CALFED BAY-DELTA PROGRAM

CALFED SCIENCE FELLOWS PROGRAM

Sea Grant California

In cooperation with the

California Sea Grant College Program

FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE [x] Postdoctoral Researcher [ ] Ph.D. Graduate Student

PROJECT NUMBER _____

PROJECT TITLE Heterotrophic bacteria and the foodweb of the low salinity zone and salt marsh habitats of the San Francisco estuary

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Will animal subjects be used? [ ] Yes [x] No

APPROVAL DATE: _____ PROTOCOL #: _____ PENDING: _____

Does this application involve any recombinant DNA technology or research? [ ] Yes [ ] No

2. Proposed Research

Heterotrophic bacteria and the foodweb of low salinity zone and salt marsh habitats of the San Francisco Estuary

Introduction

This proposal seeks to address specific agency needs related to understanding the pelagic organism decline (POD) outlined by CALFED in University of California – CALFED Science Fellows Program solicitation. The following proposal, an evaluation of the relative importance of phytoplankton and bacterial abundance and production in open water and salt marsh tributaries, seeks to address two questions: 1. Do Suisun Bay and Suisun Marsh (e.g. channels and restored marshes) show different trends in the communities and habitat of aquatic organisms? 2. How important are microbial food sources to the metazoan food web in Suisun Bay versus the food web in Suisun Marsh?

I am currently a member of a multi – PI / multi institution research program (funded by CALFED – W. Kimmerer Lead PI) entitled "Foodweb support for the endangered Delta Smelt and other fish in Suisun Bay and the western Delta of the Sacramento – San Joaquin River" (Foodweb Program). The objective of the Foodweb Program is to develop a conceptual model of the foodweb in the open water habitats of the low salinity zone (LSZ) of SFE, a region of the estuary that has been identified as critical habitat for many of the declining fish species. My contribution to the Foodweb Program is a collaborative assessment (with George McManus- University of Connecticut) of the microbial loop and an evaluation of the contribution of phytoplankton versus bacterial production in forming the base of the estuarine foodweb. Through California Sea Grant – CALFED Science Fellows Program I propose to build on my experience working in the open water habitats of Suisun Bay and the strength of the current Foodweb Program to extend measurements of microbial parameters in Suisun Bay for comparison with the salt marsh tributaries of Suisun Marsh.

Background

Evidence of Declines in the Estuarine Foodweb in Suisun Bay

The LSZ includes areas of the western Delta and Suisun Bay, an area identified as critical habitat for the threatened Delta Smelt (Hobbs et al., 2006). Several important changes in the LSZ pelagic foodweb have been documented over the last two decades and there are several reasons to believe that food for Delta Smelt and other threatened fishes is in short supply (Müeller-Solger et al. 2002). Perhaps one of the more striking changes in Delta is the sharp decline in phytoplankton between 1975 -1995 (Jassby et. al., 2002). The decline in phytoplankton has been accompanied by changes in the species composition of the copepods and like phytoplankton, zooplankton abundance in the San Francisco Estuary is low compared to other estuaries (Kimmerer and Orsi 1996). With declining phytoplankton biomass the organization of the pelagic foodweb in the LSZ may shift from the traditional autotrophic pathway to a less efficient foodweb based on heterotrophic bacterial biomass and fueled by exogenous dissolved organic matter (DOM). If changes in the LSZ foodweb are in fact influencing the success of key estuarine species then a more complete understanding of material flows within the estuarine foodweb is a critical component in an effective management plan. While higher levels of the estuarine foodweb may provide important indicators of overall ecosystem decline, changes in lower trophic level function may hold the key to understanding the factors contributing to system decline.

The Foodweb Program was initiated in 2006 to develop a conceptual model of the foodweb in the LSZ of the San Francisco Estuary. The foodweb Program is organized to examine these lower trophic levels and consists of 6 components (PIs responsible for each component- highlighted components indicate Parker's involvement).

1. **Nutrients and coupled carbon and nitrogen productivity by phytoplankton (Dugdale, Wilkerson, and Parker)**
2. **Bacteria production, abundance and inorganic N use. Dynamics of DOM (Parker)**
3. Primary production, biomass and community structure (Carpenter)
4. Microzooplankton rates and abundance (McManus)
5. Macrozooplankton rates and abundance (Kimmerer)
6. *Corbula* rates and abundance (Thompson)

Heterotrophic Bacteria and the Microbial Loop

A likely scenario for compensation by the pelagic foodweb for reduced phytoplankton biomass is a shift to greater reliance on heterotrophic bacteria and the microbial loop. The role that heterotrophic bacteria play in the biogeochemistry of marine systems has undergone major revisions over the past thirty years. In this revised food web model bacterial biomass plays a significant role in mediating energy and material flow to higher trophic levels via dissolved organic matter (DOM) (Williams, 1981, Azam et al., 1983) albeit at lower trophic transfer efficiency compared to direct phytoplankton – herbivore link (Ducklow et al., 1986; Sherr, et al., 1987). In the open ocean it is estimated that bacterial biomass is roughly equivalent to phytoplankton (Cho and Azam, 1990) and as much as

half of primary production is required to fuel bacterial production (Ducklow 2000). Because estuaries generally support high algal growth rates and biomass, the relative importance of heterotrophic bacteria and the microbial loop is not as clear (Ietswaart & Flynn, 1995; Bouvy, et al., 1998; Revilla, et al., 2000). However, because the San Francisco Estuary (SFE) is characterized by low phytoplankton biomass (e.g. Cloern 2002) the microbial loop may represent an important source of organic matter for the foodweb.

