

EXHIBIT A: PROJECT NARRATIVE

**Understanding the Effects of Nutrient Forms, Nutrient Ratios and Light Availability on the Lower Food Web of the Delta**

**1. PROJECT PURPOSE**

Statement of the Problem

Flows of energy and materials through the food web of the San Francisco Estuary/Delta are complex and not well understood. As described in detail below, there are several lines of evidence that suggest that changes in nutrient loads, forms or ratios have impacted the food web of the Bay Delta, and may be related to changes in fish species. This proposal aims to explore the relationships between nutrients and changes in the primary producers of the food web through manipulated mesocosm experiments. Uncertainties with regard to the types of phytoplankton that respond to different types of nutrients are central to understand past changes that have occurred as well as to understanding what types of changes in the lower food web might be expected with the many proposed changes in the Bay Delta system that are under consideration.

Shifts in algal composition and food availability have been suggested as an important factor in fish decline (Müller Solger et al., 2002, Kimmerer 2004), but the complexity of factors contributing to stress on the food web, including climate change, habitat changes, increases in toxic algae and toxin loading, among others, has led to a conclusion that multiple stressors have combined to cause a population collapse of pelagic fish (Sommer et al. 2007, MacNally et al. 2010). Identifying the changes at the base of the food web that are linked to changes in their driving factors has been especially difficult (Kimmerer 2004). Phytoplankton productivity in the Bay Delta is generally low due to high turbidity and light limitation (e.g., Cole and Cloern 1984, Cloern 1991, 1996). Nutrients have been considered to be in excess, but not at levels that cause eutrophication (Hager and Schemel, 1992; Cole and Cloern 1984, Kimmerer 2004). Yet, nutrients may shape community composition in complex ways; they do not have to be "limiting" to be important drivers of plankton communities, and thus of food webs. Recent findings are suggestive that elevated nutrients, particularly chemically reduced forms of nitrogen (N), may be inhibitory, rather than stimulatory for phytoplankton production (Wilkerson et al., 2006, Dugdale et al., 2007). Moreover, these results suggest that some members of the phytoplankton community are more sensitive to inhibition than others.

The quality (form) of N has long been recognized to influence the relationship between primary producers and fish. Within the field of oceanography,  $\text{NO}_3^-$ -based food webs are thought to lead to fish (export) production while those based on  $\text{NH}_4^+$  more generally support retentive or microbial food webs in nutrient-depleted marine systems, based on the classic concept of "new" and "regenerated" production (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Glibert, 1998). However, the extent to which this dichotomous control of food webs applies in nutrient-enriched estuarine and coastal systems is unclear. The Bay Delta receives significant inputs of "new" N in reduced form and therefore the question remains as to whether total nutrient load or form controls food webs when loadings are high (e.g., Nixon and Buckley, 2002).

Changes in primary producers in the Bay Delta have been especially pronounced in the past decade, not only because of the increasing frequency and range of blooms of the cyanobacterium, *Microcystis aeruginosa* (Lehman et al., 2005, 2008), but because of declines in diatoms and increases in flagellates (Lehman, 1996; Brown, 2010). Three lines of evidence suggest that nutrient changes are important in this regard. First, based on experimental data, high ammonium ( $\text{NH}_4^+$ ) levels have been shown to inhibit diatom productivity, thus potentially restricting the availability of a preferred food source in the food chain that supports fish (Wilkerson et al., 2006; Dugdale et al., 2007). Second, changes in nitrogen:phosphorus (N:P) ratios of nutrients in the water have been correlated with overall declines in water column chlorophyll a (Chl *a*) of the Bay Delta in the mid-1990s (Van Nieuwenhuysse, 2007). Third, both the long-term increases in  $\text{NH}_4^+$  and the changes in N:P over time were found to be related to changes in the phytoplankton community composition, with cryptophytes and cyanobacteria becoming more important than diatoms as both the  $\text{NH}_4^+$  and the N:P ratio increased (Glibert 2010). However, direct tests of the effects of changes in nutrients have not been undertaken, and thus there are no experimental data to confirm or refute the potential impact of nutrient changes on the lower food web. There are many other stressors in the system, such as the effects of increased grazing by the invasive clam, *Corbula amurensis* (Alpine and Cloern 1992, Kimmerer 2004) and thus disentangling these effects requires direct experimentation. The analyses proposed here extend experiments previously conducted by Dugdale and Wilkerson (Wilkerson et al., 2006, Dugdale et al., 2007). The previous analyses looked at responses by the phytoplankton community after enclosure but without experimental nutrient manipulations. The previous experiments documented the differential responses of resident populations of phytoplankton from different subregions of the estuary (Suisun Bay, Central Bay and San Pablo Bay) following enclosure and a “grow-out” period.

**The critical unknown is the response of the phytoplankton community composition and productivity to changes in nutrient stressors.**

#### Scientific Questions

The overarching question to be addressed in this effort is: *Do the phytoplankton of the Bay Delta respond to changes in nutrient loads, forms or ratios, and if so, are the changes measured experimentally comparable to those observed over time and do they change with light availability?* The specific questions therefore are:

1. Does the phytoplankton community composition change over time (days) when exposed to nutrient conditions that have been altered in terms of nitrogen form (as  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  or urea) or in terms of nitrogen:phosphorus availability?
2. Do the responses by phytoplankton to altered nutrient conditions differ under different light regimes?
3. Do different ambient phytoplankton communities vary in their response to different nutrient and light regimes; does seasonality affect the response?
4. Are there physiological differences between the phytoplankton underlying any responses to nutrient and light regimes?
5. What is the response of the bacterial community compared to the phytoplankton community?

6. If changes in phytoplankton community are documented over time under altered nutrient conditions, do these changes compare with the long-term trends in changes in nutrient loading and phytoplankton community composition observed for the Bay Delta?

### Hypotheses

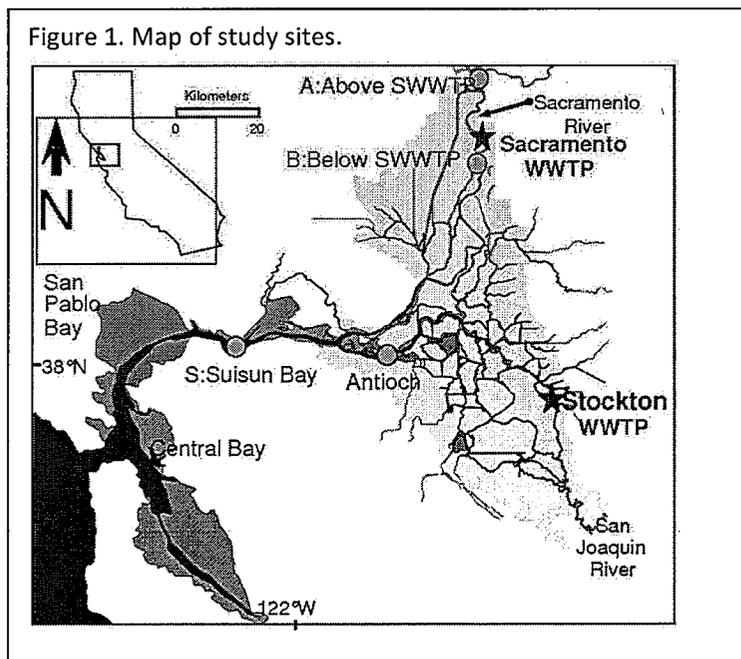
*The overarching hypothesis is that algal functional groups differ in their response to nutrients, and thus alterations in nutrient form or composition will ultimately be related to algal biodiversity. Algal biodiversity affects the food web. The specific hypotheses are:*

