EXHIBIT A: Attachment 1 –Project Narrative

Habitat Values of the Native SAV, *Stuckenia pectinata*, in the Low Salinity Zone of San Francisco Estuary

Primary Investigator: Katharyn Boyer

Revised proposal submitted April 27, 2011 to the Delta Science Program

Project Purpose

The northern San Francisco Estuary and Delta are home to a number of fish species of special interest, including delta smelt, sacramento splittail, chinook salmon, green and white sturgeon, and steelhead. The particular habitat and dietary needs as well as constraints to the success of these native fish species are a major focus of research in the region, yet there are a number of important gaps in our understanding (e.g., Brown 2003). **The purpose of this project is to investigate the value of native submerged aquatic vegetation (SAV) beds in providing habitat that may enhance food web support for native fish species in the low salinity zone of the San Francisco Bay-Delta.**

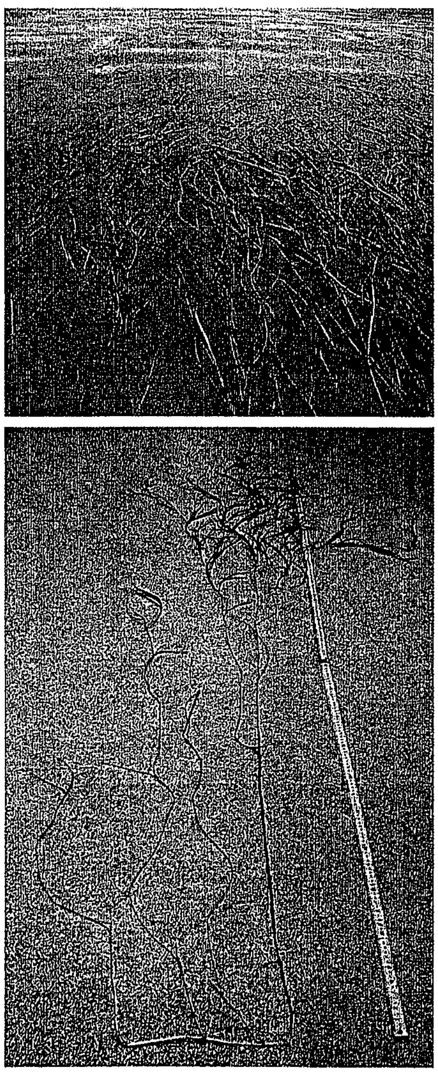

Figure 1. Native SAV beds dominated by *Stuckenia pectinata* are prominent features in the shallow subtidal zone of Suisun Bay and the west Delta, forming relatively open canopies up to 3m tall (preliminary data).

These native SAV beds, composed primarily of *Stuckenia pectinata* (sago pondweed; Fig. 1), are an extensive feature in the shallows of Suisun Bay and the west Delta, yet almost nothing is known of their seasonal patterns, the invertebrate communities they support, or how their physical structure or food resources may influence use by native fishes. This is surprising considering their regional extent, size, position through major migratory paths of native fishes, and persistence through time (Fig. 2). We hypothesize that the position of these plant canopies in the shallow water column makes associated food resources, such as epifaunal invertebrates, readily accessible to native fishes in this region of the estuary. SAV beds positioned along islands in this region are likely to increase habitat and food resource options of adjacent wetland and channel habitats.

SAV generally has a poor reputation in the Delta region due to the negative effects of invasive SAV species, primarily *Egeria densa* (Brazilian waterweed), on both native fishes and recreational uses (Nobriga et al. 2005, Brown and Michniuk 2007). We suggest that the timing is right to begin to investigate the roles of native SAV beds in the low salinity zone, not only for their potentially important current support of food webs of native fishes, but because they are likely to increase up-estuary with drought or sea level rise and concomitant increases in salinity. Further, accidental or intentional actions (such as breaching levees for restoration purposes) that flood subsided lands may also increase salt penetration while also achieving shallow subtidal elevations suitable for these plants. Habitat value for native fishes might be enhanced through active restoration of these plants in these newly subtidal areas.

The objectives of this project are to: 1) characterize seasonal patterns in habitat characteristics of SAV beds in four locations in Suisun Bay and the western Delta, with comparisons to four non-native *Egeria densa*-dominated beds), 2) document seasonal changes in the epifaunal invertebrate community composition and abundance in *Stuckenia* versus *Egeria* beds, 3) utilize stable isotope analyses to evaluate food web relationships within and among the beds, and 4) begin preliminary evaluation of fish use of *Stuckenia* beds through limited sampling inside and outside of beds.