Phytoplankton versus Bacteria in SFE

Perhaps the most direct approach for assessing the significance of bacterial biomass for the estuarine foodweb is to compare their activity with that of phytoplankton. Previous work in the northern SFE suggests that bacteria contribute significantly to overall estuarine production. For example, Rudek and Cloern (1996) compared plankton respiration and production and concluded that the northern estuary was net heterotrophic. Similarly, Hollibaugh & Wong (1996) made comparison of bacterial production with previously reported rates of primary production and found them to be roughly equivalent in Suisun Bay during the period before the introduction of the exotic clam, *Corbula amurensis* to the bay; bacterial production was nearly 5-fold greater than primary production after the clam's introduction. These earlier studies are compelling and do suggest that the bacterial biomass may be represent the base of the foodweb in the LSZ. Parallel estimates of phytoplankton and bacterial activity are still lacking and may provide critical information about the external factors driving their relationship to the foodweb.

Bacteria and Higher Trophic Levels

The microbial loop is generally considered an inefficient pathway in part because of the additional trophic linkages (with respiratory losses) required for bacterial biomass to reach higher trophic levels (Sherr et. al., 1987). Roughly half of bacteria in SFE are associated with particles (Hollibaugh & Wong, 1996). This may have important consequences for trophic pathways as larger aggregates containing bacterial carbon may be directly consumed by larger organisms leading to an overall increase in efficiency of microbial loop. Amino acid incorporation rates are similar between free and particle associated bacteria and because of little difference in bacterial species composition and diversity between free and particle bound cells, it is thought that there biogeochemical significance is similar (Hollibaugh et al 2000) There is some indication of seasonality in the presence of particle-associated bacteria in Suisun Bay which may influence microbial loop functioning.

Organic Matter in Suisun Bay

Conditions of net heterotrophy, which have been described for several estuaries, including SFE, (e.g. Hoch and Kirchman, 1993, Jassby and Powell, 1994, Revilla, et. al., 2000, Parker, 2005) are fueled by exogenous organic matter (OM) (Carlsson, et. al., 1999, Conan, et. al., 1999). Considerable uncertainty exists in terms of the classification of compounds and nutritional quality of exogenous OM, even though dissolved organic matter (DOM) is the largest reservoir of organic carbon in the aquatic environment. Until recently, exogenous DOM was considered essentially an inert water constituent. The bioavailability of OM depends on the terrestrial sources and land use practices (Stedmon et al., 2006), with OM from anthropogenic sources more bioavailable than those originating from natural landscapes (Seitzinger et al., 2002). In addition, organic carbon and nitrogen can become uncoupled due to supply as well as differences in nutritional requirements of phytoplankton and bacteria (Parker, 2005). Sobczak et al. (2002) found that the majority of organic matter in the Delta is delivered by freshwater flow via the Sacramento - San Joaquin system but was largely refractory and supported ecosystem respiration rather than production. Previous studies in Suisun Bay found that bacterial production co-varied with freshwater flow suggesting that bacteria were responding favorably to exogenous organic matter supply (Hollibaugh & Wong, 1996, Murrell et al 1999). Murrell et al (1999) examined bacterial ectoenzyme activity in Suisun which also suggested that bacteria were actively metabolizing carbohydrate-rich terrestrial OM. Even though the organic matter inventory of the LSZ is dominated by exogenous loading, *in situ* OM production appears to play a disproportionate role in fueling bacterial production (Sobczak et. al., 2005). As a result, habitats that support phytoplankton growth (i.e. shoal environments or areas with longer residence times) may provide intermittent pulses of bioavailable OM for bacteria. Hollibaugh and Wong (1996) hypothesized that the variability they observed in bacterial production rates in the northern estuary were in fact the result of periodic pulses of labile organic matter superimposed on relatively low production rates supported by refractory DOM.

Suisun Marsh

Expansive salt marsh habitats are an important habitat type in the northern SFE and the site of substantial management activity. Suisun Marsh, the largest contiguous brackish marsh system in the west coast of North America has been extensively modified since the 1800s; it is estimated that >90%of salt marsh habitats in the SFE have been filled or dyked (Nichols, 1986). A movement is currently underway to restore wetlands within the SFE with 35 projects either planned or in progress (Kay, 2001). Unintentional inundation of dyked wetland habitats may also occur through levee failures or through predicted sea level rise, these modifications to water flow and transport

of material into and out of salt marsh habitats will certainly influence ecosystem function (Miller et al, 2001) in ways that are yet to be identified.

Salt marshes are generally considered highly productive areas and provide nursery habitats for many estuarine species. They are also a potentially large source of dissolved, colloidal and particulate organic matter supply to open water habitats of Suisun Bay. There has long been an interest in determining the relative contribution of plant versus algal derived organic matter for estuarine foodwebs (Peterson & Howarth, 1987) in open water and in sediments. Stable isotope studies suggest that foodwebs access local OM supply with variable contribution of from terrestrial and algal origin (Deegen & Garriett, 1997).

