Foodweb Support For The Threatened Delta Smelt And Other Estuarine Fishes In Suisun Bay And The Western Sacramento-San Joaquin Delta

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PROJECT PURPOSE

This project addresses two distinct but related topics:

1. Food-web support for the threatened delta smelt, and
2. Potential mechanisms underlying relationships of abundance or survival of some fish to freshwater flow

These two topics are related through the productivity of the foodweb of the estuarine Low-Salinity Zone (LSZ) or oligohaline zone. This region, which encompasses a salinity of ca. 0.5 - 6 psu, is a key region of the estuary and the rearing area for numerous estuarine-dependent fishes.

Topic 1: The threatened delta smelt (*Hyponcles transpacificus*) is now the principal species of concern for management of freshwater flow and diversions in the Sacramento-San Joaquin Delta, and the principal target for restoration in the upper San Francisco Estuary. The abundance of this federally-listed threatened species has been low since the early 1980s, and it has not recovered to the point where it can be considered for delisting; indeed, the 2004 abundance index was the lowest on record. Potential reasons for its low abundance are many, but evidence points to the direct and indirect effects of export pumping of freshwater in the south Delta, toxic substances, and low food supply as likely contributing factors (Bennett, submitted). We believe that the feeding environment of delta smelt may be implicated in the continued low abundance of this species. Delta smelt feed for their entire lives on zooplankton, principally copepods, mainly in the brackish waters of the western Delta and Suisun Bay. As discussed below, copepod abundance is depressed in this region.

Topic 2: Previous work on the responses of the estuarine ecosystem to interannual variation in freshwater flow has demonstrated a decoupling between the abundance of lower trophic levels and that of fish and shrimp (Kiørner 2002a, b, 2004). This decoupling may imply that variability in foodweb support is unimportant to variability of higher trophic levels, but there are some important pieces missing from the puzzle. Chief among these is the fact that the supply of labile organic matter from freshwater to the LSZ varies with freshwater flow, and this flux has not been accounted for in analyses of the estuarine foodweb.

Our research project is aimed at understanding and possibly improving the foodweb supporting delta smelt and other estuarine species. *This project will address the following key questions regarding this foodweb, focusing on the Low-Salinity Zone of the northern estuary:*

1. How do benthic grazing, available solar irradiance, and the concentrations of and composition of nitrogenous nutrients interact to influence the species composition and production of phytoplankton?
2. How does bacterial production respond to changes in particulate and dissolved organic carbon (POC & DOC) delivered primarily through river flow?
3. What is the role of the microbial foodweb in supporting higher trophic levels?
4. To what extent is copepod production dependent on these alternative energetic pathways (phytoplankton and bacterial production)?
We will try to answer the following questions:

1. **To what extent is abundance and biomass of phytoplankton, particularly diatoms, controlled by benthic grazing, light limitation, salinity stress, or nutrient composition in the LSZ?** It has seemed clear since 1988 that benthic grazing was having an overwhelming effect on phytoplankton in Suisun Bay, particularly given the low growth rates possible in that turbid water. However, it is not clear whether clams have the filtration capacity to suppress the development of blooms during all seasons. We do not anticipate an "either-or" answer to this question; rather, we propose to determine the relative importance of these two mechanisms and examine their inter-dependence.

2. **What is the relative importance of each of the alternative trophic pathways in Figure 1?** This question is of very broad interest among aquatic scientists. We have a good chance of answering it here for several reasons. The first is the extensive background information available on the northern estuary, from which we can draw general information as well as specifics. The second is the intensive, ongoing monitoring programs run by the Interagency Ecological Program and the USGS. The third is our study design, which includes all of the key elements of the foodweb, and will allow us to measure the relevant material fluxes on samples from the same water body at the same time.

**Study site**

The LSZ, generally located in eastern Suisun Bay and the western Delta (Figure 6), has been the subject of considerable research focusing on phytoplankton (Arthur and Ball 1979, Cloern et al. 1983), hydrodynamics and the interaction of vertical position of larval fish and zooplankton with the flow field (Kimmerer et al. 1998, 2002, Bennett et al. 2002), and on the impacts of the introduced clam *Potamocorbula amurensis* (Kimmerer et al. 1994). It is the center of abundance of several zooplankton and fish species including delta smelt and several of the species that respond strongly-to freshwater flow (Kimmerer and Orsi 1996, Kimmerer 2004).

Low-salinity regions of estuaries are sites of fundamental chemical and biological transformation (Morris et al. 1978), commonly associated with a turbidity maximum (ETM, Postma and Kalle 1955, Kimmerer et al. 1998) and high microbial activity, particularly by bacteria associated with organic particles (Hollibaugh and Wong 1996). In the San Francisco Estuary, turbidity maxima are produced by bathymetric features (Schoellhammer 2001), and stratification and gravitational circulation are uncommon when the LSZ is in the shallow Suisun Bay (Kammerer et al. 1998). Nevertheless, turbidity is higher in the LSZ than either landward or seaward of it (Kimmerer et al. 1998), apparently because of interactions between tidal currents and the mean flow (Schoellhammer 2001). Thus, the LSZ in this estuary has some features in common with other estuaries (the salinity regime, turbidity maximum) but not others (stratified flow, gravitational flow).
PROJECT DESCRIPTION

We plan a collaborative program of field and laboratory research focused on lower trophic levels in the Low-Salinity Zone of the western Delta and Suisun Bay. Fundamentally we hope to understand better how the degraded foodweb supports fish, principally delta smelt. The research will comprise four major components each addressing one of the research questions posed above.