1. Elevated loading of  $\text{NH}_4^+$  depresses phytoplankton growth, but is disproportionately detrimental to diatoms.
2. When nutrient availability is skewed in the direction of high N:P, and where N forms are chemically reduced ( $\text{NH}_4^+$  or urea), cyanobacteria will be favored.
3. When nutrient availability is skewed in the direction of low N:P, and where N forms are chemically reduced ( $\text{NH}_4^+$  or urea), dinoflagellates will dominate.
4. When nutrient availability is balanced (Redfield ratio), and where N forms are dominated by oxidized forms ( $\text{NO}_3^-$ ), diatoms will be favored.
5. Reducing the irradiance level will favor heterotrophs (bacteria) and possibly those phytoplankton that can benefit from using particulate nutrients (mixotrophic flagellates).
6. The starting inoculum (i.e., seasonally and spatially varying natural assemblages) will alter the magnitude of the change observed.

## 2. BACKGROUND AND CONCEPTUAL MODEL

### Physical Setting

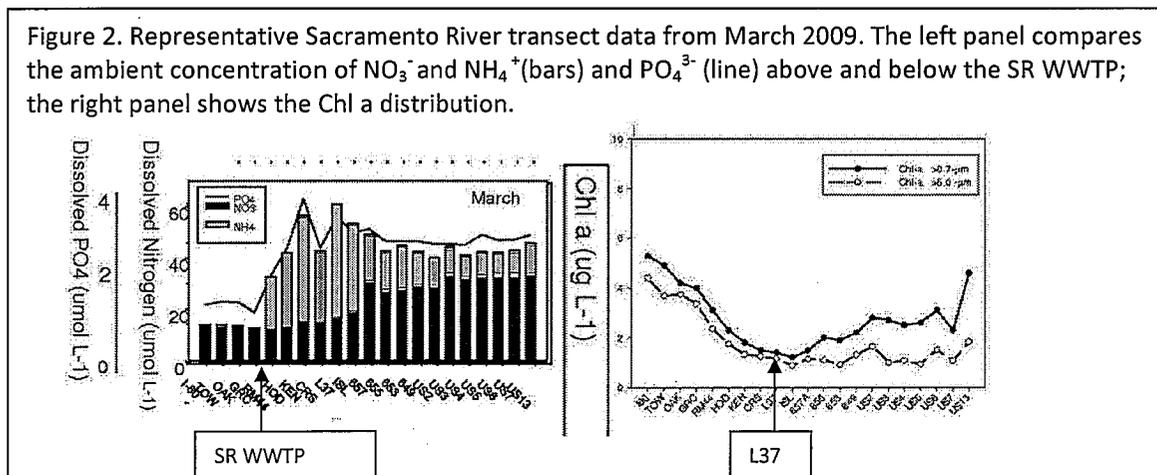
This proposal focuses on the upper Sacramento River, Suisun Bay and the lower San Joaquin at Antioch (Fig. 1). These regions have vastly different ambient nutrients, in quantity and form. The two sites in the Sacramento River will encompass above and below the Sacramento Regional Wastewater Treatment Plant (SR WWTP). The SR WWTP discharges 12 metric tonnes of  $\text{NH}_4^+$  day<sup>-1</sup> in the Sacramento River (Van Nieuwenhuyse 2007, Glibert 2010) and thus is the single most important nutrient source to this river. Antioch was selected as a contrasting site, somewhat removed from the direct influence of SR WWTP effluent and where blooms of *Microcystis* have been regularly documented each summer in recent years (Lehman et



al. 2005, 2008). Nutrients overall are lower in Suisun Bay, but concentrations of  $\text{NH}_4^+$  are still elevated compared to those of  $\text{NO}_3^-$ .

### Trends in Nutrients

Trends in nutrients and size fractionated chlorophyll *a* (Chl *a*) are illustrated by recent surveys of the Sacramento River down to Suisun Bay. Above the City of Sacramento (i.e., route I80 and TOW), the river nutrients are characterized by high levels of  $\text{NO}_3^-$  and low levels of  $\text{PO}_4^{3-}$  with  $\text{NH}_4^+$  near analytical detection level (Fig. 2; March 2009 data shown only). At river mile (RM44), the region of the SR WWTP outfall,  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  increase to high concentrations. These concentrations continue to increase downstream reaching a maximum of about 40  $\mu\text{M-N}$ , near location L37.  $\text{NH}_4^+$  concentrations then decline and  $\text{NO}_3^-$  concentrations increase, likely due to nitrification (Schemel and Hager, 1986; C. Kendall, pers. comm.). Our (Dugdale/Wilkerson) preliminary data show a U-shaped pattern of Chl *a* abundance along the river transect, with maxima in the river above the SR WWTP and in Suisun Bay (as well as seaward to San Pablo Bay), but a minimum near station L37, essentially a mirror image of the  $\text{NH}_4^+$  distribution. Thus, these data are suggestive of an inhibition of Chl *a* by  $\text{NH}_4^+$  in this region. The impact on the community diversity is unknown although size fractionated Chl *a* suggest that larger cells (i.e., > 5  $\mu\text{m}$  diameter) make up to 60% or more of the population for most of the transect, above L37. Preliminary fluoroprobe and flow cytometer data suggest that diatoms dominate where the higher Chl *a* concentrations and lower  $\text{NH}_4^+$  levels occur.



