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INVESTIGATING THE LOWER TROPHIC LEVELS OF THE SUISUN BAY FOOD WEB: A COMPOUND-SPECIFC ISOTOPE APPROACH

1. INTRODUCTION

The San Francisco Bay (SFB) Estuary is a large, strongly tidal, heavily modified estuary with a residence time on the order of months (Figure 1). Over the past decades and especially over the last six years, many species of fish, including the federally-listed threatened species Delta Smelt, have declined dramatically in abundance (Sommer et al., in press). Several theories as to the cause of this decline have been proposed, including export pumping of freshwater in the south Delta, toxic substances, and low food supply (Sommer et al., in press; Bennett, 2005).

Simultaneous declines in Delta phytoplankton, native zooplankton, and native fish populations suggest a possible trophic linkage and that a decline in food resources is an important factor in the decline of juvenile fish (Bennett and Moyle, 1996). Planktonic food webs can be dominated by herbivorous, multivorous or microbial webs according to the relative abundance and trophic status of their components (Legendre and Rassoulzadegan, 1995). Bioassays and labeled uptake experiments indicate that within the upper SFB estuary phytoplankton are the most bio-available form of organic matter (Sobczak et al., 2002, 2005; Müller-Solger et al., 2002) to lower trophic levels despite much higher concentrations of detrital organic matter (Jassby and Cloern, 2000). However, as many species of zooplankton, for example, are opportunistic, they may be able to survive on detritus, the microbial food web, or some combination thereof (Kimmerer et al., 1998; Lee, 1999; Kimmer et al., 2002; Holst et al., 1998).

Water management practices can significantly impact primary production and the delivery of organic matter to the Delta. For example, increasing the flow of the San Joaquin River through the Delta is being actively discussed; one beneficial potential effect would be to deliver the high phytoplankton and zooplankton concentrations found in this productive river to the much less productive central and western parts of the Delta and Suisun Bay during the summer. Delivery of organic matter to these environments can, in turn, impact detritivores and other secondary producers such as bacteria; and the degree to which an increase in the flux of labile organic matter (resulting from increased river flow, for example) is transferred into the food web in presently unknown. The zooplankton species present in the Delta changes temporally, often for multiple reasons (IEP_POD work plan 2007). Because individual species have different feeding strategies and because river flow directly and/or indirectly controls the magnitude and quality of organic matter available to these the organisms, there is a need to directly relate water management strategies to shifts in zooplankton community composition and abundance. Our research goal in this proposal is to obtain a coarse "time-series" of food sources being utilized by the dominant zooplankton inhabiting the central portion of the upper SFB estuary (Suisun Bay). Based on this series of 'snapshots' delineating dominant food sources to organisms in the Bay, we hope to further assess the impact of particular water management strategies on zooplankton community dynamics. Here we propose to focus our efforts here the upper estuary (Suisun

Bay) as it is a critical habitat for the threatened Delta Smelt (Hobbs et al., 2002) and is likely to be strongly affected by proposed changes to the San Joaquin River. Furthermore, biomass appears to more limited in Suisun Bay than in the lower estuary (IEP_POD 2006). Several previous and ongoing studies have focused their research on goals similar to those proposed above. However, the research approach we propose to apply here, measuring *compound-specific* isotope signatures of particular biomarkers in various organic matter fractions in the Bay, is an approach that has not been previously applied in this environment. To re-iterate, our primary research questions are:

What sources of organic matter support the dominant zooplankton population within Suisun Bay at different times of the year?

Will changes in water management practices modify the type and magnitude of food sources available to support zooplankton production?

2. RATIONALE

Isotopic signatures can be used to distinguish between sources of organic matter and trophic levels. The stable carbon (^{13}C) and nitrogen (^{15}N) isotope signature of primary producers often differ, depending on the inorganic nutrients they have incorporated and their metabolisms. The traditional approach of measuring the isotopic signature of bulk organic matter fractions is difficult in the SFB estuary because of the number of potential sources and their overlapping isotopic signatures (Canuel et al., 1995; Cloern et al., 2002). Furthermore, using a specific biomarker (lipids, for example) to follow the trophic transfer of organic matter can also be complicated in estuarine systems where both riverine and marine phytoplankton and bacteria can serve as potential food sources. Although bulk parameters and single biomarker compounds may not be individually sufficient to identify specific sources of organic matter to the SFB estuary, when combined they are often more diagnostic (Canuel et al., 1995; Canuel, 2001). The simultaneous use of multiple isotopic tracers can also help overcome some of the limitation imposed by multiple sources having similar signatures of certain isotopes (Bauer et al., 2002).