Of the Proposal Solicitation Package's Priority Research Topic List, Topic 1, Native Fish Biology and Ecology is central to this proposal, as we intend to assess the potential for native SAV beds to support native fish species through provision of habitat and food resources. We will characterize the habitat attributes of these native SAV beds, measuring canopy architecture, temperature, salinity, light penetration, and food quality and quantity in these beds. We will conduct preliminary evaluation of fish use through sampling inside and outside of *Stuckenia* beds with assistance from scientists at UC Davis.

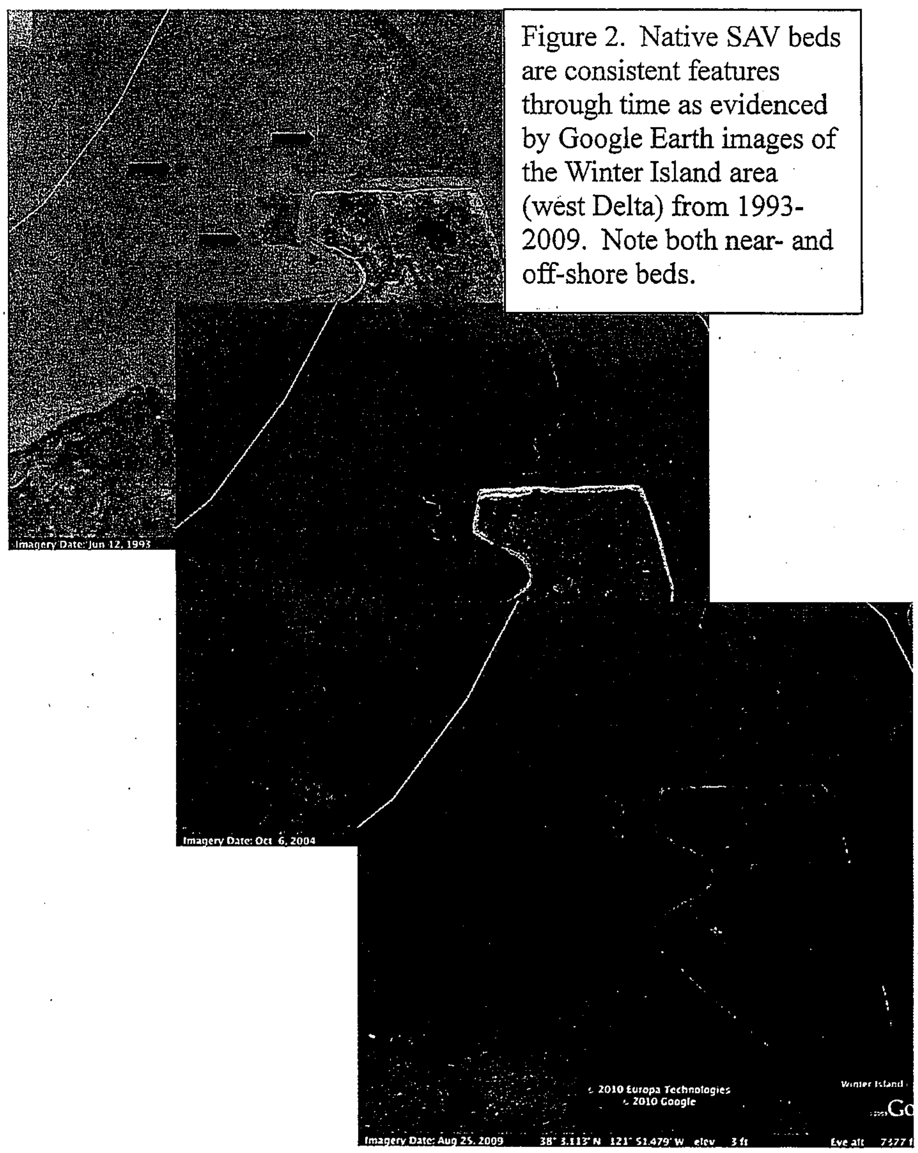

Figure 2. Native SAV beds are consistent features through time as evidenced by Google Earth images of the Winter Island area (west Delta) from 1993-2009. Note both near- and off-shore beds.

This work is also closely related to Topic 2, Food Webs of Key Delta Species. We will identify and quantify the invertebrate assemblages that potentially contribute to native fish diets. Further, we will use stable isotopes to envision the food web relationships within these native SAV beds. If possible during preliminary fish sampling, we will include tissues from captured fish in the stable isotope analysis and will conduct gastric lavage to assess gut contents.

Background and Conceptual Models

Our research group has been working in the lower San Francisco Estuary (San Pablo, Central, and South Bays) on many different aspects of ecology, restoration and conservation of the SAV species, eelgrass (*Zostera marina*), including evaluation of its habitat functions and food web relationships (e.g., Boyer and Wyllie-Echeverria 2010, Carr et al. 2011, Carr et al. in review). Our colleagues working up-estuary have sent photos and Google Earth images showing the native SAV beds in Suisun Bay and the west Delta, encouraging our interest in developing a study to evaluate their roles. It has become apparent that very little is known about these beds, including species composition and structural characteristics of the SAV, composition and relative abundance of associated assemblages, and use by native fishes.