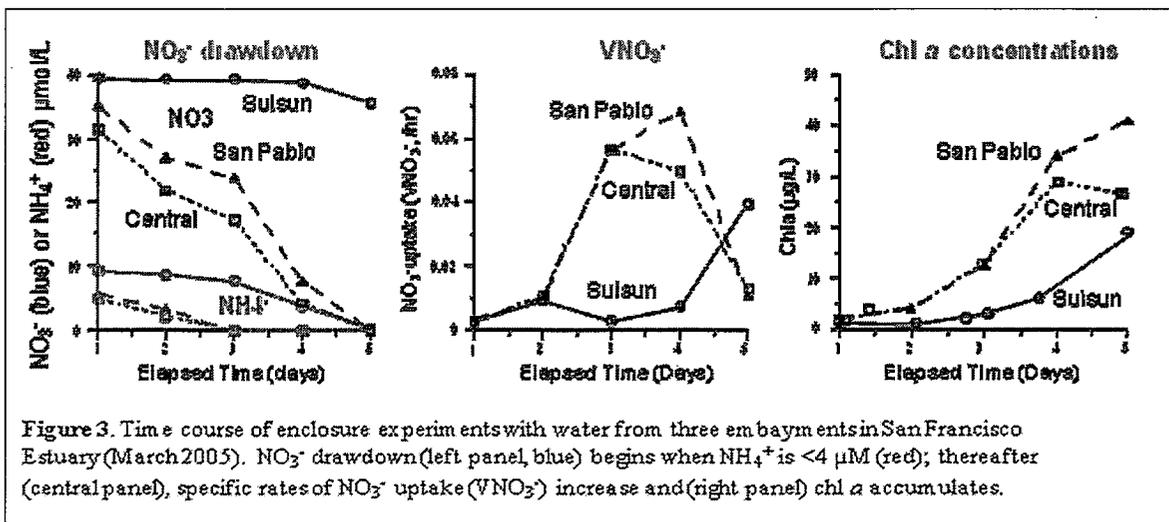
Previous studies on phytoplankton production and nutrient uptake in Suisun Bay, San Pablo Bay and Central Bay lend support to this notion. These bays exhibit a continuum from freshwater waters to the north with high nutrient levels, to the more oceanic, lower nutrient levels of Central Bay (Wilkerson et al. 2006) and have relatively low mean seasonal Chl *a* concentrations, ranging from 1.2-6.5  $\mu\text{g L}^{-1}$ . All three bays are light limited due to high turbidity (Cole and Cloern 1984, Cloern 1991, 1996) but typically have spring blooms. However, Suisun Bay exhibits primary production and nutrient uptake processes that are unlike those of San Pablo and Central Bays: they are significantly lower in value (Wilkerson et al. 2006) and it rarely has spring blooms. It

has been hypothesized that diatom growth is inhibited there due to the higher levels of  $\text{NH}_4^+$  observed there (Dugdale et al. 2007).

### Previous Laboratory and Field Results

Although  $\text{NH}_4^+$  may be a preferred N form by phytoplankton under N limitation, **it can be inhibitory at high concentrations, a phenomenon that has been well described in the physiological literature** (e.g., Syrett, 1981). Interactions between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake have long been known (Dortch 1990). Some of the first observations that  $\text{NH}_4^+$  is assimilated by algae first, and only then is  $\text{NO}_3^-$  get assimilated, were from batch culture experiments in the 1930s-1950s (e.g. Ludwig 1938, Harvey 1953).  $\text{NH}_4^+$  suppression of  $\text{NO}_3^-$  uptake has been documented in phytoplankton isolates (e.g. Caperon and Ziemann 1976, Dortch et al. 1991, Lomas and Glibert 1999b, Maguer et al. 2007) and in natural communities (e.g. Conway 1977, McCarthy et al. 1977, Collos et al. 1989, L'Helguen et al. 1993, Cochlan and Bronk 2003, L'Helguen et al. 2008). Dortch et al. (1991) noted "...the apparent enormous species variation in the interaction between  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake..." Furthermore, the importance of nutrient preconditioning, growth rate (Dortch and Conway 1984, Dortch et al. 1991), and irradiance (Bates 1976) in regulating  $\text{NH}_4^+$ / $\text{NO}_3^-$  interactions have been recognized. Two important conclusions have emerged from previous studies: **Phytoplankton preconditioned to  $\text{NH}_4^+$  are more susceptible to  $\text{NH}_4^+$  inhibition of  $\text{NO}_3^-$  uptake, especially under low light; and  $\text{NH}_4^+$  inhibition increases with decreasing growth rate** (Lomas and Glibert 1999b). Diatoms prefer and, under some conditions, physiologically require,  $\text{NO}_3^-$  over  $\text{NH}_4^+$ , unlike many other algae which preferentially use  $\text{NH}_4^+$  over other N forms (McCarthy et al., 1977; Syrett 1981; Berg et al., 2001; Glibert et al., 2004, 2006).  $\text{NO}_3^-$  is used in the energy balance of these cells as a photoprotective mechanism (Lomas and Glibert, 1999a,b).

In "grow-out" experiments conducted in San Francisco Estuary waters, this phenomenon was documented (Dugdale et al. 2007; Fig. 3). In Suisun Bay,  $\text{NH}_4^+$  of 10  $\mu\text{M-N}$  resulted in cessation of  $\text{NO}_3^-$  uptake by diatom-dominated assemblages. Once  $\text{NH}_4^+$  uptake by other algae decreased  $\text{NH}_4^+$  to < 4  $\mu\text{M-N}$ ,  $\text{NO}_3^-$  uptake and diatom production resumed. Diatoms thus thrived on  $\text{NO}_3^-$ , but could not be sustained when  $\text{NH}_4^+$  levels were high.



The same general pattern of apparent inhibition of diatom growth by elevated  $\text{NH}_4^+$  can be inferred from the time series of nutrients and phytoplankton over many years in Suisun Bay (Glibert 2010). As  $\text{NH}_4^+$  increased in the mid-1980s, coincident with the increased loading from the SR WWTP (Van Nieuwenhuysse 2007, Jassby 2008, Glibert 2010), the abundance of diatoms declined (Fig. 4). The competitive advantage shifted to phytoplankton taxa that could more efficiently use reduced forms of N. Among the phytoplankton groups that replaced diatoms in this system were cyanobacteria, green algae and flagellates (Lehman et al. 1996, 2004, Brown 2010, Glibert 2010).

Cyanobacteria and many flagellates have been shown to have a preference for chemically reduced forms of N in many natural systems (e.g., Berg et al., 2001; Glibert et al., 2004, 2006). For example, measurements of the rates of uptake of different forms of N in Florida Bay have shown clearly that the relative community dominance by

diatoms was related directly to the proportion of N taken up as  $\text{NO}_3^-$ , while that of cyanobacteria was directly proportional to that of  $\text{NH}_4^+$  or urea (Glibert et al. 2004; Fig. 5).

As with  $\text{NO}_3^-$  uptake,  $\text{NH}_4^+$  also inhibits urea uptake by phytoplankton in culture and in natural assemblages (e.g. Rees and Syrett 1979, Lund 1987, Lomas 2004, Jauzein et al. 2008a). For example, urea uptake rates generally decrease after the addition of  $\text{NH}_4^+$  or with increasing ambient  $\text{NH}_4^+$  concentrations (see review by Solomon et al. 2010). Urea uptake rates decreased after the addition of  $20 \mu\text{M-N NH}_4^+$ , but not after the addition of  $20 \mu\text{M-N NO}_3^-$  in field incubations of Baltic seawater (Tamminen and Irmisch 1996). Urea uptake was found to be inhibited or repressed by  $\text{NH}_4^+$  at concentrations higher than  $1\text{-}2 \mu\text{M-N}$  in Oslofjord, Norway (Kristansen 1983),  $40 \mu\text{M-N}$  in the Neuse Estuary, NC (Twomey et al. 2005), and  $5 \mu\text{M-N}$  in Chesapeake Bay (Solomon 2006). **The role of urea, if any, in**

Figure 4. The change over time (monthly sampling) in  $\text{NH}_4^+$  and in diatom abundance in Suisun Bay. Data are from [www.bdat.ca.gov](http://www.bdat.ca.gov) (IEP data portal) and were compiled by Glibert (2010).