Hypothetical models of marsh restoration suggest a smooth "trajectory" of restored ecosystem function approximating natural marsh function within ca. one decade however, results from restoration monitoring do not always show directional change (Craft, et al , 1999). Several research stations have been monitored within the Suisun Marsh. For example the San Francisco National Estuarine Research Reserve conducts water quality monitoring at the Rush Ranch Reserve and CALFED has funded programs such as the Integrated Research in Wetlands Monitoring (IRWM) program which examined patterns in productivity and nutrients at natural and restored salt marsh locations (R. Dugdale project participant). To better manage salt marsh habitats and evaluate restoration efforts as well as understand the consequences of inadvertent marsh inundation, models of estuarine food webs must consider how habitat modification affects the balance of autotrophy and heterotrophy.

Applications of Bacterial Growth Efficiency to Evaluate Bacterial Uptake of DOM

Bacterial growth efficiency (BGE) is an index of the bacterial production achieved per unit of carbon consumed (i.e. BP/(BP+R)) and provides a means to evaluate lability of organic matter supply. BGE also provides information about the efficiency of supply of organic matter to the microbial loop. Estimates of BGE vary considerably from 0.1 – >0.4 with higher rates found in eutrophic systems. Accurate measures of BGE are critical for interpretation of bacterial production results (Hollibaugh & Wong, 1996). For example, an increase in BGE from 0.25 to 0.40 results in 1.5-fold lower total carbon demand by bacteria produce the same carbon production. To my knowledge no direct estimates of BGE have been made for LSZ severely limiting interpretation of bacterial carbon demand there.

Another proposed application of BGE measurements is in the evaluation of salt marsh restoration (http://ciceet.unh.edu/bulletins/newellBacteria.html). Del Giorgio & Newell have suggest that "BGE provides more fundamental information than current measures of plant and animal abundance because BGE is clearly highly influenced by changes in the sources and magnitude of organic carbon inputs induced by either marsh degradation or restoration." (CICEET Progress report, http://ciceet.unh.edu/news/newellMethods/). The investigators have found that restored marshes exhibit lower BGE compared with undisturbed sites and are evaluating change and recovery to New Jersey salt marshes using this tool. Models of sea level rise and inundation of wetlands predict a decrease in respiration with smaller effects of production and an overall loss of organic matter inputs to the marsh (Miller et al 2001). Microbial biogeochemistry of restored versus natural sites are different (Thompson et al., 1995).

Integrating Previous Studies

The large number studies that have been completed in the northern estuary and referred to above, provide a tremendous opportunity to integrate our current knowledge of OM cycling and microbial rates to investigate how the interplay between autochthonous and exogenous OM supply and the organization of the microbial foodweb influence the success of higher trophic levels. It appears that the management of freshwater flow and the composition and modification of marsh habitats are external drivers of phytoplankton and bacteria and through these functional groups influence ecosystem function.

Project Objectives and hypotheses

The proposed research has three objectives each with a set of hypotheses:

1. To evaluate the contribution of bacteria versus phytoplankton for organic matter supply to the estuarine foodweb in open water and restored vs. natural salt marsh habitats.
    A. A gradient exists in the ratio of phytoplankton: bacterial production driven by proximity to exogenous organic matter sources. Upstream station and stations within the salt marsh will be more heterotrophic compared with downstream stations.
    B. Primary production is more variable than bacterial production at all stations with variation in primary production driven primarily by water residence time.
    C. Spatial gradients in particle-associated bacteria exist in the LSZ with the highest concentrations of particle-associated bacteria found at salt marsh sites where there is high exogenous OM supply.

2. To determine the bioavailability of autochthonous and exogenous dissolved organic matter (DOM) for bacteria and the relative importance of these sources of DOM in each of the habitat types listed above.
    A. Salt marsh tributaries are sources of labile dissolved organic matter (from both terrestrial and autochthonous sources). These areas support higher rates of bacterial production compared to open water sites.
    B. Natural and restored salt marsh sites will support different levels of bacterial production and produce different OM concentrations will
    C. Phytoplankton exudates will fuel higher rates of bacterial production compared with bacterial production on exogenous OM from open water, natural or restored salt marsh habitats.

3. To investigate bacterial growth efficiency (BGE) in the three habitat types of Suisun Bay and marsh and evaluate the use of BGE as an indicator of marsh restoration success.
    A. BGE will be higher in salt marsh tributaries compared with open water habitats.
    B. BGE will vary and increase with periodic blooms in phytoplankton in salt marsh tributaries. i.e. phytoplankton DOM is responsible for higher BGE.
    C. Distinct differences between BGE estimates in natural versus restored salt marsh habitats will reflect microbial functioning. Higher BGE will be found in natural marshes compared to natural sites.

Only limited interpretation of preliminary results from the Foodweb Program 2006 field season have been made at this time (at the time of this writing I am still actively engaged in the field effort) but provide some illustrations of how to address these questions and identify unknowns.

Is the foodweb in the low salinity zone supported primarily by phytoplankton or bacteria?

Preliminary Results from 2006

I have been estimating both bacterial production and primary production weekly in the open water environments of the LSZ since mid March (Table 1 - results for March - June). Within the photic zone (ca. 2 m) the ratio of bacterial production to primary production varied widely and averaged 1.2 (range 0.2 – 8.1). Assuming an average water column depth of 7 m the ratio increases to 4.3 (range 0.8 – 10.8). These estimates are consistent with earlier studies mentioned above; however, they also show tremendous spatial and temporal variation that may provide insight into underlying mechanisms responsible for the relationship. My results indicate that stations at 0.5 and 2 psu are more heterotrophic than 5 psu suggesting a gradient in the relative contribution of phytoplankton. Consistent with studies mentioned above, the degree of heterotrophy appears to be more function of higher variability in primary production (range <50 – 260 mg C m^2 d^{-1}) than bacterial production (320 – 1178 mg C m^2 d^{-1}).