Our frame of reference in this study will be predominantly salinity rather than geography. That is, we propose to sample for plankton at stations defined by selected salinity ranges rather than at fixed stations. Planktonic organisms are incapable of swimming against tidal currents, and are less likely to be oriented to geography than to salinity (Laprise and Dodson 1993, Kimmerer et al. 1998). Estuarine fish, too, appear to orient to salinity ranges (Baxter et al. 1999, Kimmerer 2004). In addition, we are interested in the chemical and biological conditions in the transition between the freshwater environment and brackish water (Morris et al. 1978).

Although it is appropriate to sample the water column at stations defined by salinity, the same is not true for the benthos. We need information on how grazing by the clam *Potamocorbula amurensis* affects biota in the LSZ. Since the effect of the clam operates at a time scale longer than a tidal cycle (e.g., turnover time of water column chlorophyll), effects of clams on the overlying water column are smeared out over a broad region by tidal dispersion (see Kimmerer and Orsi 1996, Jassby et al. 2002). Therefore sampling for clams will be by a stratified random sampling design in Suisun Bay and the western Delta. The calculated grazing rate in the LSZ will be an average of rates determined in sub-regions of this area, weighted by the time the LSZ spends in each area. In this way we will be able to link variability at two different temporal scales.

The proposed research will provide a quantitative basis for the model depicted in Figure 1. This model will be developed using available data as a framework, including data to develop advective and dispersive fluxes of materials and organisms.

We list here several hypotheses to be tested, organized by the fundamental research questions above. Although stated as hypotheses, in many of these cases we aim not so much to test these hypotheses formally as to compare among competing (or complementary) models of how the system works (Hilborn and Mangel 1997). The complexity of the LSZ foodweb (Figure 1) suggests that multiple mechanisms operate simultaneously, and that therefore simple yes/no hypothesis tests must be supplanted with quantitative estimates of model parameters. Except where noted, these hypotheses apply during times when delta smelt are likely to be in the LSZ (Figure 2). The hypotheses incorporated in these models are:

1. **Phytoplankton dynamics**
   a. Suppression of the spring bloom varies with freshwater flow into the LSZ through its transport of NH$_4$
   b. Lysing of freshwater phytoplankton in brackish water contributes DOC to the LSZ foodweb.
2. **Benthic grazing effects**
a. Suppression of the spring bloom varies with freshwater flow into the LSZ through its influence on biomass of clams
b. Summer diatom biomass continues to be suppressed by clam grazing.

3. **Bacterial production**
   a. Bacterial production is controlled by the supply of phytoplankton-derived DOC
   b. Bacterial production is controlled by clam grazing

4. **Microbial foodweb**
   a. Microzooplankton production is fueled by bacteria
   b. Microzooplankton production is fueled by phytoplankton

5. **Copepod production**
   a. Copepod production is supported by local phytoplankton production
   b. Copepod production is supported by exogenous carbon

**Overall work plan**

We propose a staged sequence of field work, laboratory studies, and synthesis. Our field program will focus on the spring bloom (April-May), and on the mid- to late-summer period of low phytoplankton biomass and production. We anticipate two years of field work, with an initial emphasis on evaluating longitudinal gradients and a subsequent focus on two salinity regimes; the third year will be used for analysis and synthesis. Laboratory work will use incubations for various measurements, including nitrogenous nutrient interactions, phytoplankton production, bacterial production, microbial foodweb production, and consumption of various components by copepods.

During years 1 and 2 we plan weekly cruises to the LSZ during spring (mid-March to mid-May), then every three weeks through August, using RTC’s 38-foot research vessel, R/V Questuary: Spring trips will focus on bloom dynamics, and summer trips on alternative food sources for copepods. Two additional trips will be taken to sample clams. The clam cruises will also require a small boat operating simultaneously to collect in shallow regions inaccessible to the Questuary. We also plan to take 8 trips by small boat during each year to fill in samples between the main cruises. In general, cruises will depart early enough to return samples to RTC for processing and incubation; this approach worked well during our recent EPA-funded study of Suisun, San Pablo, and Central Bays.

Because much of our work depends on the timing of specific events (e.g., spring bloom or no bloom, spring minimum in copepod biomass, summer movement of delta smelt), we will be flexible about cruise dates and adjust them as necessary. Information on conditions in the LSZ will be monitored through continual checks of online data for salinity and temperature, and examination of data as they become available from the DFG 20mm survey for late larval delta smelt. Our sampling will be partially scheduled and partially event-driven. To the extent possible we will also coordinate our cruises with monitoring surveys of the USGS vessel R/V Polaris and the DWR vessel R/V San Carlos, as well as DFG vessels taking fish samples.

**Detailed approach**

Water column data (temperature, salinity, fluorescence, PAR, transmittance) will be recorded using a Seabird SBE-19 CTD equipped with WETSTAR fluorometer and beam
transmissometer and PAR sensor. Water column samples for phytoplankton, bacteria, and microzooplankton will be taken using 10 L Niskin bottles mounted on an SBE-33 carousel or with a bucket. Surface water will be used to fill 3.8 L cubitainers that will be immediately returned to RTC along with samples for ambient nutrients, phytoplankton, bacteria, and microzooplankton. If significant stratification in water column properties is observed, additional samples will be taken deeper in the water column.

Task 1. Phytoplankton dynamics

Nutrients will be measured on freshly collected samples from field or enclosures. NO$_3$ and PO$_4$ concentrations will be determined according to the procedures of Whitledge et al. (1981), and Si(OH)$_4$ following the protocol outlined in Bran and Luebbe Method G-177-96 using a Bran and Luebbe AutoAnalyzer II. If samples are frozen before analyses they will be thawed 24 hours prior to analysis to avoid polymerization effects on Si(OH)$_4$ measurements, and thus poor reproducibility (Macdonald et al. 1986). NH$_4$ will be measured spectrophotometrically according to the phenolhypochlorite method of Solorzano (1969) using a Hewlett Packard Model 8452A diode array spectrophotometer and 10 cm cell.