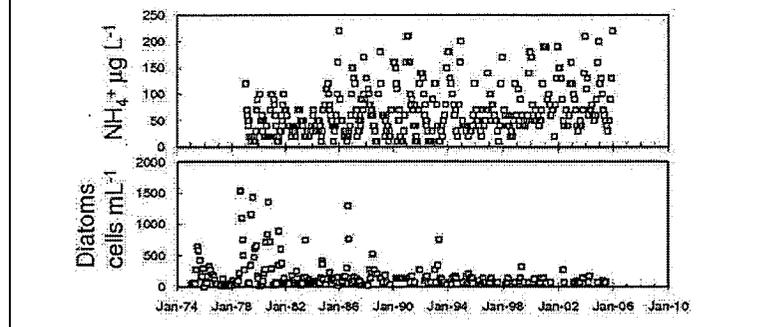
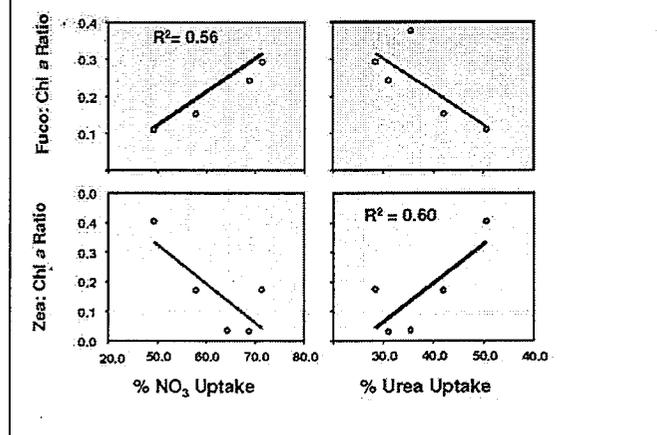


Figure 5. Comparison of the rates of N uptake by form (as  $\text{NO}_3^-$  or urea) and the relative composition of the phytoplankton community (determined by pigment abundance) off the FL coast. Zeaxanthin is indicative of cyanobacteria and fucoxanthin is indicative of diatoms. From Heil et al. (2007).



**plankton dynamics of the San Francisco Bay Delta is unknown.** Ambient concentrations that have been measured to date are low ( $< 2 \mu\text{M}$ ; Dugdale/Wilkerson, unpub. data).

### Complexity of Nutrient and Food Web Changes in the Delta

Changes in the loading of  $\text{NH}_4^+$ , however, are not the only nutrient changes that have occurred over time in the Bay Delta. One of the largest changes in nutrient loading occurred in the mid-1990s when the N:P ratio roughly doubled (Van Nieuwenhuysse 2007, Glibert 2010). This change, as shown here for the Sacramento River near Hood (Fig. 6), was the result of changes in effluent discharge from the SR WWTP (Glibert 2010), and was most likely a result of the removal of P in domestic detergents that occurred around that time (Litke, 1999). Collectively, over time, the changes in nutrient loading has led to an increase in  $\text{NH}_4^+$ , but a decrease in the P available for phytoplankton.

These changes in N form and N:P ratio over time are coincident with 1) decreases in Chl *a* overall; 2) changes in the phytoplankton composition with a decreasing proportion of diatoms; and 3) a change in the food web. As diatoms declined in the system, so too did the copepod *Eurytemora affinis*.

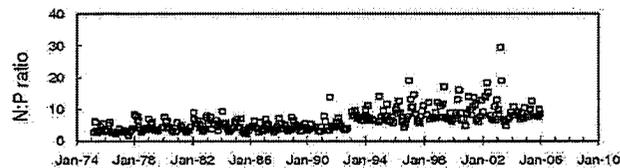
The invasive copepods

*Pseudodiaptomus forbesi* and *Limnoithoina tetraspina* increased in abundance when cryptophytes and green algae increased in abundance (Glibert 2010). While the copepods *P. forbesi* and *L. tetraspina* are considered invasive, they do also respond to food availability. *L. tetraspina*, for example, does poorly when feeding on diatoms and feeds on flagellates and ciliates (e.g., Kimmerer 2004, Bouley and Kimmerer 2006). These relationships are strongly suggestive that **different forms of N and ratios of N:P have major influence on the lower food web.**

Changes in both productivity (rates of growth) and phytoplankton composition are thought to be major determinants of the amount and “quality” (i.e., species composition) of the higher food web in the Bay Delta (e.g., Cloern and Dufford 1995, Mueller-Solger et al. 2002, Glibert 2010). Nutrient changes over time have been correlated with changes in fish populations (Glibert 2010). The elemental composition of fish has been the subject of a considerable number of ecological studies, from fish bioenergetics to whole system nutrient models (e.g., Kraft, 1992; Vanni, 1996; Sterner and George, 2000). Fish composition and fish size previously have been related to nutrient availability. Sterner and George (2000) speculated that the P content of fish “relates to their ‘boniness’”. Clearly there is much to be examined with regard to the ecological stoichiometry of all the components of the food web and how changes in the nutrient availability may be related not only to the food web of the Bay Delta. This proposal aims to focus specifically on those changes at the base of the food web.

Understanding how nutrient changes may alter the “quality” and productivity of phytoplankton communities does not eliminate the potential for other “stressors” to be important in driving the

Figure 6. The change over time (monthly sampling) in N:P ratio ( $\mu\text{g L}^{-1}:\mu\text{g L}^{-1}$ ) in the Sacramento River. Data are from [www.bdat.ca.gov](http://www.bdat.ca.gov) (IEP data portal) and were compiled by Glibert (2010).



food web of the Bay Delta system. For example, In Suisun Bay, the historic decline in phytoplankton abundance (measured as Chl *a*) has also been suggested to be the result of grazing by the introduced clam, *Corbula amurensis* (Cloern and Alpine 1991, Kimmerer 2004). While clams may continue to keep phytoplankton Chl *a* low due to their filtering, the decline in diatoms witnessed before the clam invasion (in 1982; Cloern and Alpine 1991) is better explained by the inhibitory effect of elevated  $\text{NH}_4^+$  loading at that time than due to the clam invasion (Glibert, 2010). Clams also have been shown to consume *E. affinis* nauplii (Kimmerer et al., 1994), but *E. affinis* was already in decline before clams became well established. Moreover, the clam population is at a minimum seasonally during the spring which is the period when diatoms are most common. Thus, *Corbula* may be holding overall Chl *a* levels low, while community composition and growth rates are likely influenced to a greater degree by nutrients.