I assessed the bacterial community abundance and production for "free-living" (<1.2 µm) and particle associated bacteria (>1.2 µm) using the technique of Crump et al (1999). It appears that free bacteria may represent a higher proportion (75% at 0.5 and 2 psu and ca. 90% at 5psu) than previous estimates for the SFE. These results do not suggest temporal variation in free versus particle-associated bacteria however there does appear to be strong spatial differences.

Proposed study extension

Salt marsh habitats may support both higher rates of phytoplankton production via increased nutrient loading, longer residence times and improved light conditions or higher bacterial production and respiration via increased loading of OM of variable nutritional quality. Some of these factors co-vary (i.e. nutrient and OM supply) while other factors should be negatively related (i.e. DOM supply and residence time). The relationships between factors may not be as clear (i.e. delivery of labile OM and nutrient supply). Through a combination of seasonal sampling and manipulation experiments I will examine the relationships between these factors and their subsequent effect on the ratio of bacteria and phytoplankton. Comparisons of the contribution of free and particle-associated bacteria will be assessed in open water versus salt marsh sites to evaluate the potential for more efficient trophic transfer of bacterial biomass to higher trophic levels.

Does autochthonous or allochthonous organic matter fuel bacterial production?

Preliminary results from 2006

DOC and DON concentrations at 2 psu decreased from March to June 2006 but did not appear to co-vary with Delta outflow (Fig 1 - March – June). These results are also supported by a poor correlation between Delta outflow and inorganic nutrient concentrations (data not shown), suggesting that other factors, in addition to river flow, at least modulate the concentration of OM to Suisun Bay. The C:N ratio of the DOM is relatively constant over the study period (C:N ratio ca. 22± 1 to 24 ± 1) suggesting that DOC and DON concentrations are coupled. The C:N ratio is consistent with reports from other estuarine systems and were relatively enriched in organic C compared with C:N requirements of heterotrophic bacteria (Goldman & Dennett 2001). This would imply that

bacteria balance C:N ratios either through uptake of additional N (e.g. NH_4) or respiration of carbon. It also implies that the concentration of bulk DOM is a poor predictor of bacterial activity.

Bacterial production, assessed weekly at three salinities within the LSZ, was more similar at 0.5 psu and 2 psu and somewhat lower at 5psu (Fig 2). Stations 0.5 psu and 2 psu each showed a distinct peak in bacterial production (0.5 psu – May 23, 2 psu – May 9) however, the temporal offset in increased activity suggests that while these locations generally had similar bacterial activity, the underlying mechanism that controls bacterial production at these salinities may have been different.

The ability of phytoplankton OM to support higher bacterial production was tested in a series of phytoplankton salinity tolerance / lysis experiments (Fig 3). In these experiments, freshwater phytoplankton were exposed to 3 higher salinity environments for 12 hours. Periodic sampling was conducted to examine DOC production, phytoplankton visual inspection and bacterial production to determine whether phytoplankton lysis was occurring (Fig.). Bacterial production did not appear to respond with the increasing salinity treatments even though initial indications from visual inspection of phytoplankton indicate severe salinity stress to cells. This suggests that phytoplankton exudates were not sufficient to stimulate increased bacterial growth.

Proposed study extension

I will examine spatial and temporal variability in particulate and dissolved organic carbon and nitrogen in salt marsh and open water sites and investigate whether Suisun Marsh may act as a modulator of DOM concentrations observed in Suisun Bay through supply. I will use C:N ratio of the organic matter pools as one tool to assess potential bioavailability. I will also investigate how phytoplankton supply of OM may influence bacterial production and growth efficiency in each of these habitat types.

How efficient are bacteria at assimilating organic matter and what external controls influence bacterial growth efficiency?

Preliminary results

Despite considerable variability in bacterial production, weekly measurements of BGE were similar throughout the period and averaged 51 ± 1% (Fig 4). These estimates of BGE are high relative to previously reported values but within the range anticipated BGE (del Giorgio, 2000). This suggests that the nutritional quality of dissolved organic matter (DOM) fueling bacteria in the low salinity zone was high and the composition of organic matter supply was similar throughout the period studied.

Proposed study extension

I propose to measure BGE at open water and salt marsh habitats and evaluate the nutritional quality of OM (part of objective 2). In addition, seasonal and spatial estimates of BGE will provide critical information necessary for evaluating bacterial production estimates on a seasonal and spatial basis. Finally, I propose to investigate the feasibility of using BGE as an index of marsh restoration work by determining whether distinct differences in BGE are apparent between natural and restored marsh sites.

Approach / Plan of Work

The proposed research will be completed over 2 years. During Year 1 field studies will be organized within the framework of the Foodweb Program (the last field season of that program) and will be dedicated to weekly (3 stations in open water habitats) and biweekly (2 stations in salt marsh) surveys to resolve spatial and temporal variability in biological activity and water chemistry. Year 2 field studies will consist of monthly mesocosm experiments (of 5 to 21-d duration) carried out at RTC using water collected at each of the habitat types. These experiments will be conducted to examine further the fluxes and bioavailability of DOM from autochthonous and exogenous sources and bacterial- phytoplankton coupling via DOM. Marsh sites will be identified in coordination with community research mentors at the USGS and DWR. Potential sites include Montezuma Slough and the Blacklock Restoration Site as well as Browns tract and Sherman Island. These sites were included in a previous CALFED award to Drs. Siegel, Bollens et. al., including Dr. Dugdale (IRWM),as ‘natural’ and ‘restored’ wetlands.