In this proposal we will use radiocarbon signature ($\Delta^{14}C$) as one tracer of organic carbon source as it has a greater sensitivity and dynamic range (-1000 ‰ to +200 ‰) than stable carbon isotope ($\delta^{13}C$) signature (35 ‰ to 12 ‰) and can distinguish between estuarine OM sources that would not otherwise be distinct (McCallister et al., 2004; Raymond and Bauer 2001; Bauer et al., 2002). Soil and terrestrial organic matter can have radiocarbon ages that are decades to thousands of years old – reflecting plant residues that has been fixed on land over the past decade, or soil organic matter (Raymond and Bauer, 2001). Our specific goal is to use this tracer to primarily distinguish vascular plant inputs and terrestrial organic inputs into the Bay's food web, since these sources will have $\Delta^{14}C$ values that are unique from carbon that is newly fixed in the estuary. We will focus on zooplankton as our recorder of food sources sustaining lower tropic levels; and by comparing the $\Delta^{14}C$ signature of zooplankton to potential carbon sources including phytoplankton, bacteria, dissolved and particulate organic matter (DOC and POC), we hope to distinguish the extent to which some these sources contribute to zooplankton productivity. To assess the importance of these various carbon sources to zooplankton we

propose to make these measurements in the Sacramento and San Joaquin Rivers, in Suisun Bay, and in the Suisun marsh.

Lipid biomarkers have already been shown to be useful in distinguishing organic matter inputs to benthic clams in the Suisun Bay (Canuel et al., 1995). In particular, fatty acids are useful as dietary biomarkers because they can be diagnostic of different prey groups and certain fatty acids are passed conservatively from prey to predator (see Dalsgaard et al., 2003 for review). Moreover, they provide insight into the nutritional quality of the organic carbon that is consumed (Brett and Müller-Navarra 1997; Müller-Solger et al., 2006). For example, the lipid profile of zooplankton is important to its consumer as fish cannot synthesize certain essential fatty acids and must instead obtain them from their diet (e.g. zooplankton). Diatoms contain $C_{20:5(n-3)}$ fatty acids (eicosapentaenoic acid; EPA) while dinoflagellates have $C_{22:6(n-3)}$ fatty acids (docosahexaenoic acid; DHA) (Viso and Marty, 1993) Alternatively bacterial biomarkers include odd and/or branched fatty acids (Sargent et al., 1987; Kaneda, 1991; Pranal et al., 1996), while vascular plants are characterized by even-numbered, long chain saturated fatty acids (>n – C_{20}). The approach of using the lipid biomarker signature of copepods to deduce their feeding strategies has been very successful in other locations (e.g. Stevens et al., 2004). When combined with measurements of the $\delta^{13}C$ signature of these individual biomarker lipids, this approach can be very powerful. For example, although both freshwater and marine diatoms produce EPA, we expect that the $\delta^{13}C$ of EPA will distinguish between riverine and Bay phytoplankton sources (e.g. $\delta^{13}C_{fresh\text{-}phyto}$ = -32 to -26 ‰; $\delta^{13}C_{marine\text{-}phyto}$ = -27 to – 15 ‰; Cloern et al., 2002). In this study we propose to use the isotopes of biomarkers of known prey origin to determine their relative contribution to organic matter and to zooplankton diet.

Nitrogen isotopic signatures ($\delta^{15}N$) of individual amino acids can provide another dimension of information, particularly when considering trophic level interactions. Typically, bulk nitrogen isotopes are used to infer trophic level positions, with a general assumption of a approximately 3 - 4 ‰ increase in $\delta^{15}N$ signature between prey and predator (Minagawa and Wada, 1984); but these relationships can quickly become confusing in a complex system (Cloern et al., 2002). When they are consumed, individual amino acids are metabolized to different extents by zooplankton (e.g., McClelland and Montoya, 2002). Certain amino acids become strongly fractionated as trophic-level increases (e.g. alanine, glutamic and aspartic acid) while others are transferred more or less conservatively (e.g. glycine, phenylalanine, serine and tyrosine). These relationships have been found to hold for rotifers, size-fractionated zooplankton and krill (McClelland and Montoya, 2002; Schmidt et al., 2006). Thus there is the potential to use the $\delta^{15}N$ signature of some amino acids to infer their source, while using others to infer the trophic status of the consumer. The $\delta^{15}N$ signature of primary producers can vary spatially, but terrestrial organic matter is typically depleted in ^{15}N relative to estuarine organic matter (Michener and Schell, 1994; Deegan and Garritt, 1997). Here we propose to measure the $\delta^{15}N$ signature of alanine (modified) and phenyalanine (conserved) in bacteria, freshwater phytoplankton, marine phytoplankton, and zooplankton to determine the transfer of N between these various organisms. The $\delta^{15}N$ signature of bulk DOM and POM within the bay, the rivers, and the marsh will also be measured. In addition, the recent study by Wankel et al., (2006) provides the necessary data on $\delta^{15}N$ signatures of inorganic N reservoirs in the region.