In field collected samples and in some enclosure experiments, phytoplankton samples will be counted. Phytoplankton will be identified to genus or to species where possible, and their density will be determined using two methods, one for picoplankton and one for larger phytoplankton. Picoplankton (*Synechococcus* spp.) density will be determined by filtering an appropriate volume (10-50 ml) of water onto a 0.6 $\mu$m pore size Nuclepore filter, fixing it with 4% paraformaldehyde for 20 min, then freezing the filter at - 80°C until cells are counted using blue or green excitation with a Zeiss epifluorescence microscope at 1000 X magnification. For larger phytoplankton, a 250 ml volume of water will be preserved with Lugols solution, and cells will be concentrated by centrifugation (Sukhanova 1978) then identified to species and quantitatively counted with a Sedgwick Rafter counting chamber (Guillard 1978) at 400X.

Another estimate of diatom abundance is to measure biogenic silica which will be measured in water filtered on 2 um pore sized nucleopore filters using a protocol modified from Brzezinski and Nelson (1989).

$^{15}$N and $^{14}$C uptake incubations will be carried out on shipboard samples and during enclosure experiments in 280ml polycarbonate bottles, maintained for 24 hours in filtered SFE water-cooled incubation tables, under window screening to expose them to selected light levels. Typically DIN inoculations will be made with trace additions of either K$^{15}$NO$_3$ or K$^{15}$NH$_4$Cl (99 atom% $^{15}$N). In some experiments, unlabelled KNO$_3$ or NH$_4$Cl will also be added for incubations. Upon completion of the incubations, samples will be collected by filtration onto pre-combusted (450°C for 4 hours) GF/F filters, frozen until analysis for $^{15}$N enrichment (Wilkerson and Dugdale, 1992) with a PPZ Europa 20/20 mass spectrometer system. The transport rate ($p$) and specific uptake rate ($V$) will be calculated according to Dugdale and Wilkerson (1986). The uptake kinetic parameters $V_{max}$ and $K_s$ and $K_{LT}$ will be calculated after linear transformations or iterative curve fit programs are applied (e.g. Kudela et al. 2000, Cochlan et al. 2001). To evaluate NO$_3$ uptake when ambient NO$_3$ concentrations are saturating, it may be necessary to repeat the experiment after one to several days, to allow the phytoplankton within the cubitainer samples to reduce the ambient NO$_3$ to lower levels. NH$_4$
regeneration rates will be measured at selected stations and enclosures by a $^{15}$N dilution method using the diffusion-concentration method of Slawyk et al. (1990) and calculated using the equations of Blackburn (1979) and Caperon et al. (1979) as outlined by Slawyk et al. (1990).

Phytoplankton primary productivity will be measured using the $^{14}$C light-dark bottle method (JGOFS IOC 1996) with modifications for use with Bay phytoplankton. Twenty p.Ci of $^{14}$C in the form of bicarbonate will be added to 250-ml bay water samples. Bottles will be placed in a flow through water table under light conditions similar to those of the LSZ. Following a 24-h incubation, 100 ml of the contents of each replicate will be filtered onto a GF/F glass fiber filter. The filters will be fumed over HC1, placed in scintillation cocktail, and counted in a scintillation counter. Chlorophyll a will be determined by _in vitro_ fluorometry using a Turner Designs Model 10 fluorometer and the protocol of Arr and Collins (1992) on samples filtered onto Whatman 25 mm GF/F filters. Dissolved CO$_2$ (DIC) in the water (required to calculate C fixation) will be measured using a Monterey Bay Research Institute-clone DIC analyzer with acid-sparging and NDIR analysis (Walz & Friederichl 996) following preservation with HgCl$_2$.

Enclosure experiments will be used to study the interaction of light, nutrients, and salinity on phytoplankton growth and contributions to DOC (Table 1). The enclosures will be 3.8L polycarbonate cubitainers. These enclosures have been very successful both in SFE (our preliminary studies) and in coastal waters (e.g. Kudela et al., 2000). The enclosures will be transported to RTC and held in filtered bay-water cooled incubators under natural light. Enclosures will be sampled daily over 5 days for chlorophyll, nutrients, diatom abundance and species composition, $^{14}$C fixation, DOC, and $^{15}$NO$_3$ or $^{15}$NH$_4$ uptake.

To determine the salinity at which freshwater phytoplankton lyse, water from the Delta with the natural phytoplankton assemblage will be subjected to a salinity of 0 (control), 1, or 2 psu, ambient nutrients, and adequate light (50% of surface irradiance). This salinity range was chosen because it is representative of what phytoplankton noinially encounter in the LSZ, and freshwater phytoplankton have been found to lyse in 0.1-1.0 psu (Morris et al. 1978). At intervals of 1, 6, 12 and 24 hours, we will measure primary and bacterial production, chlorophyll, and cell lysis as determined by quantitative microscopic counts and examination. Bacterial productivity will be measured as described under Task 3. To examine whether nutrient availability affects salinity tolerance of phytoplankton, and therefore potential to lyse, similar experiments will be run with high N03, high NH4, and adequate light (50% of surface irradiance).
Table 1 Summary of enclosure experiments for phytoplankton dynamics.