Year 1: Station sampling

In order to build upon my existing dataset collected through the Foodweb Program, open water stations will be similar to those sampled in 2006 and based on salinity. During year 1 five stations will be visited regularly from mid march – August. Three stations will consist of open water habitats will be selected based on salinity (0.5psu, 2psu, and 5psu) and will be sampled weekly as part of the Foodweb Program using the RV Questuary. These weekly cruises are now routine and allow for assessment of water properties including temperature, salinity and turbidity, inorganic nutrients, DOC and DON, phytoplankton and bacterial (free and particle associated) rates and stocks. As

part of the larger foodweb program these results will be evaluated with results from microzooplankton and macrozooplankton. Salt marsh sites (natural and restored) will be sampled biweekly by small boat and will include sampling for temperature, salinity, turbidity, inorganic nutrients, DOC and DON. Phytoplankton and bacterial (free and particle associated) rates and stocks will be measured and estimates of BGE. I have used a similar sampling approach in salt marsh habitats of Delaware Bay to resolve temporal variation in phytoplankton derived DOM being utilized by bacteria (Parker, 2004) Higher trophic levels (i.e. zooplankton) will not be included in the salt marsh surveys.

Year 2: Enclosure experiments

Year 2 will be dedicated to a series of mesocosm experiments (5 – 21d) to further constrain fluxes and bioavailability of DOM and bacterial growth efficiency on the substrates from autochthones and exogenous sources. Mesocosms of 1 – 100-L will be employed and will consist of unmanipulated bioassay experiments similar to those described in Sobczak, et al 2002) and manipulated experiments such as those described in Normann et al (2001) to evaluate bacterial production and growth efficiency on exogenous and autochthonous DOM. I will directly measure bacterial production using ^{3}H-leucine and will also employ stable isotope tracers techniques ($^{1}H^{13}CO3$ and $K^{15}NH_4$) to investigate the assimilation of inorganic carbon and nitrogen by phytoplankton and subsequent uptake of immediate release products by bacteria (Parker, 2004, 2005). The methods described in Parker 2005 relied on size fractionation to separate phytoplankton from bacteria which may prove difficult in SFE, however preliminary results from 2006 suggest that particle associated bacteria may constitute a smaller fraction of the bacterial community (figure) and I will evaluate the contribution of picoautotrophs and correct for their contribution to apparent bacterial uptake..

Detailed Methods

Routine Biogeochemical Sampling

Temperature and salinity will be made using a Seabird SBE-19 CTD during sampling from the RV Questuary. A YSI probe will be used for temperature and salinity measurements during sampling from small boat. Light attenuation coefficient, k, will be determined by secchi disk using the equation of Cloern 1990). Samples for chemical and biological analysis will collected just below the surface ((assuming a well mixed water column) Primary nutrient concentrations (NO_3, NO_2, PO_4, and Si) will be analyzed with a Bran and Luebbe AutoAnalyzer II according to the procedures of Whitledge et al. (1981) for all but Si which will use Bran and Luebbe (1999). Separate 25 ml samples will be collected for manual colorimetric determination of NH_4 (Solorzano 1969). Diissolved inorganic carbon (DIC) will be measured using an MBARI-clone DIC analyzer with acid-sparging and NDIR analysis (Parker, et al, submitted).

Bacterial respiration and BGE

Bacterial respiration will be assessed by measuring changes in dissolved inorganic carbon and dissolved oxygen (DO) concentrations in 24-hr dark bottle incubations. Bottles will be maintained at ambient temperature and phytoplankton will be excluded by filtration (1.0 µm) before incubations are started. Six replicate bottles will be prepared for initial and final DIC determination, excluding the highest and lowest values in as described by Rudek & Cloern, 1996. The precision of DIC analysis using the MBARI –clone system is routinely ca. 0.1- 0.3% allowing statically significant respiration rates to be calculated (Parker, et al., 2006). Respiration measurements are typically made using changes in DO (Preen & Kirchman, 2004). I will use this approach to validate my results. (Bacterial growth efficiency will be calculated according to delGiorgio and Cole (?) as bacterial production (BP) / BP + bacterial respiration (BR). The sum of BP + BR represents total bacterial carbon demand and should be equivalent to the disappearance of dissolved organic carbon.

Dissolved organic carbon and total dissolved nitrogen

Dissolved organic carbon and total dissolved nitrogen (TDN) will be determined using a high temperature combustion (HTC) instrument (Sharp et al, 2002, 2004). Samples for DOC/ TDN analysis will be filtered through baked (450 C, 4-hr) GF/F filters with filtrate dispensed and frozen in 25 ml glass ampoules. Prior to analysis, DIC is removed from the samples with the addition of trace metal grade HCl. The RTC recently received a NSF major equipment grant to acquire a HTC instrument on site. Additionally, advisement and DOM analysis may be performed at the laboratory of J. H. Sharp (dissertation advisor - see letter of recommendation). DON will be calculated as the difference between TDN and DIN determined by colorimetric methods described above.