A final measure of the role of freshwater organic carbon within the Suisun Bay food web will be provided by sulfur isotopes. Similar to carbon and nitrogen isotopes, the $\delta^{34}S$ signature of primary producer biomass reflects the characteristics of the water in which it was produced. In addition, sulfur isotopes undergo little fractionation (<1 ‰ per step) with food assimilation. Therefore, it has been suggested that the combination of sulfur and carbon isotopes provides the best statistical chance for distinguishing between different food-sources (Connolly et al., 2004). The $\delta^{34}S$ signature of estuarine and marine primary producers typically exhibits low variability because they use seawater sulfate (^{34}S = +18 ‰), whereas marsh and terrestrial plants typically derive sulfur from a more variable, and typically isotopically depleted (-10 to +5‰) inorganic sulfur pool (Deegan and Garritt, 1997). Here we propose to measure the bulk $\delta^{34}S$ signature of phytoplankton and zooplankton to distinguish the consumption of riverine versus estuarine phytoplankton by zooplankton in Suisun Bay.

3. POTENTIAL ORGANIC MATTER INPUTS

3.1. Terrestrial organic matter

As outlined previously, this proposal seeks to quantify the relative importance of the various sources of organic matter incorporated into the food web of Suisun Bay, and relate these findings to water-management strategies.

On average, tributary-borne organic matter is the largest carbon source to Suisun Bay and the entire SFB estuary; this terrestrial input contributes more than four times the amount of organic carbon than the next most important source, phytoplankton production (Jassby and Cloern, 2000). Results from bioassay and uptake experiments suggest, however, that this organic matter is largely unavailable to biota and unable to support secondary productivity (Sobczak et al., 2002; Müller-Solger et al., 2002). Instead, phytoplankton-derived organic matter appears to be the major bioavailable input to the Bay, even though it constitutes less than 5% of the total organic matter in deep-river channel habitats (Sobczak et al., 2002).

While it has been shown that terrestrial organic matter is less bioavailable than phytoplankton biomass, it is unlikely that this carbon is entirely excluded from the food web. In fact, the estuary is net heterotrophic (Sobczak et al., 2005), requiring the de facto utilization of some portion of the allochthonous material by microheterotrophs, and several copepods and rotifers in this area are capable of consuming detritus (Kimmerer et al., 1998; Lee, 1999; Kimmer et al., 2002; Holst et al., 1998). There is a persistent additional source of organic matter near Suisun Bay, possibly from the San Joaquin River, in the form of DOC (e.g. dissolved humic substances, and dissolved carbohydrates; Murrell and Hollibaugh, 2000). Furthermore, microbial metabolic measures have been shown to correlate with freshwater flow in the San Francisco Bay, with slow rates occurring under low-flow conditions due presumably to decreases in the delivery of organic matter (Murrell et al., 1999). Together, these pieces of evidence suggest that dissolved and particulate organic detritus could have an impact on Bay food webs, particularly if this carbon is consumed by bacteria and then made available to higher trophic levels.

The incorporation of terrestrial organic matter into the larger food web may occur in two ways. First, zooplankton may directly incorporate detritus delivered by the rivers, with an

ultimate source from agricultural fields or soil organic matter. Both sources will have unique $\Delta^{14}C$ signatures – for example, until the last few years, the $\Delta^{14}C$ signature of atmospheric CO_2 was significantly enriched (>200‰) relative to marine DIC and so terrestrial plants should reflect this enrichement; on the other hand, soil organic matter is likely to have $\Delta^{14}C$ signatures that are significantly depleted relative to marine DIC ($\Delta^{14}C$ values < -50‰) (Raymond and Bauer, 2001). Therefore, zooplankton relying on these different carbon sources – vascular plants, soil organic matter and marine organic matter – should be distinguishable based on their $\Delta^{14}C$ signature. Vascular plants produce diagnostic long chain, unsaturated fatty acids and their presence would also indicate the reliance of zooplankton on terrestrial organic matter. Additionally, estuarine/marine organic matter sources are expected to have a constant ^{34}S ratio, reflecting sulfate utilization.

Alternatively, zooplankton may only indirectly rely upon terrigenous organic matter - after it has been shunted through the microbial loop. In this case, the lipids that would ordinarily be diagnostic of vascular plants (long chain unsaturated fatty acids) will be destroyed and replaced by those diagnostic of bacteria (branched and/or odd fatty acids). The ^{14}C signature of the zooplankton will still allow us to distinguish between the carbon sources identified above. Additionally, as explained above, $\delta^{34}S$ signatures will identify freshwater sources as well.