Climate variability and changes in river flow may also affect phytoplankton composition through nutrient changes. For example, under periods of low flow, the point source discharges of  $\text{NH}_4^+$  represents a greater fraction of the total N load in the upper Sacramento River, while under high flow there is greater dilution of the effluent  $\text{NH}_4^+$  by other riverine nutrients. As reported from the long-term time series of nutrient data (Glibert 2010), under very low flow conditions (1987-1993), the ratio of  $\text{NO}_3^-:\text{NH}_4^+$  changed to a greater degree from upstream (Sacramento River) to Suisun Bay, suggesting a greater degree of nitrification was occurring when flow was low.

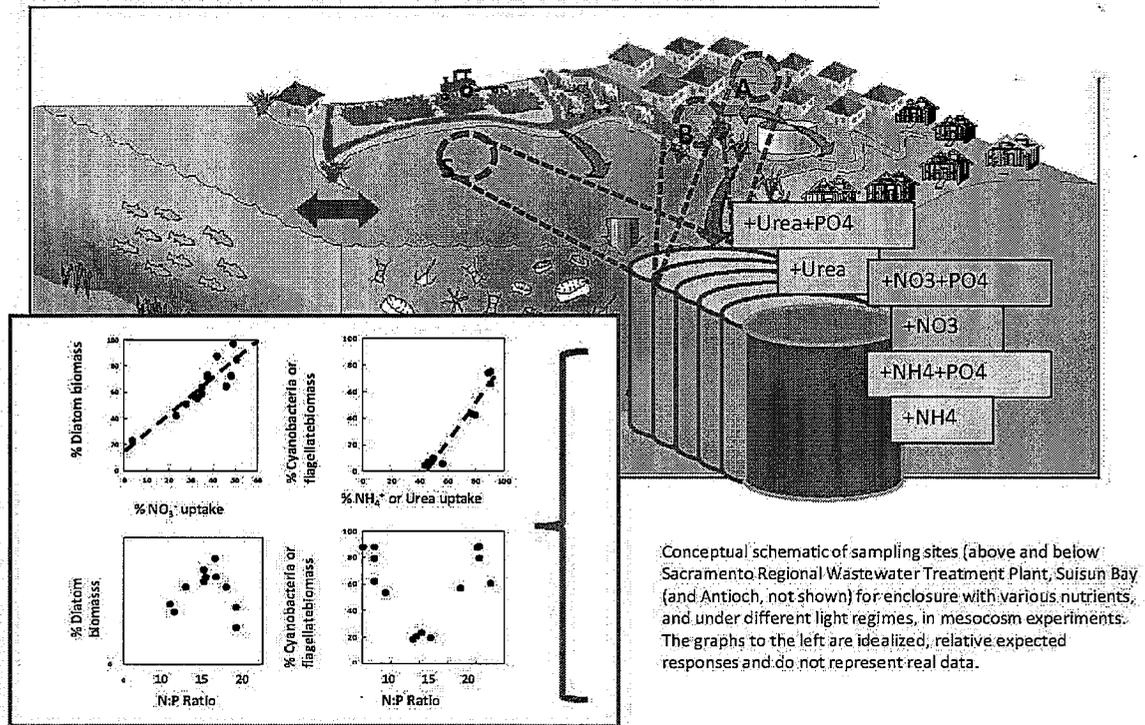
***In sum, changes in the availability of reduced and oxidized N forms (e.g.,  $\text{NH}_4^+$  or urea vs.  $\text{NO}_3^-$ ), as well as the relative availability of N to P, are thought to affect microbial biodiversity, which, in turn, influences the food web. While there is much known about phytoplankton N metabolism, there is very little known about how  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , or urea uptake and metabolism vary among different phytoplankton species exposed to varying N concentrations or forms, and especially about how such relationships may change with P availability. Moreover, these relationships are also affected by the light availability, as heterotrophic processes may begin to dominate over autotrophic processes at low light levels. Even less is understood at the current time about the collective interactions of these factors. The ability to quantitatively link individual taxa-level responses to individual and combined nutrient and light changes is a necessary step toward understanding ecosystem scale rate processes. Algal functional groups differ in their physiological regulation of N uptake, and changes in physiological regulation when reduced N forms are abundant strongly influence algal biodiversity.***

#### Conceptual Model

Our conceptual model is that changes in N form, N:P ratio and light availability will alter the phytoplankton community composition. As noted above, we hypothesize that when  $\text{NO}_3^-$  is proportionately abundant relative to  $\text{NH}_4^+$  (and the N:P ratio is suitable), diatoms will dominate, but when  $\text{NH}_4^+$  is proportionately abundant, cyanobacteria or flagellates will become dominant. Reduced light availability will lead to communities that have higher bacterial abundance, and/or higher proportions of flagellates that are able to alter their nutrition to mixotrophy (Burkholder et al. 2008). A direct experimental test of whether nutrient loads, forms and ratios can drive different phytoplankton community composition has never been undertaken in this system. By conducting experimental manipulations with different ambient communities (above and below the SR WWTP, Suisun Bay and the San Joaquin at Antioch), and at different seasons, we will directly test whether algal community composition is responsive to changes in N form, N:P and

light (Fig. 7) and how such responses differ when the ambient populations and conditions change.

Figure 7. Conceptual diagram of proposed experiments and anticipated responses from manipulated mesocosm experiments. The sites of sample collection range from the upper Sacramento River (above and below the SR WWTP, "A" and "B") to Suisun Bay ("S") and Antioch (not shown); each represents a site with differing ambient phytoplankton community composition. Moreover, by varying season, the effects of other naturally varying physical factors will be taken into account, as will varying physiological states of the communities.



### Scientific Objectives

Our scientific objectives are thus to:

1. Conduct a series of mesocosm experiments in which the changes in biomass, community composition (phytoplankton only) and rates of production of phytoplankton and bacteria in response to altered nutrient and light conditions will be measured;
2. Compare responses of similarly conducted experiments with sample water collected from different sites and seasons to assess how different assemblages, representing different communities and different physiological states, respond;
3. Synthesize these responses together with other available data from previous related projects and long-term data into a broad understanding of how different algal groups respond to, and have responded over time, to perturbations in nutrients;

4. Integrate these findings with existing knowledge to comprehensively explain the implications of anthropogenic nutrient loading on coastal ecosystems to management groups and in outreach efforts.

### 3. APPROACH AND SCOPE OF WORK

#### Tasks and Schedule

The scope of work has been split into 7 tasks. Task 1 will be ongoing throughout the project and is required by the Delta Science Program to be the administrative task and includes synthesis and communication of findings. Tasks 2-7 are experimental, but are differentiated by the site of water collection, season, and year for the experiments and have specific timelines.

#### **Task 1. Project Management**

This task represents project oversight, data handling, preparation of reports, communicating results to Delta Science Program and the other funding authorities. This task also includes outreach activities and synthesis of results, publication, and communication. It involves most of the personnel, although Task 1-specific salary funds for SFSU are not requested. The lead institution is the University of Maryland Center for Environmental Science.