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<tr>
<td>LSZ water with ambient nutrients, high NO₃, high NH₄, adequate light (50% of surface irradiance, LPD)</td>
<td>Determine NH₄ inhibition of NO₃ uptake and chl accumulation. Quantify time scale and lag for NO₃ depletion, measure NH₄ regeneration rate.</td>
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<tr>
<td>LSZ water with ambient nutrients, high NO₃, low NH₄, adequate light (50% LPD)</td>
<td>Quantify the time scale and intensity for NO₃ depletion and bloom development by phytoplankton with optimal conditions. Measure NH₄ regeneration</td>
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<td>Different light conditions (0 to 100%LPD) ambient nutrients, high NO₃, high NH₄ or low NH₄.</td>
<td>Light conditions required for NO₃ uptake to increase, measure light impact on time scale for NO₃ uptake.</td>
</tr>
<tr>
<td>LSZ water with ambient nutrients, salinity 0, 1 or 2 psu, adequate light (5i)% of surface irradiance).</td>
<td>Determine salinity at which freshwater phytoplankton lyse or release DOC. Measure productivity, DOC, cell lysis.</td>
</tr>
<tr>
<td>LSZ water with high NO₃, high NH₄, 0, 1 or 2 psu, adequate light (50% of surface irradiance).</td>
<td>Nutrient effects on salinity tolerance and potential to lyse. Measure productivity, DOC, cell lysis.</td>
</tr>
<tr>
<td>Different NH₄ additions: LSZ water with ambient nutrients, high NO₃, adequate light (50% LPD)</td>
<td>Test the threshold NH₄ concentration, quantify any inhibition of NH₄ on NO₃ uptake</td>
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We will also monitor dissolved organic carbon concentrations at the beginning and end of the enclosure experiments to assess any gross changes in the DOC pool over the course of the experiment. Samples will be filtered (0.2 µm) and frozen (-80°C) until being processed using a DOC analyzer at UC Santa Barbara. Because we assume that the products of phytoplankton lysis (DOC) will be labile, we do not anticipate any significant accumulation of DOC over the time scale at which we will be sampling. However, increases in bacterial abundance and production will indicate whether increases in DOC supply have occurred.

Task 2. Benthic biomass and grazing

Forty samples, distributed randomly within strata that are representative of known habitat types for *Potamocorbula* (depth, salinity, and substrate) will be collected at the beginning of the spring sampling period and at the end to evaluate the grazing rate in the LSZ during the "spring bloom period". An additional 5 stations will be sampled monthly (in addition to 4 stations currently sampled by the USGS) to confirm the grazing rate trajectory between spring and summer and to calculate secondary production at those locations for both years. Four sites are located in the channel and the remaining five in the shallow water (4 in Grizzly Bay and 1 in Honker Bay which is poorly represented since it has not had a seasonally persistent *Potamocorbula* population since 1993). Samples will be collected with a 0.05 m² van Veen
grab, sieved on a 0.5mm screen, preserved in foilinalin, and transferred to 70% ethyl alcohol stained with Rose Bengal. A subsample of live bivalves will be collected in each habitat to estimate weight as a function of length; animals will be sized, dried, weighed, ashed and reweighed to determine ash-free-dry-weight which is convertible to carbon weight, Cloern and Canuel 1993). Bivalves will be removed from preserved samples and measured, and tissue dry weight will be estimated from the length-weight regressions. Biomass will be calculated for each sample and grazing rate estimated as described in Thompson (in press).

In addition to the samples for biomass described above, seasonal samples will be collected at five historical stations to estimate secondary production to evaluate previous hypotheses that Potamocorbula production is fueled by a combination of phytoplankton and bacterial producers. These new data will be compared to secondary production rates during previous years (since Potamocorbula was introduced in 1986) when we have secondary production estimates but no measurements of bacterial or phytoplankton production. We will use these new secondary production data to ‘riminalize’ our previous estimates of bacterial consumption rates. This will allow us to evaluate the influence of Potamocorbula consumption on bacteria and phytoplankton and ultimately the long-term trends in zooplankton biomass.

Secondary production will be calculated using the Crisp (1971) method which is made possible by the semi-annual spawning period and the presence of distinctive cohorts. Errors for all methods will be calculated as described by Bevington and Robinson (1992).

Nitrogen remineralization rates will be estimated from our grazing and production rate estimates using assimilation efficiencies from the literature (Werner and Hollibaugh 1993). The enclosure experiments in Task 1 will determine the potential of the ambient phytoplankton to reduce upper estuary ammonium concentrations to non-inhibitory level, with the effects of clam grazing and remineralization excluded. We will then examine these data to determine whether the early spring clam population has the capacity to overwhelm the growth of diatoms through a combination of grazing and excretion.

Task 3. Response of bacterial production to inputs.

Bacterial biomass and production will be measured during field sampling as well as in enclosure experiments to estimate the relative importance of bacterial biomass in supporting the LSZ foodweb. The basic approach will be to estimate bacterial production using radioactive tracers and bacterial biomass by direct counts and then convert these estimates to carbon units to make comparisons with other components of the microbial food web.

Estimates of bacterial production will be made using tritiated leucine (Kirchman, 1984) with the microcentrifuge method described in Kirchman (2001), which requires smaller incubation volumes and therefore significantly less radioactive isotope than traditional methods. Bacterial leucine incorporation will be converted to bacterial carbon assimilation by empirically derived Carbon : leucine conversion factors. Carbon : leucine conversion factors will be made by simultaneous estimates of bacterial abundance and leucine incorporation in incubations in which grazers have been excluded by filtration (<1 µm). Increases in cell numbers will be directly related to leucine incorporation.
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).

Task 4. Microbial foodweb

In most marine and estuarine food webs, microzooplankton (20 - 200 µm in length) are the principal herbivores (Calbet & Landry 2004). Although copepods and other mesozooplankton can also be significant direct grazers on phytoplankton, it is now recognized that the link from phyto- to microzoo- to mesozooplankton is significant in the overall flows of energy and material in estuarine and coastal ecosystems (Gifford & Dagg 1988, Gifford 1991, Gifford & Dagg 1991). In addition, small particle feeders such as ciliates and other protozoan microzooplankton can pass detrital and bacterial organic matter up the food chain to metazoan grazers as part of the "microbial loop", a pathway through which dissolved organic matter is utilized in marine and estuarine food webs (Fenchel 1988, McManus 1991, Vezina & Savenkoff 1999): For a detritus-dominated ecosystem such as Suisun Bay and the Western Sacramento-San Joaquin Delta, this pathway may be particularly significant (Murrell et al. 1999).