One way to differentiate between the above processes, in addition to the lipid biomarkers, will be through ^{14}N amino acid isotopes. The ^{15}N ratio of those amino acids that do not change significantly during trophic transfer (e.g. phenylalanie) will still retain a terrestrial plant composition after it is consumed by zooplankton. If consume directly, isotopic increases in amino acids such as glycine will be indicative of a single trophic level transfer. But if terrestrial detritus is shunted through the microbial web, a much larger trophic level increase will be observed.

3.2 Fresh-water phytoplankton

In addition to delivering large amounts of terrestrial carbon, the Sacramento and San Joaquin Rivers contribute fresh-water phytoplankton to the Suisun Bay. Phytoplankton are present in both rivers (Cloern et al., 2002) and high concentrations can build as a result of flooding the Yolo Bypass (Schemel et al., 2003). A reliance by zooplankton on fresh-water phytoplankton will be evident in several ways. First, phytoplankton-specific lipids (e.g. EPA, DHA) will have 13C compositions that differ from those of estuarine phytoplankton ($^{13}C_{fresh\text{-}phyto}$ = -32 to -26 ‰ $^{13}C_{marine\text{-}phyto}$ = -27 to – 15 ‰; Cloern et al., 2002). River DIC also tends to be more enriched in ^{14}C compared to that of estuarine DIC (+110 to +150 ‰ vs. +50 to +100 ‰; Raymond and Bauer, 2001) and, in fact, is expected to be the most enriched source of ^{14}C available. Nitrogen isotopes of fresh-water algae are also depleted in ^{15}N compared to algae found in the estuary (2 to 8 ‰ vs. 7 to 14 ‰; Cloern et al., 2002). Finally, sulfur isotopes will reflect a "fresh-water" source.

3.3. Estuarine-marine Phytoplankton

As already discussed, the Suisun Bay food web is believed to depend heavily upon estuarine-marine phytoplankton. We will use phytoplankton specific biomarkers to determine the extent to which they are taken up by zooplankton and their contribution to organic matter

(DOC and POC) within the bay. As previously explained, the ^{13}C composition of diagnostic fatty acids (e.g. EPA) will help to distinguish between estuarine-marine phytoplankton and freshwater phytoplankton. ^{34}S will also provide insight into the role of freshwater vs marine organic matter input. If zooplankton predominantly consume estuarine phytoplankton, we expect the ^{15}N ratio of their amino acids to be closely linked that of local phytoplankton (7 – 14 ‰).

3.4 Marsh Organic Matter

Much of the tidal wetlands of the Sacramento-San Joaquin Delta have been levied removing them from tidal and floodwater inundation. To our knowledge, an accounting of the organic matter output from the Suisun marsh has yet to be made. General estimates based on other estuaries were unable to determine if the tidal wetlands would be a net source or sink of organic matter (Jassby and Cloern, 2000). Flooding of the wetlands to create fish habitat or due to levee breach is expected to increase phytoplankton production and may mobilize vascular plant organics.

In other estuaries, marsh organic matter is ^{14}C-depleted and ^{13}C-enriched relative to the organic matter in the estuary (McCallister et al., 2004). As part of this proposal, we sample at least one area of the tidal marsh during different seasons to determine the isotopic composition of the organic matter that is exported into the Suisun Bay. Incorporation of marsh-derived organic matter will be evident through vascular plant biomarker. Marsh plants also typically have ^{34}S ratios that reflect the inorganic sulfur sources within the soil and are thus lighter and variable than estuarine phytoplankton.

3.5. Microbial Food Web

The potential importance of bacteria as a food source to upper trophic levels has been discussed above. Bacterial input into upper trophic levels will be evident in several ways. First, they produce diagnostic lipids (branched and/or odd fatty acids) that can be found up the food web. Similar to distinguishing fresh-water versus estuarine phytoplankton, the ^{13}C content of microbial fatty acids will help to determine their food source. Secondly, ^{15}N signatures of biomass that has passed through the microbial loop often will have a large isotopic increase, since it has been heavily re-worked. We therefore expect zooplankton relying upon the microbial loop to have a higher ^{15}N ratio than they would if they were relying upon phytoplankton production entirely. Finally, we expect the radiocarbon signature of bacteria within the bay to differ from that of phytoplankton production. Bacteria are likely utilizing several sources of detrital organic matter, potentially from the rivers, from phytoplankton, and from the marsh. Thus, the radiocarbon signature of zooplankton may also provide insight into their food consumption.