**Task 2.** Experimental studies comparing the responses of the phytoplankton and bacterial communities above and below the Sacramento Regional Wastewater Treatment Plant *in summer*

**Task 3:** Experimental studies comparing the responses of the phytoplankton and bacterial communities above and below the Sacramento Regional Wastewater Treatment Plant *in spring*

**Task 4:** Experimental studies comparing the responses of the phytoplankton and bacterial communities above the Sacramento Regional Wastewater Treatment Plant and in Suisun Bay *in summer*

**Task 5:** Experimental studies comparing the responses of the phytoplankton and bacterial communities above the Sacramento Regional Wastewater Treatment Plant and in Suisun Bay *in spring*

**Task 6:** Experimental studies comparing the responses of the phytoplankton and bacterial communities above the Sacramento Regional Wastewater Treatment Plant and in the lower San Joaquin at Antioch *in summer*

**Task 7:** Experimental studies comparing the responses of the phytoplankton and bacterial communities above the Sacramento Regional Wastewater Treatment Plant and in the lower San Joaquin at Antioch *in spring*

#### Scientific Approach for Tasks 2-7

Each spring and summer, water will be collected from the region of study (i.e., upper Sacramento River, Suisun Bay, lower San Joaquin) using the R/V Questuary. Water will be

characterized using a Seabird SBE-19 plus CTD, at the surface and at two other depths for temperature, salinity, and turbidity. Samples will be collected for later determination of inorganic nutrients ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{Si}(\text{OH})_4$ ), organic nutrients (DON, DOP), inorganic carbon (DIC), Chl *a* (by size class), algal pigment composition, cell size spectra by flow cytometry, and bacterial abundance. These are essentially the same approaches used in the studies of San Francisco Estuary by the Dugdale/Wilkerson/Parker team for the past 10 years, with some additional parameters. Light attenuation, *k*, will be calculated by linear regression of log transformed PAR from shipboard Licor 4II PAR sensor versus depth. Turbidity (ntu) will be measured with a D & A OBS turbidity sensor.

Water will be collected in large carboys for return by boat to the Romberg Tiburon Center to initiate the mesocosm experiments. Mesocosms are experimental tools that provide an “intermediate scale” of analysis, adding more realism than laboratory culture studies, but lacking the complexity of field-based experiments (Peterson et al. 2009). Mesocosm experiments will be conducted to understand how algal physiology and biomass in varying natural communities, at physiological and taxonomic levels, changes over time in response to varying nutrient conditions. These experiments are similar to “bioassays” in that biomass responses (i.e., change in Chl *a* or bacterial abundance) to nutrient manipulations will be measured. They differ from “bioassays”, however, in that in addition to biomass responses, the change in algal composition, as well as *the changes in rates* of algal and bacterial productivity and N uptake, will also be measured. The mesocosms will be 20 L in size.

Mesocosm experiments will be conducted twice in each project year. The enrichment treatments will vary depending on ambient conditions, but we will strive to assess the effects of: 1) control (no additions); 2) plus  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , or urea; 3) the same N treatments +  $\text{PO}_4^{3-}$  (see Table 1). The actual amounts of N and P to be added will be determined based on the ambient conditions, and thus not all additions will be appropriate for all stations (for example, if  $\text{NH}_4^+$  is already elevated, no further addition of that substrate will be made). The amount of  $\text{PO}_4^{3-}$  to be added will also depend on ambient conditions. Mesocosms will be run at two levels of irradiance: 50% and 10% of natural irradiance. Experiments will be run for up to 10 days, but responses likely will be greatest in the first few days (as illustrated above in Fig. 3). The total mesocosm treatments to be tested in each experimental trial will be ~16. Replication of all treatments will be undertaken, emphasizing the biomass parameters. Ultimately, these experiments are designed to ask: *Do taxonomic changes occur over a scale of days that reflect the differences in physiological responses to different N forms, different N:P ratios or different levels of light availability?*

Supplementing the measurements over time will be measurements of the ambient rates of uptake from the sample water at the time of collection (note that it will require up to a full day following sample collection for all mesocosms to be set up as analysis of ambient nutrients will be required). The measurements to be made at the time of sample collection will also involve varying substrate concentration and light level.

*Table 1. Proposed detail for nutrient additions for each set of experiments to be conducted each year. Experiments will be repeated spring and summer. "A" indicates site above the WWTP; "B" just below the WWTP; "S" for Suisun Bay and "SJ" indicates the lower San Joaquin at Antioch (for locations see Map, Fig 1). "LL" indicates low light treatment. All treatments and nutrient addition levels are subject to change depending on ambient conditions at the time of sample collection and are provided as **examples only**.*

Treatment number	Yr 1: Above "A" and below "B" WWTP	Yr 2: Above "A" and Suisun "S"	Yr 3: Above "A" and San Joaquin @Antioch "SJ"
1	A-control	A-control	A-control
2	A-LL-control	A-LL-control	A-LL-control
3	A-+30 $\mu\text{M NH}_4$	A-+30 $\mu\text{M NH}_4$	A-+30 $\mu\text{M NH}_4$
4	A-LL+30 $\mu\text{M NH}_4$	A-LL+30 $\mu\text{M NH}_4$	A-LL+30 $\mu\text{M NH}_4$
5	A-+30 $\mu\text{M NH}_4 + \text{PO}_4$	A-+30 $\mu\text{M NH}_4 + \text{PO}_4$	A-+30 $\mu\text{M NH}_4 + \text{PO}_4$
6	A-LL+30 $\mu\text{M NH}_4 + \text{PO}_4$	A-LL+30 $\mu\text{M NH}_4 + \text{PO}_4$	A-LL+30 $\mu\text{M NH}_4 + \text{PO}_4$
7	A-+ 10 $\mu\text{M urea}$	A-+30 $\mu\text{M NO}_3$	A-+30 $\mu\text{M NO}_3$
8	A-LL+10 $\mu\text{M urea}$	A-+30 $\mu\text{M NO}_3 + \text{PO}_4$	A-+30 $\mu\text{M NO}_3 + \text{PO}_4$
9	A-+30 $\mu\text{M NO}_3$	S-control	SJ-control
10	A-LL+30 $\mu\text{M NO}_3$	S-LL-control	SJ-LL-control
11	A-+30 $\mu\text{M NO}_3 + \text{PO}_4$	S-+30 $\mu\text{M NH}_4$	SJ-+30 $\mu\text{M NH}_4$
12	A-LL+30 $\mu\text{M NO}_3 + \text{PO}_4$	S-LL+30 $\mu\text{M NH}_4$	SJ-LL+30 $\mu\text{M NH}_4$
13	B-control	S-+30 $\mu\text{M NH}_4 + \text{PO}_4$	SJ-+30 $\mu\text{M NH}_4 + \text{PO}_4$
14	B-LL-control	S-LL+30 $\mu\text{M NH}_4 + \text{PO}_4$	SJ-LL+30 $\mu\text{M NH}_4 + \text{PO}_4$
15	B-+ $\text{PO}_4$	S-+30 $\mu\text{M NO}_3$	SJ-+30 $\mu\text{M NO}_3$
16	B-LL+ $\text{PO}_4$	S-+30 $\mu\text{M NO}_3 + \text{PO}_4$	SJ-+30 $\mu\text{M NO}_3 + \text{PO}_4$

Each mesocosm will then be sampled daily (every other day after the first several days) for temperature turbidity, inorganic and organic nutrients, DIC, chlorophyll (size fractionated), algal taxa and pigment composition, cell size spectra using flow cytometry, primary productivity, bacterial productivity, and rates of N uptake. The schedule of sampling is detailed in Table 2.