We will measure two important fluxes related to the flow of energy from microbes to metazoans: microzooplankton herbivory and bacterivory. To estimate herbivory, we will use two independent approaches. We will measure herbivory directly using the dilution technique (Landry & Hassett 1982). This method requires minimal disruption of delicate nano- and microzooplankters and has become the standard for measuring this flux. It will also allow us to measure direct ingestion of detrital POC by microzooplankton. However, there are indications that some of the key assumptions of the dilution method may not hold in turbid estuarine environments (Murrell & Hollibaugh 1998, Dolan et al. 2000). Thus, our second approach will be to measure microzooplankton species composition and abundance and estimate herbivory using published specific clearance rates. This approach will also give much finer temporal and spatial resolution than would have been possible with the dilution method alone.

To estimate bacterivory, we will use the fluorescently-labeled bacteria (FLB) method (Sherr et al. 1987, Pace et al. 1990). In this method, monodisperse, fluorescently-stained bacteria are added to a sample from the environment. Disappearance of the FLB's is monitored over time to estimate the mortality rate of the natural bacterioplankton. Because the FLB's are unable to grow, they are not subject to viral lysis and hence their disappearance should be due only to grazing. Since the FLB's are not a good proxy for particle-attached bacteria, we will estimate the grazing mortality of the latter by difference, using the results of the bacterial growth measurements being made for task 3 (i.e. particle-attached bacterial mortality = growth of unattached bacteria + growth of particle-attached bacteria - mortality of unattached bacteria). Because we will have separate growth measurements for unattached and attached bacteria, we will be able to test the implicit assumption that these rates are in balance on short time scales. As with the herbivory estimates, we will independently estimate bacterivory using abundances of bacterivores (small heterotrophic flagellates and bacterivorous ciliates, i.e., respectively nano- and microzooplankton) and published clearance rates.
All of the measurements for task 4 will be coordinated closely with those for task 3 (bacterial dynamics) so that we will have as complete a picture as possible of stocks and fluxes within the microbial food web, as well as links to higher trophic levels.

Task 5. Copepod production

The hypotheses for copepod production will be examined through a combination of field and laboratory work in collaboration with researchers on other tasks. We want to know how much of the copepods’ growth is based on phytoplankton and how much on bacteria, either directly or through the microbial food web. This suggests a variety of approaches, since the various measurements required must be made by different methods and at different scales (duration of experiments, container size, copepod density, use of various labels, termination method). Experiments designed to measure uptake (e.g., feeding on phytoplankton vs. feeding on bacteria) will be run as close to simultaneously as possible so that results can be compared.

A principal focus in this task will be to contrast the cyclopoid Limnoithona tetraspina with the calanoid copepod Pseudodiaptomus forbesi and, if present, Eurytemora affinis and Sinocalanus doerrii. Copepods will be collected by gentle net tows using a mesh suitable for the species being examined: 120 urn for L. ‘tetraspina and 250 urn for calanoid copepods. Samples will be diluted immediately upon capture into 20-liter insulated carboys for transport to the laboratory. Copepods will be sorted as soon as feasible using a dissecting microscope, and will be kept and incubated at a temperature close to ambient.

Grazing and production by copepods will be measured using techniques that have been applied either in the Kimmerer or McManus laboratories. Grazing on phytoplankton will be measured in incubations using the disappearance of chlorophyll, and grazing on phytoplankton and ciliates will be measured by direct cell counts (Bouley in prep.). Bottle sizes, densities within the bottles, and duration of experiments will all be varied to ensure an adequate signal:noise ratio and to account-for any bottle effects.

Grazing on bacteria will be determined using short-term labeling experiments. The purpose of short-term experiments is to minimize egestion and excretion of label, which should begin -1 hour after ingestion. Water with particle-attached bacteria will be collected from the field by gently dipping with buckets (Hollibaugh and Wong 1996). Tritiated leucine or thymidine will be added and uptake will be allowed for -1 hour. Copepods will then be introduced to the containers and allowed to feed for 15-30 minutes. Copepods will then be removed and processed for scintillation counting. Samples of the water taken before and after copepods are introduced will be filtered on GF/F filters and also on 5 p.m Nuclepore filters using gravity flow to concentrate bacteria-laden aggregates. These samples will also be counted and the activity per ml (total and on aggregates) will be determined. Clearance rate on bacteria will then be determined as the rate of increase of copepod activity divided by activity per unit volume. Several controls and ancillary experiments will be required for this approach. Some bottles will be treated with antibiotics to reduce bacterial uptake of label, to determine the extent of direct uptake by copepods. Some bottles will have copepods added that have been killed using carbon dioxide to determine uptake in the absence of feeding. In addition, copepods will be incubated in containers that have been filtered to remove aggregates before labeling. Several range-finding experiments will be necessary to establish the appropriate experimental duration, volume, and density. In addition, various alternative methods will be
tried to keep particles in suspension, including the use of a plankton wheel with end-over-end
rotation at 1 rpm.

"Cascade" experiments will be conducted to evaluate copepod ingestion of detrital POC
directly and as a tool for 'integrating the results of individual experiments (Calbet & Landry
1999). In these experiments, 1 L bottles of <200 um screened water are incubated (24h) with
no copepods, ambient concentrations of copepods, 2X ambient copepods, 4X ambient
copepods, and 8X ambient copepods. Adding more copepods should increase the rate at which
detrital POC disappears if the copepods are eating the POC, unless there is a stronger
cascading effect of copepods eating protozoa that are eating the POC. In the latter case, adding
more copepods prevents the POC from being ingested by reducing protozoan populations, and
there should be more POC in treatments with more copepods. The endpoint of these
experiments will include measurements of chlorophyll, POC, and bacterial abundance in
several size fractions.