4. TRACING WATER MANAGEMENT CHANGES THROUGH THE FOOD WEB

Several changes in water management strategies will alter the quality and quantity of organic matter being delivered to the Bay, often with the intent to increase food resources. If

these projects are successful, they will alter the dietary signature of zooplankton in predictable ways.

Flooding of the Yolo Bypass promotes fresh-water phytoplankton production, especially if it is flooded multiple times during the winter and especially the spring season (Schemel et al., 2003). If zooplankton are able to utilize this increase in labile biomass, their ^{14}C and ^{34}S signatures should become lighter, as should the ^{15}N of concerved amino acids. Evidence of bacterial input (e.g. lipids, very heavy ^{15}N glycince ratios) should not be evident.

Increasing the flow of the San Joaquin River through the Delta is also being actively discussed by water resource managers. The intent is to counteract reverse river flows caused by State and Federal water project operations to avoid fish entrinment and to help deliver the high phytoplankton and zooplankton concentrations found in this productive river to the much less productive central and western parts of the Delta and Suisun Bay during the summer. The biomarker results in zooplankton would be similar to those described above. If this increase in the San Joaquin River flows also increases the input of vascular plants or soil organic matter, the ^{14}C of zooplankton biomass would be depleted. Differentiating between two different 'directions' of ^{14}C (enriched due to increase freshwater phytoplankton versus depleted due to increase in terrestrial organic matter) could be achieved through reliance on ^{15}N isotopes of 'source' amino acids, which will differ between the two sources. Further, an increased reliance on river-borne organic matter will be evident in any case through ^{34}S isotopes.

5. RELATIONSHIP WITH OTHER CALFED PROJECTS

The questions posed here are very similar to another CALFED-funded project currently under way ("Foodweb Support for the Threatened Delta Smelt and Other Estuarine Fishes in Suisun Bay and the Western Sacramento-San Joaquin Delta;" PI: Kimmerer) and, combined, the two approaches will allow for an overall much greater understanding of the system than if either were performed alone. The approach of Kimmerer et al. includes weekly sampling to measure controls on phytoplankton (grazing, light limitation), uptake rates, and zooplankton mesocosm experiments to determine feeding habits. They do not plan to incorporate the natural abundance of isotopes in their research. Their research will help us to eliminate potential organic matter inputs and thus constrain our isotopic mass balances. Our research will be able to provide clear constraints on the maximum and minimum percentages different food sources contribute to zooplankton diet through isotopic mass balances. It will provide direct confirmation that food sources are consumed by zooplankton even under complex conditions. I intend to closely interact with members of the Kimmerer team, including two current Calfed Science Fellows, Dr. Lindsay Sullivan and Dr. Alex Parker, associated with this project and have already had initial conversations.

The lipid measurements proposed here will also provide information on the nutritional value of the available organic matter for zooplankton and of zooplankton for fish as has been shown in a previous CALFED-supported project ("Food resources for zooplankton in the Sacramento-San Joaquin River Delta;" PI: Müller-Solger, Müller-Solger et al., 2006). Several of these PUFA's have been identified as key constituents determining the quality of food for

zooplankton and fish in freshwater and marine systems (Arts & Wainman 1999, Brett and Müller-Navarra 1997), including the essential ω3- fatty acids eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) and their precursors alpha linolenic acid (ALA, 18:3ω3) and stearidonic acid (SDA, 18:4ω3). The concentrations of these compounds has been examined in *P. forbesi* and *E. affinis,* but is largely unknown for other Delta zooplankton species (Müller-Solger et al., 2006). These compounds are of principle interest to this proposal and the data we obtain will contribute to determining similar nutritional information for other species or groups.

6. METHODS

6.1. Sampling Scheme

The first year of the project will be used to determine a baseline value for all proposed analyses and to assess zooplankton diet. Since the compound-specific isotopic analyses we are proposing are time-intensive and expensive, we will only sample twice during the first year (Spring and Summer). We will conduct all of the described isotopic measurement for these two sample sets and use these results to direct our sampling efforts in following years.

We will sample more frequently during the second year (4 times) and third year (3 times) but we will also limit our biomarker purification efforts to focus on the suite of analyses that provide the most diagnostic source information. We hope to determine the appropriate sub-set for this focus following our research efforts during the first year. However, samples will be archived allowing us to return to them for additional analyses if necessary.