Table 2. Schedule of daily sampling of each mesocosm.

Day 0	Day 1	Day 2	Day 3	Day 4	Day 5/6	Day 7/8	Day 9/10
Nutrients	*	*	*	*	*	*	*
Total and size-fractionated Chl <i>a</i>	*	*	*	*	*	*	*
Phytoplankton pigments (HPLC)	*		*		*		*
Phytoplankton composition	*	*	*		*		*
Rates of NO <sub>3</sub> , NH <sub>4</sub> and Urea uptake	*	*	*		*		*
Primary production	*	*	*		*		*
Bacterial numbers and production rates	*	*	*		*		*

### Detailed Methods

#### *Nutrients and dissolved inorganic carbon*

Dissolved inorganic and organic nutrient and carbon concentrations will be analyzed following procedures in Whitley et al. (1981 - NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>-3</sup>), Bran and Luebbe (1999 - Si(OH)<sub>4</sub>), Solórzano (1969 - NH<sub>4</sub><sup>+</sup>), Revilla et al. (2006 - urea), Friederich et al. (2002) and Parker (2005) and Parker et al. (2006 ;DIC), Sharp et al. (2004 - DOC, TDN), Bronk et al. (2000-total N, DON), and Solórzano and Sharp (1980-TP, DOP). Particulate organic carbon and nitrogen (POC and PON) will be determined by elemental analyzer.

#### *Phytoplankton biomass and composition*

Phytoplankton biomass will be estimated from extracted Chl *a*, size fractionated for cells >5 µm and <5 µm and > 75 µm in *Microcystis* conditions, using the protocol of Arar and Collins (1992) and a Turner Designs Model 10 fluorometer. Typically in the Bay Delta the larger cells represent the diatom population (Cloern and Dufford, 2005) The size distribution of fluorescent particles will be made using a CytoSense flow cytometer in 20 ml samples (Dubelaar and Gerritsen 2000). Phytoplankton samples will be preserved with acidic Lugol's solution. Light microscopy with phase and epifluorescence will be used to identify and enumerate abundant algal taxa.

Samples will also be collected on filters, as for Chl *a*, and flash frozen for later analysis by high performance liquid chromatography (HPLC; Van Heukelem and Thomas 2001). Both accessory pigments and microscopic enumerations will be used to indicate the presence of dominant groups of algae. Although unambiguous interpretation is difficult using only pigment data, in conjunction with microscopic identifications, it is possible to use zeaxanthin as an indicator of cyanobacteria, fucoxanthin as an indicator of diatoms and some chrysophytes, and peridinin as indicative of photosynthetic dinoflagellates (e.g. Jeffrey and Wright 1994, Jeffrey and Vesk 1997, Ansotegui et al. 2001). The application of software such as CHEMTAX (Mackey et al. 1996) will be explored, but will depend on availability of appropriate calibration factors for the relevant species (Lewitus et al. 2005). Lewitus et al. (2005), for example, found that there was a tendency for and overestimation of diatom biomass and an underestimate of some raphidophyte species with CHEMTAX. By combining these analyses with microscopic verification, much more confidence in phytoplankton composition will be gained.

Heterotrophic bacterial production will be estimated by the microcentrifuge method of Kirchman et al. (2001) using  $^3\text{H}$ - leucine. Bacterial abundance will be determined from slides prepared with a DNA-binding stain and visualized by epifluorescence microscopy (Hobbie et al. 1977).

#### *Phytoplankton productivity and Nitrogen Uptake Measurements*

Rates of C uptake (primary production) will be estimated with dual labeled  $^{15}\text{N}/^{13}\text{C}$  stable isotope tracer techniques (Slawyk et al. 1977, Legendre and Gosselin 1996) to yield simultaneous N and C uptake rates from a single sample.  $^{13}\text{C}$  estimates of primary production are reliable and consistent with the  $^{14}\text{C}$  method in estuaries (Parker 2004). Uptake rates of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and urea will be determined by  $^{15}\text{N}$  isotopic tracer techniques. For all  $^{15}\text{N}$  uptake experiments, water will be inoculated with either  $\text{Na}^{13}\text{CO}_3$  and  $\text{K}^{15}\text{NO}_3$ , or  $\text{Na}^{13}\text{CO}_3$  and  $^{15}\text{NH}_4\text{Cl}$  or  $^{15}\text{N}$ -urea (99 atom% enriched). Levels of addition will vary by experiment, but will not be less than 10 atom%. Immediately following inoculation, one sample will be filtered onto a precombusted GF/F filter (450°C, 4 hr) by gentle vacuum to determine the initial values. Incubations will be conducted at ambient temperature and light intensities of the mesocosm (50% or 10% surface irradiance), and will be terminated after incubation of several hours. Incubations will be terminated by gentle vacuum filtration onto precombusted GF/F filters. Isotopic analyses will be determined using a Europa 20/20 mass spectrometer (Wilkerson/Dugdale lab) or a Sercon mass spectrometer (Glibert lab). N and C uptake rates will be calculated based on isotopic enrichment according to Dugdale and Wilkerson (1986).

#### Equipment and Facilities

The collaborating laboratories are well equipped to conduct these experiments. Dugdale and Wilkerson each have an analytical chemical/biological laboratory at the Romberg Tiburon Center, San Francisco State University (RTC) with appropriate equipment. Instrumentation and analytical capability includes muffle furnaces, spectrophotometers, fluorometers, balances, micropipettes, filtration racks, and pumps. Wilkerson also has a designated radioactivity area for  $^{14}\text{C}$ ,  $^3\text{H}$  and  $^{32}\text{Si}$  uptake analyses. Small boats and the R/V Questuary are available for water sampling. Jointly shared equipment (image processing and production laboratory, microscopes, scintillation counters, etc) are also available at RTC. For the mesocosm experiments, extensive

water tables and plexiglass incubator facilities are available. RTC monitoring site SFBeams (CTD, weather, fluorometer, transmissometer, PAR) is available with real-time data.