Results of these experiments will be interpreted by incorporating information from the
phytoplankton and bacteria measurements. We will be able to calculate the consumption rate
of the copepods (from their production rate and gross growth efficiency from the literature),
and will then use clearance rates on alternative food sources from individual experiments to
estimate the fraction of ingestion that is due to each source. Ingestion will then be portioned
into the various pathways shown in Figure 1. This partitioning will differ among species, and
with the relative abundance of alternative food sources, since copepods are opportunistic
feeders.

**Task Synthesis**

All participants in this project have agreed that in addition to the work outlined above, they
will participate in a synthesis effort to combine the entire suite of results into a coherent story
about the foodweb of delta smelt. This synthesis will begin at project inception: we will re-
examine and analyze the available data on phytoplankton to calculate rates of transport of
freshwater phytoplankton to the LSZ. To a first approximation this can be calculated from the
net river-derived flow and freshwater chlorophyll concentrations, but we will also estimate
dispersion using available results from hydrodynamic models and salt flux studies.

The flux data will be used to interpret the data being developed by the various tasks. After the
first year's results have been calculated we will hold a working session to place initial bounds
on the fluxes in Figure 1. These calculations may lead us to refine our approach in Year 2.
After the Year 2 data are available the synthesis will enter a more formal stage, culminating in
a quantitative model of carbon flow in the LSZ. A synthesis paper will be prepared reporting
these results. In addition, a report will detail the significance of these findings for management
of the estuary, as discussed above.

**Feasibility**

The feasibility of a research project such as this depends on the capabilities of the participants,
the organization of the team, the availability of suitable facilities, and the availability of the
necessary equipment. The leaders of our research team include scientists with decades of
research experience in a wide variety of environments, and talented and energetic postdoctoral
associates. Previous experience suggests that this team can accomplish the proposed tasks. In
addition, most of the members of this team have worked with at least one other member on significant research projects. The team's organization (see Management below) is relatively straightforward and this has been successful in projects of similar scope in the past.

Facilities and equipment at RTC  The bulk of the laboratory work will be done at RTC. RTC's main laboratory building has a total area of some 20,000 ft$^2$ of improved space and an open bay of 12,000 ft$^2$. We have just begun phase II of the renovation of this building, a $3.2$ million project that will add new laboratories, offices, classrooms and needed infrastructure. An additional bay-front building (12,000 ft$^2$) houses the Ecology and Aquarium Facility with space for seawater tables and tanks. The RTC seawater system has been upgraded with a 300 foot tethered intake line, and water delivery to the main building and mixing facilities are being planned. In addition to a large research wet lab (1500 sq. ft.) with running bay water, the Center has constructed an animal culture room (700 sq. ft.), which is temperature- and light-controlled.

General use equipment includes constant temperature rooms, balances, spectrophotometers, centrifuges, refrigerators and freezers (-80 & -20°C), fume hoods, a PCR theiniocycler, and a laminar flow hood. A Zeiss Axioskop epifluorescent microscope is available for phytoplankton, picoplankton and bacterial counts and a Turner fluorometer is on hand for chlorophyll a measurements. We recently acquired a low background liquid scintillation counter (Winspectral Guardian LSC from PerkinElmer) and a Europa gas chromatograph-stable isotope analyzer (20-20 mass spectrometer, ANCA GSL elemental analyzer and Agileht 6890N GC combination, and previously a Technicon II Autoanalyzer for nutrient analysis. These instruments are housed in a joint-use Nutrient Analysis Laboratory with additional instruments to measure rates of nutrient uptake, carbon and Si(OH)$_4$ uptake with scintillation counting, and nitrogen uptake with mass spectrometry. Additionally, a molecular biology research laboratory was constructed three years ago at the Center. RTC students and staff have access to other equipment available at specialized laboratories at SFSU (e.g. the GIS Facility in the Geography Department, the Electron Microscope facility and the Conservation Genetics Laboratory in the Biology Department).

RTC owns and operates a 38' aluminum-hulled shallow draft research vessel capable of a maximum speed of 20 knots and equipped with an A-frame, adequate a/c power, a hydraulic system, a hydrographic winch with conducting cable, instrumented rosette sampling system, depth sounder/bioacoustic sampler, acoustic doppler current profiler (ADCP), differential global positioning system (GPS), and a data acquisition computer system. In addition, RTC has a number of smaller vessels such as the new Twin Vee Powercat and a 17-foot Boston Whaler.

Peuuiitting  Field personnel will obtain Scientific Collector's Permits issued by the State. We expect zero take of all native fish species of special status during field sampling.

Relation to other projects, current and pending  This project does not depend on other projects for successful completion. Kimmerer is currently lead PI of a CALFED ERP-funded project, "Determining the mechanisms relating freshwater flow and abundance of estuarine biota" with E. Gross and W. Bennett. This project is the first formal step in unraveling the basis for the "fish-X2" relationships. It has two components: first, we are using the 3-dimensional hydrodynamics model TRIM3D to explore the response of the estuary to changing freshwater
flow. In particular we are focusing on the distribution of physical habitat as defined by salinity, depth, temperature, and other characteristics, with the goal of assessing the possibility that increasing physical habitat with increasing flow could underlie some of the fish-X2 relationships. The second component is an effort to develop a plan, working with members of the IEP Estuarine Ecology Team, for a program of research to determine what mechanisms are actually operating in the estuary. That work will finish by June 2006, i.e., early in this project. Although the plan is not yet complete, several themes have emerged from the discussions to date and will be in the final plan. *One of these is the need to look more carefully at the base of the foodweb for potential effects of flow.*

Dugdale and Wilkerson are funded by Sea Grant to determine the role of ammonium inhibition in SFE in suppressing phytoplankton blooms. The project is focused on Central Bay with the specific hypothesis that reducing ambient NH$_4$ to less than 4 µM makes the high ambient levels of NO$_3$ accessible to SFE primary producers, resulting in healthy diatom-dominated ecosystems. Enclosure experiments are being made with Central Bay water to understand the interaction between NH$_4$ inhibition of NO$_3$ uptake and light limitation in the initiation/fouination of diatom blooms in SFE, sufficiently well to predict the effects of variation in NH$_4$ inputs and dilution. The results of this research will be used to test and improve an existing simulation model of phytoplankton production with NH$_4$ inhibition; incorporating kinetic parameters for light and nutrient uptake obtained from the enclosure experiments.