We propose to sample Suisun Bay, a tidal marsh location, the Sacramento River and the San Joaquin River during all years. Several research groups regularly sample the deep water channels of Suisun Bay and we will rely upon cruises of opportunity to obtain the necessary samples. We have already contacted Jim Cloern (USGS), and he has agreed to provide a berth on his monthly cruises, which travel the length of Suisun Bay and to the mouth of the Sacramento River. The rivers and the tidal marsh will be sampled from Department of Water Resources sampling locations: Sacramento River at Hood (C3A); San Joaquin River near Vernalis (C10); Suisun Bay at entrapment zone (point where electro-conductivity is 2000 µS/cm); Suisun Marsh (NZ032). The two river sites are continuous monitoring stations which include instruments that continuously measure total and dissolved organic carbon concentrations (see http://www.baydelta.water.ca.gov/emp/ and http://www.wq.water.ca.gov/mwqi/toc/toc.cfm for more information) This provides us with the additional benefit of having long term water quality data from the same location as our samples.

In each location samples will be collected for DOM, POM, bacterial biomass, phytoplankton, microzooplankton and mesozooplankton. Water samples will be collected with Niscan bottles or acid washed Teflon bottles and filtered through a GF/F filter to isolate POM from DOM. The filter and water samples will be frozen for later POC, $PO^{14}C$, DOC, $DO^{14}C$ analysis (see below). Bacterial and phytoplankton biomass will be passing water through three filters in series (coarse 45 µm screen followed by a 1.3 µm filter followed by a 0.2 µm filter) using a teflon hose and compressed air-driven diaphragm pump. The 50 µm to 1.3 µm size

fraction will be processed for phytoplankton-related biomarkers, and the 1.3 μm to 0.2 μm size fraction will be used to sample for bacterial parameters.

To ensure that the 1.3 μm filter is dominated by phytoplankton – and not detritus or bacteria – an aliquot of the total cell-extract will be analyzed for chlorophyll *a*, phaeophytin, D-alanine, and L-alanine. Fresh phytoplankton will contain high concentrations of chlorophyll *a* relative to its degradation product, phaeophytin (Sachs et al., 1999). In addition to L-alanine bacteria produce the D-enantiomer of alanine, which is present in peptidoglycan; eukaryotic organisms produce primarily the L-enantiomer. We expect the 1.3 μm filter to contain few to no no bacteria and thus low concentrations of D-alanine.

Zooplankton will be sampled with a 45 μm net and frozen until further processing in the lab. They will be visually identified and grouped with the help of Anke Müeller-Solger (see letter of support). If enough individual adults belonging to the same species can be identified, they will be sorted and grouped together. Remaining zooplankton will be sorted into two subclasses: microzooplankton (including protozoans and rotifers, <200 microns) and mesozooplankton (including copepods, 200 μm to 2 mm). Groups of zooplankton will be rinsed, freeze-dried and ground for chemical and isotopic analyses. If enough individuals are present, the mesozooplankton fraction will be further sorted into the narrowest taxonomic groups possible. Groups of zooplankton will be rinsed, freeze-dried and ground.

6.2. Bulk Stable Isotopes and Elemental Ratios

Aliquots of biomass from each filter and zooplankton size-class will be analyzed for stable isotope and elemental ratio measurements using a coupled elemental analyzer-isotope ratio mass spectrometer, available through the Unified Laboratory Facilities (ULF) at SIO.

6.3. Radiocarbon of bacteria, phytoplankton and zooplankton

The remaining filter material will be processed for radiocarbon measurements of bacteria (0.2 μm) and phytoplankton (1.3 μm). Biomass will be dried *in vacuo* and oxidized to CO_2 by dry combustion with CuO and Ag at 850°C in double quartz tube. The generated CO_2 will be quantified at SIO and submitted for $\Delta^{14}C$ measurements at the National Ocean Science Accelarator Mass Spectrometry Facility (NOSAMS) at Woods Hole (www.nosams.edu) or the Center for Accelerator Mass Spectrometry at the Lawrence Livermore National Laboratory (cams.llnl.gov). Individual zooplankton considered representative of each class will be ground, dried *in vacuo*, and oxidized as above.

6.4. Lipid Analysis

Duplicate filters (1.3 μm and 0.2 μm) will be extracted in 1.5 M $NaClO_4$ (sodium perchlorate), to lyse cells and release cellular components into solution for compound purification (Blair et al., 1985). This solution will be extracted at neutral pH with chloroform, water and phosphate buffer according to a modified Bligh and Dyer (1959) method to isolate the total lipid fraction. Proteins will remain in the water fraction and the organic/water interface.

Zooplankton will be ground in methanol and similarly extracted. Organic phases will be concentrated under N_2 and total lipid extracts will be separated by silica gel column chromatography to separate neutral, glycol-, and polar lipids. High performance liquid chromatography-mass spectrometry (HPLC-MS) will then be used to separate and identify aliphatic hydrocarbons, aromatic hydrocarbons, ketone compounds and sterol and alcohol compounds (Tolosa and Mora, 2004). Individual lipids of interest will be collected via fraction collector for isotopic measurements.