Bacterial Production and Biomass

Estimates of bacterial production will be made using ^{3}H- leucine (Kirchman, 1985) with the microcentrifuge method described in Kirchman (2001). Preliminary results from 2006 show that saturating concentrations of leucine in the LSZ are 40-60nM (data not shown). Incubations will last 30-60 minutes and will be

carried out in the dark at ambient water temperature. Leucine incorporation will be converted to carbon units using empirically derived carbon : leucine conversion factors (Hollibaugh & Wong, 1996). Experiments to determine carbon : leucine ratios (Kirchman, 1982?) for the LSZ were conducted in 2006 (data not shown). Estimates of bacterial biomass will be made using direct counts with an epifluorescence microscope after addition of a fluorescent DNA stain (Hobbie et al. 1977). Cell counts will be converted to carbon biomass using previously published values (Fukuda et al. 1998).

Primary production

Primary production and nitrogen assimilation will be measured using the stable isotope tracer approach (Legendre et al 197?) Samples will be inoculated with $K^{15}NO_3$, and $^{15}NH_4Cl$ (99 at%) for 4 -24-hr with particulate collection on baked (450 C, 4-hr) GF/F filters. Isotopic composition and particulate organic carbon and nitrogen will be determined using a Europa 20-20 isotope ratio mass spectrometer with uptake rates calculated from the equations of Dugdale & Wilkerson (1986). Size fractionated stable isotope tracers techniques ($^1H^{13}CO3$ and $K^{15}NH_4$) will be used as an additional tool to investigate the assimilation of phytoplankton –released DOM by bacteria (Parker, 2005). The technique uses pre- and post-incubation size fractionation (>1µm - phytoplankton, <1µm-bacteria) and provides estimates of bacterial uptake of ^{13}C and ^{15}N labeled phytoplankton exudates as well as estimates of bacterial uptake of DIN. Bacterial NH_4 uptake was assessed during the 2006 field season and the results indicate that evaluating bacterial activity using size fractionation may be possible in SFE.

Output/ Anticipated Products and or Benefits

The questions that will be addressed through this work are of very broad interest among aquatic scientists in other estuarine/salt marsh systems as well as having the potential for significant influence in the management of the Delta to restore ecosystem function. Because of the extensive background information and the intensive, ongoing monitoring programs run by the Interagency Ecological Program and the USGS there is a great deal of information that we can draw from. Understanding how this foodweb works will help management in two ways. First, it will provide a reason why the best efforts at restoration and water management may not always provide a measurable benefit to Delta Smelt. Together with the larger Foodweb Program (see Kimmerer, Lead PI), results from this project may provide tactical and strategic operations by which protective efforts are focused on times when Delta Smelt will receive the maximum benefit. After Year 1 a comprehensive evaluation of temporal and spatial dynamics of phytoplankton and bacterial rates will be completed. Products of Year 2 will be an evaluation of underlying mechanisms responsible for spatial/temporal trends documented in year 1.

Findings from this study will be disseminated through presentations at workshops/conferences and in peer reviewed publications. I anticipate presenting results at the Biennial State of the Estuary Conference in 2007 and the CALFED Science Conference in October 2008. In addition, I will attend the National Estuarine Research Federation Conference in fall 2007 and either the California Estuarine Research Conference or ASLO Ocean Sciences Meeting in 2008. In addition to annual progress reports I anticipate publication of one research article in the IEP newsletter in 2007 as well as two peer reviewed publications which will be prepared in 2008 (e.g. Estuaries and Coasts and California Estuarine and Watershed Science).

This project will benefit the participants as well as addressing the goals of the CALFED Bay Delta Science Program. First, as the Science Fellow I will have the opportunity to interact with state and federal agencies through informal interactions as well as through formal seminars. Through the fellowship I hope to build relationships with those involved directly with the management of the San Francisco Estuary. This type of interaction is critical for fostering positive relationships between academic and government scientists which appears to be the model for developing policy for environmental management and regulation in the future. Another important benefit to me is the opportunity to apply knowledge and techniques that I developed for the Delaware Estuary to a major west coast estuary. I believe that comparisons of major estuaries are necessary in order to develop more sophisticated conceptual models and to identify stressors affecting urbanized estuaries.

A benefit to the research mentor is the addition of several news methods developed for the laboratory. Methods for estimating bacterial production and abundance are new techniques that I have brought to Dr. Dugdale's laboratory. We recently purchased an instrument for the measurement of DIC for his laboratory and I have demonstrated its use in estuarine production and respiration studies (Parker, et al , 2006). Dr. Dugdale and I have been using mesocosm experiments successfully to investigate a range of potential stressors on the development of phytoplankton blooms in SFE and the work proposed here will both compliment and help to direct that research.

Benefits to the community mentors are collaboration between San Francisco State University, USGS and Department of Water Resources including data exchange and seminar (described below). Drs. Cloern and Mueller-Solger have collaborated on previous research to understand fluxes and reactivity of organic matter in the Delta (Sobczak, et. al., 2002) and continue to be interested in this research question.