Kimmerer is participating in two related projects: The first, submitted (with W.A. Bennett, UC Davis) in November to the CALFED Ecosystem Restoration Program, for enhancements in monitoring of delta smelt, is still pending but partial funding is expected. The RTC component of that project is to examine feeding by delta smelt, in particular their selectivity and feeding rate. In addition, Kimmerer is leading a project under this PSP involving Bennett as Well as researchers from Louisiana State University and Stanford University to develop a suite of models of delta smelt biology. Significant advantages will accrue to all projects by the broadened scope and increased opportunity for synthesis available with a number of distinct but related projects.

**Data Management**

Products will be made available as indicated below; computer codes and files will be made available upon request to any of the project team members. In addition, input and output files will be provided to the Interagency Ecological Program (TRP) which is linked to the California Environmental Information Catalog, no later than 1 year after collection of data or upon the acceptance of manuscripts.

**Expected Products/Outcomes**

Anticipated products include (Table 2):

- Presentations at the Estuarine Ecology Team, CALFED Science Conference, and at least one national conference.
- Articles in the LNP Newsletter describing progress.
At least six manuscripts submitted to peer-reviewed journals. One of these will be a synthesis prepared by the entire project team, to be submitted to the online journal San Francisco Estuary and Watershed Science.

A report to the CALFED Science Program summarizing the results and making recommendations about next steps.

Table 2. Task Table: Tasks with key personnel, deliverables, and data anticipated for each. The first row gives the deliverables and data anticipated from each task, or all tasks together in the case of the synthesis article.

<table>
<thead>
<tr>
<th>Task</th>
<th>Description</th>
<th>Key Personnel</th>
<th>Deliverables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Each</td>
<td></td>
<td>All</td>
<td>At least one paper <strong>per task</strong> in the scientific literature. One paper synthesizing results (all participate). One talk per task at the CALFED Science Conference Presentations at the Estuarine Ecology Team and other venues</td>
</tr>
<tr>
<td>1</td>
<td>Phytoplankton</td>
<td>Dugdale, Wilkerson, Carpenter, Cohen</td>
<td>As listed above; analysis of relative importance of benthic grazing and nutrient composition.</td>
</tr>
<tr>
<td>2</td>
<td>Benthic Grazing</td>
<td>Thompson</td>
<td>As listed above and under 1</td>
</tr>
<tr>
<td>3</td>
<td>Bacteria</td>
<td>Parker</td>
<td>As listed above, and a joint paper with 4</td>
</tr>
<tr>
<td>4</td>
<td>Microbial foodweb</td>
<td>McManus</td>
<td>Joint papers with 3 and 5</td>
</tr>
<tr>
<td>5</td>
<td>Copepods</td>
<td>Kimmerer</td>
<td>As listed above, and joint papers with all others</td>
</tr>
</tbody>
</table>

**PROJECT ORGANIZATION**

**Management plan**

Table 2 lists the tasks and personnel assigned to each task, along with expected deliverables for each task. The leader for each component will be responsible for ensuring that the component meets its goals. However, we also plan a synthesis paper combining the results of all components, as well as a summary report to CALFED. We believe this is an essential part of the project, and that the synthesis should lead to insights not available from any one component.

This organization provides clarity in budgeting and in assignment of responsibility: the dead person for each task is responsible for ensuring that task is completed and deliverables are provided on time. The lead PI (Kimmerer) will be responsible for gathering information and
developing semiannual Progress Reports, for liaison with contracting personnel, and for ensuring coordination among tasks.

Each of the components (tasks) is linked to the others through the passing of specific information (Figure 7). In tennis of process, this linkage will be largely informal (through email and personal contacts), with meetings of the entire project team twice yearly. Each task leader will be responsible for ensuring the information is passed in a timely manner.

Project management is not identified as a separate task. Since the PI's usually allocate their time to projects in increments of a month, separating the time spent on management would be difficult. Furthermore, in a research project it can be difficult to distinguish project management from other activities: for example, the lead PI for a project element (i.e., a task as we have defined it) spends a lot of time organizing and directing the activities of students and technicians. It is difficult and would be burdensome to attempt to separate these activities. Finally, much of the project management is actually performed by personnel supported by overhead rather than direct costs; these activities include invoicing, human resources, and contracting.

We have not provided for formal public outreach. However, SFSU and RTC are strongly committed to providing outreach and other non-traditional educational opportunities to the community. This commitment is embodied in the Education Coordinator at RTC. Opportunities are frequent for RTC scientists to engage in public speaking, lecturing to teachers or community organizations, and other such activities. We will work with the Educational Coordinator to seek such opportunities as our research unfolds, and present results of our research whenever possible.

Schedule

The schedule for this project is based on an assumed start date of 1 January 2006. However, our past experience suggests that actual start dates could be greatly delayed (i.e., over a year) by contracting difficulties. This could lead to difficulties because of the seasonal component of our proposed research. Should it appear that the start date will be pushed back too far to allow us to accomplish the first year's biological fieldwork, we will delay some of that work a year; however, provided the start date occurs by summer 2006 we will be able to start the physical-dynamics work on time.