We will also perform $\delta^{13}C$ measurements on up to 10 different lipid fractions. Fatty acids will be converted to their methyl esters using methanolic-HCl prior to gas chromatography-isotope ratio mass spectrometric (GC-IRMS) analysis. Sterols will be treated with *N,O*-bis-(trimetylsilyl)trifluoroacetic acid to convert them into their corresponding trimethylsilyl ethers prior to GC-IRMS analysis.

6.5. Isotopic ratio of individual amino acids (^{15}N)

Samples will be processed according to McClelland and Montoya (2002). The aqueous fraction from the above liquid-liquid filter extractions will be rotary evaporated to remove any traces of organic solvent, freeze dried and hydrolyzed for amino acid analysis. Aliquots of ground zooplankton samples will be dried and then hydrolyzed with 6 M hydrochloric acid for 12 hours at 110 ºC. The hydrolysate will be evaporated to dryness and re-dissolved in 0.01 M HCl. Amino acids will be purified on a cation exchange resin (50WX8 exchange resin) and eluted with 4 mL of 2 M ammonium hydroxide. The purified amino acids will be dried under N_2 and derivatized to N-pivaloyl-i-propyl amino esters (Metges et al., 1996). Amino acids will then be separated on a gas chromatograph that is directly coupled to an IRMS for determination of stable nitrogen isotope signatures

6.6. Radiocarbon of Bulk Samples

Samples will be collected for radiocarbon measurements of DIC, bulk DOC (from freshwater and low salinity environments only), and suspended POC. $DI^{14}C$ samples will be collected, stored and analyzed according to Druffel *et al.* (1992). $DO^{14}C$ samples will be processed according to Raymond and Bauer (2001). Briefly, samples will be freeze dried and oxidized as above.

Total DOC and DIC samples will be processed for radiocarbon a NOSAMS and LLNL-CAMS as above. $DI^{14}C$ samples will be purged of CO_2 after adding H_3PO_4 according to standard techniques (McNichol *et al.*, 1994). Suspended POC on GF/Fs will be acidified with 1% H_3PO_4 overnight to remove carbonates, dried *in vacuo* and oxidized to CO_2 by dry combustion with CuO and Ag at 850°C in double quartz tubes (De Jesus and Aluwihare, unpublished; according to Druffel *et al.*, 1992). All biomarker fractions and POC fractions will first be combusted and quantified at SIO before submitting to NOSAMS for measurement. All AMS and IRMS costs are included in the budget and described in the budget justification.

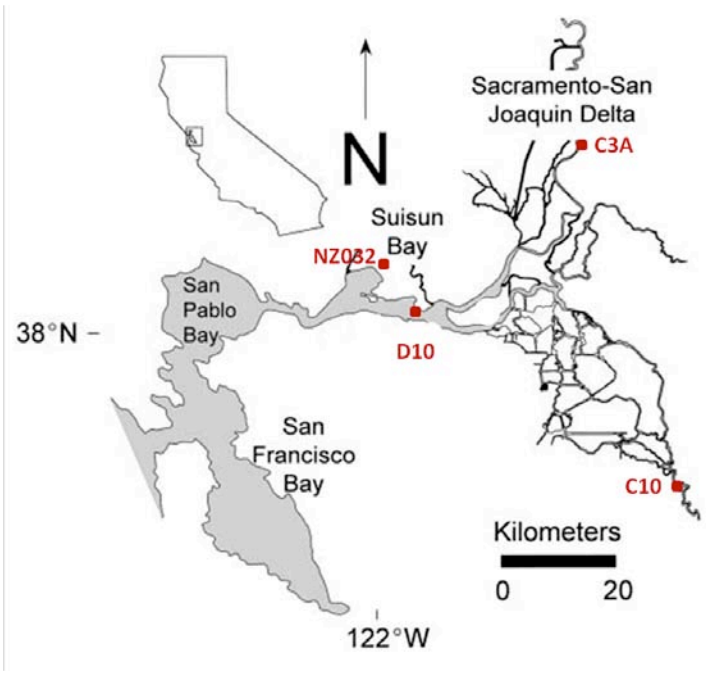


Figure 1. Map of the Sacramento-San Joaquin Delta, including planned sampling stations: Sacramento River at Hood (C3A); San Joaquin River near Vernalis (C10); Suisun Bay at entrapment zone (D10; point where electro-conductivity is 2000 µS/cm). Suisun Marsh (NZ032).