Table 1: Depth integrated primary production and bacterial production in the LSZ of San Francisco Estuary. Estimates are based on rates within the photic zone (ca. 2m) and over the whole water column (assuming 7m depth). Ratios of BP:PP for both cases.

		integrated C production (mg C m^2 h^{-1}) using Z_p		integrated C production (mg C m^2 h^{-1}) using Z_b			
	Station	**PP**	**BP**	**PP**	**BP**	**BP_p:PP**	**BP_b:PP**
23-May	**0.5psu**	176	204	176	713	1.2	4.0
	2psu	112	186	112	650	1.7	5.8
	5psu	226	125	226	439	0.6	1.9
30-May	**0.5psu**	271	231	271	808	0.9	3.0
	2psu	136	217	136	759	1.6	5.6
	5psu	198	91	198	320	0.5	1.6
6-Jun	**0.5psu**	360	271	360	948	0.8	2.6
	2psu	730	315	730	1102	0.4	1.5
	5psu	823	178	823	625	0.2	0.8
13-Jun	**0.5psu**	181	337	181	1179	1.9	6.5
	2psu	184	224	184	785	1.2	4.3
	5psu	114	235	114	824	2.1	7.2
19-Jun	**0.5psu**	229	312	229	1091	1.4	4.8
	2psu	320	116	320	407	0.4	1.3
	5psu	336	188	336	659	0.6	2.0
27-Jun	**0.5psu**	158	299	158	1046	1.9	6.6
	2psu	61	116	61	404	1.9	6.7
	5psu	50	154	50	539	3.1	10.8

Figure 1: Time series of DOC and DON measured at 2 psu in the LSZ of the San Francisco Estuary. Average C:N ratio was 23 ± 1.

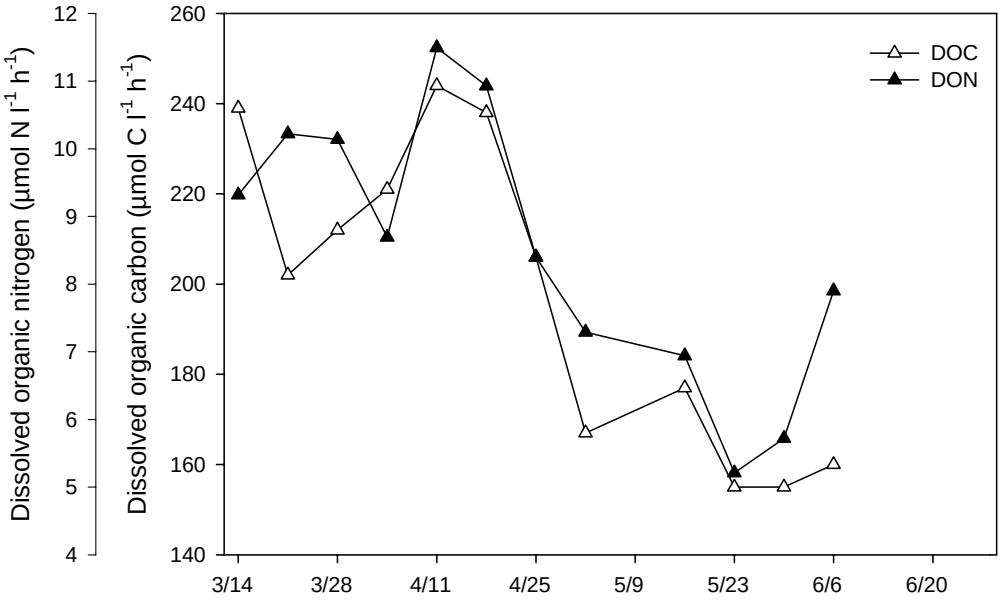


Figure 2: Time series of bacterial production at three LSZ station in the San Francisco Estuary. Bacterial production was assessed during saturated ^{3}H- Leucine incubations over ca. 0.5 -1 hr.

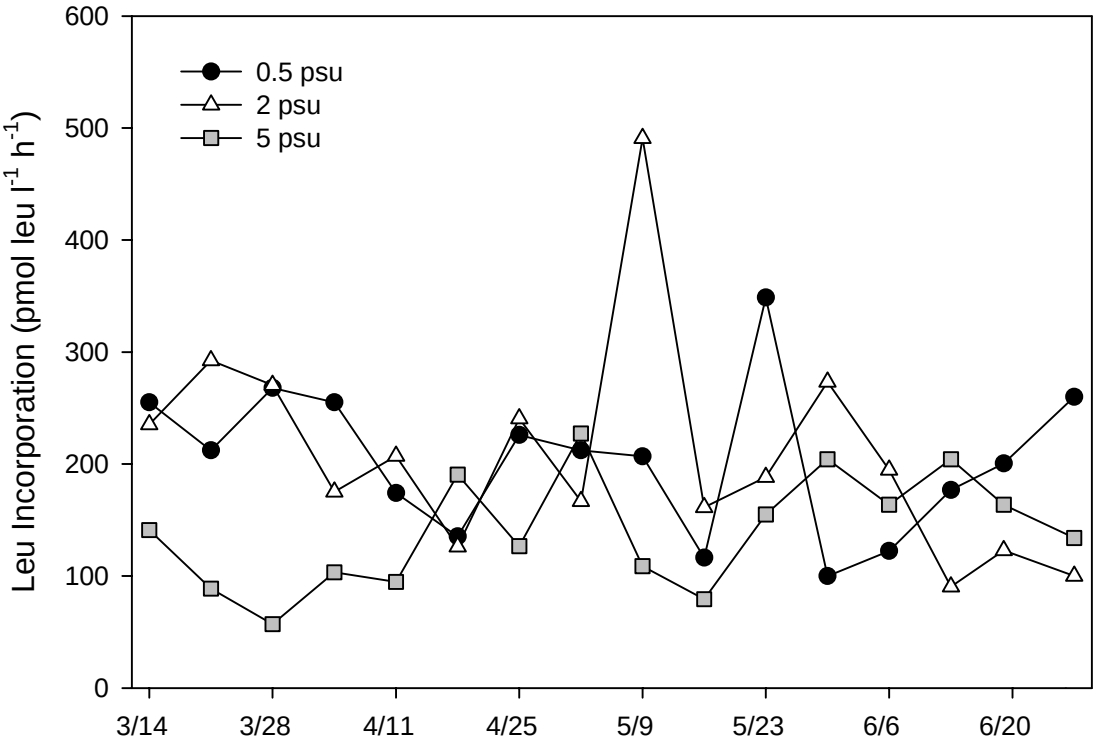


Figure 3: Bacterial production during phytoplankton salinity tolerance/ lysis experiments. Freshwater phytoplankton were exposed to 2 psu and 5 psu (0.5 psu control) with primary production, DOC production and bacterial production monitored at 3, 6, and 12-hr. There was no observed response by bacteria to increasing salinity conditions.