As with all scientific research having a field component, conditions may arise that interfere with our work or confound interpretation. Since most of our proposed work will occur in late spring and summer, it is unlikely that adverse weather conditions will have much of an effect. Furthermore, the schedule of our field work is flexible enough to allow for short delays due to storms, boat breakdowns, and other short-term exigencies.

Principal milestones are listed in Table 3. In addition, semiannual progress reports will be developed on the 6-month anniversary of project initiation and every six months thereafter. Generally the reports submitted during summer will be brief summaries of fieldwork accomplished, while those submitted in winter will include a synopsis of results to date.
Table 3. Schedule based on a start date of 1 January 2006. Dates of events are approximate.

<table>
<thead>
<tr>
<th>Date</th>
<th>Activities / Milestones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/06</td>
<td>Initial project meeting to begin planning for field season</td>
</tr>
<tr>
<td>3/06</td>
<td>Project meeting to finalize planning for field season</td>
</tr>
<tr>
<td>7/06</td>
<td>Semiannual report</td>
</tr>
<tr>
<td>9/06</td>
<td>Project meeting to discuss results and prepare presentations for CALFED Science Conf.</td>
</tr>
<tr>
<td>1/07</td>
<td>Semiannual report</td>
</tr>
<tr>
<td>3/07</td>
<td>Project meeting to finalize planning for field season</td>
</tr>
<tr>
<td>7/07</td>
<td>Semiannual report</td>
</tr>
<tr>
<td>9/06</td>
<td>Project meeting to discuss results and map out papers to be written.</td>
</tr>
<tr>
<td>1/08</td>
<td>Semiannual report</td>
</tr>
<tr>
<td>6/08</td>
<td>Draft papers circulated for internal review</td>
</tr>
<tr>
<td>7/08</td>
<td>Semiannual report; Project meeting to develop synthesis and final report</td>
</tr>
<tr>
<td>12/08</td>
<td>Final report submitted</td>
</tr>
<tr>
<td>12/08</td>
<td>Papers submitted for publication</td>
</tr>
</tbody>
</table>

* Notes:
- Semi-annual reports will be submitted every 6 months following the project start date.
- Final report will be submitted 36 months from the project start date.
- Draft manuscript will be substituted for a project closure summary report and submitted 36 months from the project start date.
- Final manuscripts will be submitted after publication.

**Justification**

This project addresses two topics central to CALFED's concerns in the Delta: the biology of delta smelt, and the effect of water management to support the current salinity standard. We believe that an understanding of foodweb support for delta smelt and other estuarine species is essential to effective management.

Delta smelt are now the principal canary in the Delta coal mine. Much of the protective activity in the Delta focuses largely on delta smelt. Yet to date there is no evidence that any of this activity is having a measurable benefit on population size or any other measure of success; indeed, the 2004 fall abundance index was the lowest on record. This implies that other factors are limiting the abundance of delta smelt.

The leading suspect among the limitations on delta smelt is food supply. Preliminary studies have indicated poor feeding condition in a large proportion of larval smelt (Bennett submitted);
the abundance of calanoid copepods on which the smelt feed is low and has become lower; food limitation seems to be rampant among the plankton in this estuary; and the base of the foodweb has been very unproductive since 1987.

We do not suggest that this situation can necessarily be fixed. Rather, we believe that 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. And second, together with proposed modeling and monitoring efforts, it may provide tactical and strategic operations by which protective efforts are focused on times when delta smelt will receive the maximum benefit. We cannot know that without understanding the foodweb of the smelt.

But delta smelt are not the only fish in the estuary. The existing salinity standard is designed to protect the estuarine ecosystem, on the basis that abundance of many estuarine-dependent species varies with outflow. If we understood the basis for those relationships, the salinity standard could possibly be made more effective or more efficient. Several of these species pass their early life stages in the Low-Salinity Zone. Phytoplankton production and biomass in the LSZ appears not to vary with flow, but the input of organic carbon from the freshwater Delta must vary with flow, and this presents a possible mechanism by which fish production could increase with flow.

MODIFICATIONS REQUIRED BY SELECTION PANEL

The Selection Panel made several requests for modification and we have accommodated them in this Scope of Work as described here:

**Budget:** As requested we have reduced the total budget by 10%. However, we are perplexed by the following remark from the Panel: "The Panel felt the overall budget could be reduced by approximately 10% given that there is an entire year dedicated to "synthesis"." This follows a paragraph commenting on the suitability of our budget for the work, which includes the statement that our year 3 budget was lower than that of the previous years because we would devote the third year to synthesis with no further field work. We are forced to conclude that the Selection Panel believes synthesis is unimportant in a multi-investigator, interdisciplinary project with a substantial budget. Of course we do not agree. We therefore choose to reduce our budget by cutting back on field work, reducing the number of days of boat time by 8, and reducing personnel time by a total of about 10 months and supplies and materials by about $7600. We have also reduced travel expenditures somewhat for the investigators in California. The Selection Panel should be aware that this change will result in a reduction in the amount of research we accomplish. Research is provisional and exploratory, and we often encounter unanticipated opportunities and obstacles. The reduction in budget will greatly limit our ability to manage these exigencies. However, we will use the last year for synthesis, and have adjusted our budget accordingly.
Project management: Project management cannot realistically be made a separate task in a University, since much of the work is done by people not paid by direct costs of the grant. We have added some language to describe how the project will be managed.

Data management: The California Environmental Information Catalog site is not really suitable for storage and retrieval of the kinds of data we will be generating, but it has links to the IEP site where we expect to deposit our data. A paragraph on our data practices has been provided.