At RTC, the following major equipment in the Dugdale and Wilkerson lab will be used:

- Braun and Luebbe Technicon 11 AutoAnalyzer for nutrient measurements
- Europa PDZ 20/20 mass spectrometer for measuring  $^{15}\text{N}$  and  $^{13}\text{C}$  enrichment and POC and PON biomass
- DIC analytical system
- Cytobuoy flow cytometer for pico-to microplankton size analysis,
- Zeiss inverted microscope

Glibert has a fully equipped laboratory at the Horn Point Laboratory (HPL), University of Maryland Center for Environmental Science. She has extensive experience conducting these types of experiments in various locations and can bring to RTC filtration racks and pumps, micropipettes, fluorometer, and incubation supplies. At HPL she has the following major equipment in her lab or in shared facilities which will be used in this project:

- Sercon mass spectrometer for measuring  $^{15}\text{N}$  and  $^{13}\text{C}$  enrichment and POC and PON biomass
- Finnigan mass spectrometer
- Agilent HPLC for pigment analysis
- Microplate reader for micro-scale analysis of urea
- Autoanalyzer, CHN analyzer, various microscopes,

#### Statistical Analyses and Quality Assurance

In addition to the data analysis approaches described above, statistical differences between sites, treatments and seasons will be compared using ANOVA. The Shannon-Weiner Diversity Index value is one approach that will be used for tracking changes in the composition of the community over time:

$$H' = - \sum_{i=1}^s p_i \log_2 p_i \quad (1)$$

$$p_i = N_i/N \quad (2)$$

where  $N_i$  is the number of individuals of species  $i$ , and  $N$  is the number of individuals in the sample and  $s$  is the number of species. Final choice of statistical treatment for the various types of data and experiments will be determined once data are available. All treatments will be fully replicated, but the level of replication will vary with individual chemical, biomass or rate measurement analyses. We will aim to replicate 30% of all  $^{15}\text{N}$  samples each year. Data from experimental mesocosms will be interpreted not only in the context of the individual experiments, but in the broader context of Bay Delta data. Not only have the co-PIs been involved in regular sampling of the rivers and have considerable data from seasonal transects, but long term data is available from the IEP web portal for broader analysis. Interpreting all experimental data in the context of ambient data, both current and past trends, will be an ongoing effort.

All PI's involved in each of these analyses have considerable experience with the respective analyses for which they are responsible. The Dugdale/Wilkerson/Parker team will have primary responsibility for inorganic nutrient analysis, primary production, and bacterial abundance and production. The Glibert laboratory will have responsibility for organic nutrients, and pigment analyses. Both the Dugdale/Wilkerson and Glibert laboratories will process the  $^{15}\text{N}$  samples, as these will be numerous and their analysis time-consuming. Intercalibrations between our laboratories will be performed on an annual basis.

RTC/SFSU carries out nutrient analyses for the USGS Polaris monthly transects, SFEI and SFRWQCB and the procedures used have been authorized as "Surface Water Ambient Monitoring Program (SWAMP) comparable by the Regional Water Quality Control Board. The following standard operating procedure manuals and method descriptions are available for nutrient analysis and guide the methods in use at HPL:

Lane, L., S. Rhoards, C. Thomas and L. vanHeukelem. 2000. Analytical services laboratory standard operating procedures. Tech Report TS-264-00. Horn Point Laboratory, Cambridge, MD 67 pp.

Overall accuracy and precision are routinely checked against standards and against spiked samples. Samples are run concurrently with standards of the respective analyte. Our research laboratories carry out high quality analyses on a routine basis and poor quality data is readily recognized by the PI's and by senior technicians.

The HPL laboratory conducts all the algal pigment calibration analyses for NASA. Protocols are well in place for shipping and storage of samples without degradation. Pigment analysis will follow procedures as recommended by:

VanHeukelem, L., C. Thomas and P.M. Glibert. 2001. Sources of variability in chlorophyll *a* detection by fluorometry and by high performance liquid chromatography. NASA Technical Report, G. Fargon (ed).

### Expected Deliverables

*Specific to Delta Science Program:* Semi-annual reports and a final report, presentations at the Delta Science annual conferences, and copies of all published material resulting from the project. In addition, outreach materials will be made available at the start and at the end of the project. P. Glibert has extensive experience producing richly illustrated materials for public audiences, as shown in the example in Fig. 8.

*Website:* A general description of the project will be placed on the websites of both collaborating laboratories. A password protected page will be set up for sharing data files.

*Presentations at Workshops, Seminars, and Conferences and Management Groups:* Project updates will be presented at the quarterly Estuarine Ecology Team meeting of the Interagency Ecological Program by Dugdale, Wilkerson or Parker. Presentations by lead PIs will also be made at the annual IEP workshops, the Delta Science conferences, as well as at national conferences such as the Coastal and Estuarine Research Federation and/or Limnology and

Oceanography meetings. Seminars and other presentations, such as to the SFB Regional Water Quality Control Board or their working groups will be made upon request.

**Publications:** The lead PIs have strong publication records and it is anticipated that results from this effort will be prepared for various journals that target the estuarine and plankton research communities, such as *Estuaries and Coasts*, *Marine Pollution Bulletin*, *Aquatic Microbial Ecology*. In addition, a publication may be considered for the on-line journal, *San Francisco Estuary and Watershed Science*.

**Outreach:** We will be involved in teacher workshops at both the Romberg Tiburon Center and Horn Point Laboratory, to improve middle and high school teachers' understanding of marine science, including hands-on activities. We will collaborate with SFSU's Science Education Partnership and Assessment Laboratory (SEPAL) Program that provides pedagogical instruction for graduate students and brings K-12 teachers together with SFSU graduate students to foster the development of science curriculum, and offers competitive graduate fellowships (\$30k/yr). There is also a teacher training and intern program at UMCES through their NSF COSEE Coastal Trends program. We will also participate in the BayQuest Education Program, a cooperative education experience run by the Bay Model Association, the US Army Corps of Engineers and RTC. Currently this program reaches 350 bay area K-12 grade students each years and runs as separate teacher training programs for as many as 70 bay area educators.

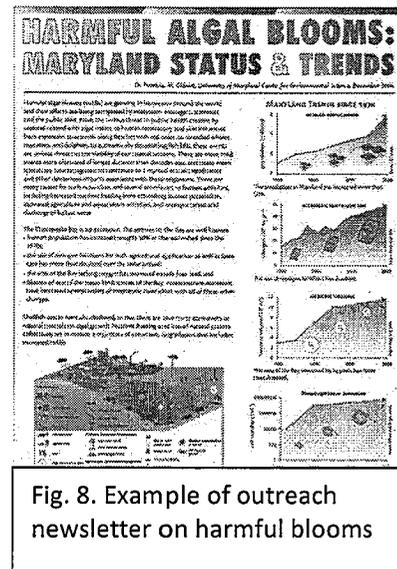


Fig. 8. Example of outreach newsletter on harmful blooms

The project's theme, of the relationship between nutrient loading and changes in primary producers, is important not only to policymakers and citizenry, but nationally and internationally. Thus, the themes and general findings of the project will be incorporated in public outreach efforts on both coasts. The importance of marine microbial diversity and the pervasive effects of cultural eutrophication in shaping benign versus harmful algal assemblages are issues that are easily communicated and thus outreach and public education efforts will go beyond regional targeted audiences.