
Domestic Travel - Field Work Travel (Local)			3,000	3,150	6,150
Subtotal Travel			500	500	1,000
			3,500	**3,650**	**7,150**
OTHER DIRECT COSTS					
Materials and Supplies (Disposable Lab Supplies)			8,000	8,000	16,000
Overhauls of 2 Flow Cytometers			5,000	0	5,000
Ship time, R/V Questuary			9,000	3,000	12,000
Other Direct Costs Subtotal	20 boat days @ $600 each		**22,000**	**11,000**	**33,000**
Paticipant Support Costs					
Graduate student tuition for 1 student			5,000	5,250	10,250
Paticipant Support Costs Subtotal			**5,000**	**5,250**	**10,250**
Subcontract for algal counts			**20,000**	**38,500**	**58,500**
Total Direct Costs			**149,527**	**129,924**	**279,451**
Indirect Costs			**36,132**	**22,793**	**58,925**
Total Costs		25%	**$185,659**	**$152,717**	**338,377**
Modified Total Direct Cost Base (MTDC)			*144,527*	*91,174*	*235,701*

BUDGET JUSTIFICATION

Overview

Rather than split the budget up into Tasks we have selected to present the overall costs in an easy format that can be split up into 2 tasks later if needed (i.e Project Management and Field Research). This is a joint project with Anke Mueller-Solger who will provide considerable agency match (see below). Her only budget requirements are for algal counts to help verify and interpret the automated Fluoroprobe results, using the same contractor as the DWR Monitoring Program for consistency. These algal identification and enumeration data are also needed by SFSU to verify the automated phytoplankton counts obtained by their Cytosense fluorometer. For simplicity of contracting this cost is included in the one SFSU budget for the entire project. Dr Mueller-Solger requires the proposed field data and shiptime that is requested in the SFSU budget, to fulfill her scope of work with the FluoroProbe.

Salaries at SFSU can only be set up and paid in monthly increments (not hourly) so salaries are reported here as monthly (or fractions of a month) amounts. To convert SFSU uses 173.33 hours to be equivalent to a month of work. Students, however are paid hourly and can work a maximum of 20 hours a week. The system that is used to pay Wilkerson and Dugdale is unique to SFSU, as percent of a semester-worth of reimbursed release time from teaching even though they do not have faculty positions. This is just the mechanism that is used-the equivalent monthly values are provided. Dugdale and Wilkerson will contribute additional time (1 months each year) at no cost to this project.

Fringe Benefits (medical and dental insurance, unemployment insurance, vacation and sick leave, retirement, Social Security; and worker's compensation) are determined in accordance with applicable SFSU policies based on the specific title and nature of each position. These range from 1.5%, to 49% of the salary amount. The indirect cost rate used here was 25% as this has been the amount set in prior CALFED proposals.

Permanent Equipment is not requested in this proposal.

Personnel

There are no new project staff in this supplemental proposal. Dr Mueller-Solger is fully supported by the DWR. Part-time support is requested for Drs, Dugdale (~0.6 mo in year 1, 0.4 mo in Year 2), Wilkerson (~0.7 mo in year 1, 0.4 mo in Year 2), and Parker (5 mo in Y1, 4 mo in Y2), and technicians Al Marchi (3 mo in Y1, 4 mo in Y2) and Vickie Hogue (3 mo in Y1, 1 mo in Y2). A full-time master's level graduate student will assist and use the data towards their thesis. All will participate in field work, transects and grow-out experiments and contribute to project management and preparation of deliverables.

Anke Mueller-Solger will take the lead in determining phytoplankton community structure and cross comparison between the FluoroProbe, CytoSense and microscopy counts. Richard Dugdale as lead PI will be responsible for the project and coordinate it with the current "Bad Suisun" CALFED project (fieldwork and grow-outs downstream) and participate on cruises as will Frances Wilkerson who will also provide logistical help-driving etc. Postdoctoral Fellow Alex Parker will oversee the fieldwork, set up grow-out experiments and participate in all cruises. He will also be responsible for rate measurement incubations and the mass spectrometry of the samples that result. In this he will be helped by technician Vickie Hogue who will prepare samples and help maintain the instrument. Ms Hogue will participate in field work and will also be responsible for all chlorophyll analyses. Technician Al Marchi will be responsible for all nutrient analyses (NO_3, NO_2, $Si(OH)_4$, PO_4 and NH_4) and DIC measurements in the lab and the CytoSense flow cytometer in the field.

Regarding project management all will work up data collected as part of this project and prepare data reports and CALFED technical deliverables (i.e. bi-annual reports, final report, project summaries for the public, requested presentations). In addition Mueller-Solger, Dugdale,

Wilkerson and Parker and the graduate student will present results at IEP meetins, EET workshops and CALFED Science Conferences and state and national level conferences.

Travel

We have requested $3000 each year to ensure that the PI's, technicians and graduate student can present the data at national meetings, and $500 for local travel to Sacramento, the field site etc.

Other: Supplies and Equipment Maintenance

We estimate that annually (Years 1 and 2), field work supplies that include disposables, isotope, chemicals for these five cruises with associated grow-out would cost $8000. We request $500 in year 1 send the two CytoSense flowcytometers to Holland for refurbishment and calibration. Ship time for the RV Questuary at $600 per day (15 days, $9K in year 1 and 5 days, $3K in year 2) is requested.

Contractual Services/Subcontract (see attached letter)

We are requesting $58,500 over the two years to apy EcoAnalysts to identify and enumerate phytoplankton for verification of the FluoroProbe and CytoSense phytoplankton analyses. This commercial company is currently contracted to supply algal counts toi DWR for their monitoring program and we have elected to use them to ensure internal consistency in enumeration, identification and protocols. Their letter of committment is attached.

Match

Matching Funds for this project from RTC, SFSU include salary support for the two PI's and car/truck use and communication and publication costs that are supplied to funded PI's by RTC. Dr R. Dugdale and Dr F. Wilkerson will contribute as matching costs, two months salary each year each PI spread over the Tasks as seems appropriate. Salary match for Anke Mueller-Solger from IEP is estimated at $32K (~4 weeks each year).