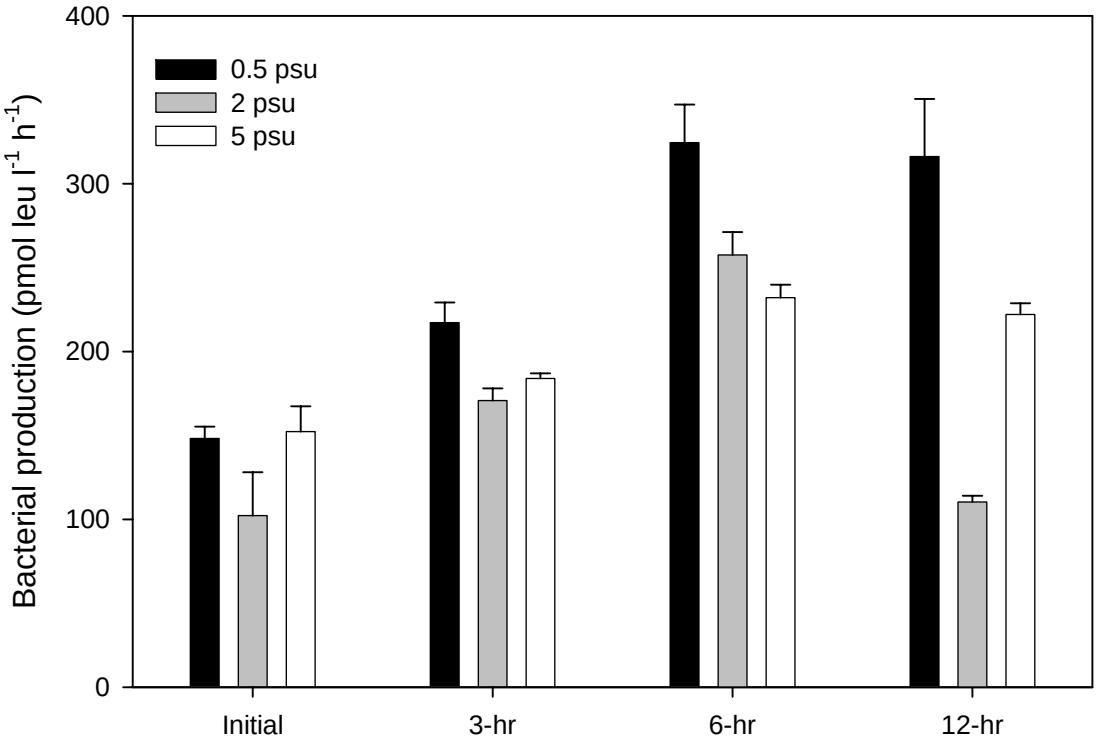
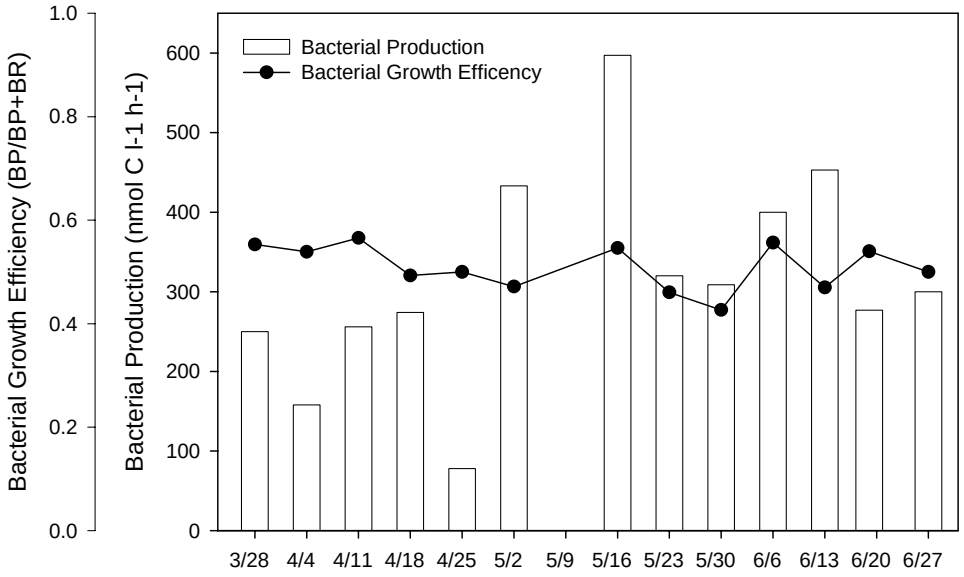


Figure 4: Bacterial production (bars) and BGE (closed circles) at 2 psu in the LSZ of the San Francisco Estuary. BGE averaged 51% over the study period.



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