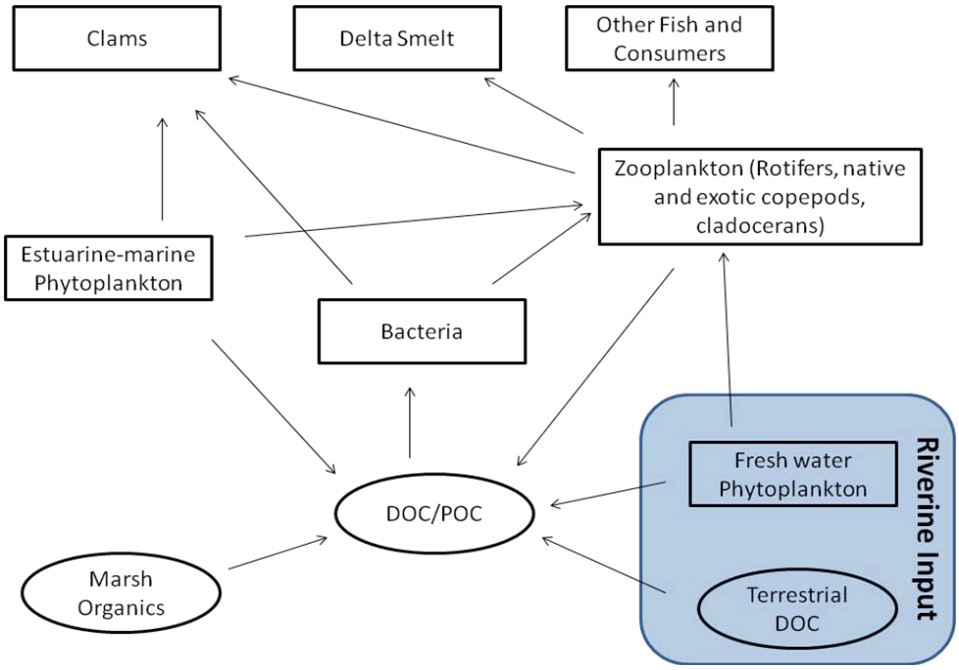
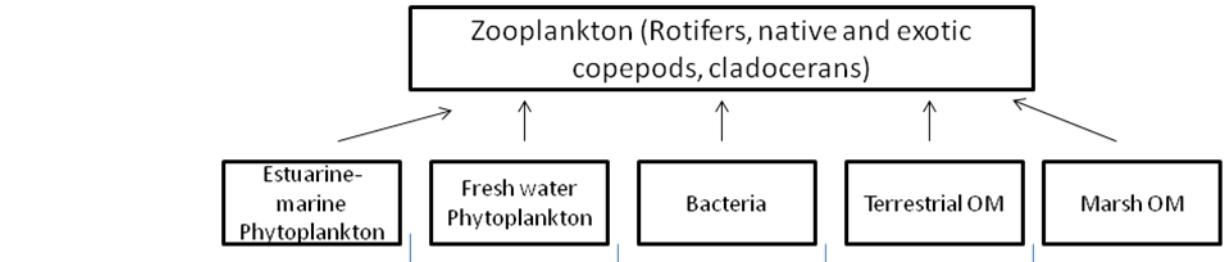


Figure 2. Simplified food web of Suisun Bay.



Lipid Biomarkers[1]	Estuarine-marine Phytoplankton	Fresh water Phytoplankton	Bacteria	Terrestrial OM	Marsh OM
	EPA and DHA	EPA and DHA	Odd/branched fatty acids	Even, Long-chain	Even, Long-chain
13C of lipids[2]	-27 to -17‰	-32 to -27‰	-27 to -17‰	-23 to -30‰	-12 to -29‰
14C of biomass[3,4]	+50 to +100	+110 to +150	-30 to +50	-30	+50
15N of source AAs[5]	7 – 14‰	2 - 8‰	7 – 14‰	-3 to 15‰	0 to 17‰
34S of AAs[7]	+18 ‰		+18 ‰	-10 to +5‰	-10 to +5‰

Figure 3. Expected values of zooplankton dietary sources.

[1] Viso and Marty, 1993; Sargent et al., 1987; Kaneda, 1991; Pranal et al., 1996
[2] Extrapolated from SFB biomass categories (Cloern et al., 2002)
[3] Terrestrial is DO14C of Sacramento River from Spiker (1980)
[4] Non-terrestrial values extrapolated from other estuarine systems (Raymond and Bauer, 2001)
[5] Extrapolated from biomass of SFB categories (Cloern et al., 2002)
[6] Extrapolated from other estuarine systems (Deegan and Garritt, 1997)

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