Generally speaking, submerged vegetation in estuaries throughout the world has important influences on biogeochemical cycling, sediment stability, and food web support (e.g., McGlathery et al. 2007; see review by Orth et al. 2006). SAV species often provide food and shelter for numerous fish and invertebrates, and serve as a nursery habitat, providing predation refuge for juvenile fishes (Orth et al. 1984; Rozas and Odum 1988; Bostrom and Bonsdorff 2000; Duarte 2000). Worldwide, they support myriad rare and endangered animals, as well as commercially important species (Hughes et al. 2009).

In the San Francisco Bay-Delta, studies of non-native SAV beds dominated by *Egeria densa*, show high fish abundances; however, the assemblages are dominated by non-native species. *Egeria* thrives in the perennially fresh, slow moving waters of the Delta, choking waterways and impeding movement of fish. It also must be removed to keep waterways open, either mechanically or using toxic copper compounds. The waterweed harbors numerous introduced freshwater predatory fish that may have negative impacts on some desirable native species (Brown and Michniuk 2005, Nobriga et al. 2005).

In contrast, the canopies of native SAV beds in Suisun and the west Delta have relatively open canopies, owing to long slender shoots with branches bearing leaves primarily in the upper 1/3 of mature shoots (Fig. 1). In a preliminary investigation of these beds in June 2010, we determined that the majority of plants in the beds are the native *Stuckenia pectinata*, formerly *Potamogeton pectinatus*, sago pondweed. Although it is not uncommon for this species to form monotypic stands in Chesapeake Bay and elsewhere, additional species may also be present (K. Boyer pers. obs., P. Baye, pers. comm.). While the abundance and species composition of fishes in *Egeria* beds has been studied (Brown and Michniuk 2005, Nobriga et al. 2005, Grimaldo et al. 2009), the role of native SAV beds for native fishes in the Bay-Delta has not been explicitly addressed.

We propose a conceptual model in which the low density cover of native submerged vegetation with abundant food provides better habitat for native fishes than either open water or dense beds dominated by the non-native *Egeria densa*. Preliminary sampling suggests that turbid waters of *Stuckenia* beds could provide some visual refuge from predation for native fish, without the dark hiding places that support non-native predators in *Egeria* beds (Nobriga et al. 2005).

Native SAV beds are a prominent and consistent feature in Suisun Bay and the west Delta, as seen by examining Google Earth images through time (e.g., Fig. 2 at Winter Island). In fact, these beds may be increasing in distribution; preliminary examination of many images suggests the beds are becoming a more prevalent habitat feature since the early 1990s.

Figure 3. We propose a conceptual model in which native SAV in the west Delta and Suisun Bay directly enhances success of native pelagic fish species of concern such as the delta smelt, sacramento splittail, and chinook salmon, through provision of structure for food resources, without the dense, dark cover that supports non-native predators in invasive *Egeria* beds. Proximity of many of the native SAV beds to tidal marshes and channels may provide multiple options for food and cover for native fishes.

Native fishes evolving in the Sacramento-San Joaquin Delta and Suisun Bay would have experienced a more variable environment than exists today with higher flow in winter (without reservoirs trapping the water) and lower in summer (without reservoirs releasing the water), and thus higher salinity conditions during the dry season (Knowles 2002, Kimmerer 2004). A more saline environment would likely have supported brackish SAV species along the wetlands of the Delta region, as opposed to the introduced SAV species that thrive in fresh conditions. A return to higher salinity conditions in the west Delta could come about through several mechanisms, including drought conditions, which could lead to increased salt penetration not counteracted by reservoir releases during the summer months; levee failures, which could lead to a greater volume of tidal waters reaching the Delta and thus greater salinity in these waters (Enright 1998); or management actions that inadvertently or deliberately reduce freshwater releases during the dry season. Higher salinity conditions are likely to have no adverse effects on native fishes that evolved in the northern Estuary, and may result in reductions in non-native freshwater SAV species and enhancement of habitat available for native SAV. Thus it is possible that future scenarios will be conducive to both an expansion in native SAV such as *Stuckenia pectinata* as well as the physical structure this habitat provides for food web support for native fishes.

While it has been hypothesized that tidal wetlands might provide food web support for pelagic fishes in the northern Estuary and Delta, there is little evidence to support this link (Brown 2003). Native SAV beds in the shallow but open waters of this region may serve this purpose to a much greater degree due to closer proximity to the migratory pathways of native fishes as well as the abundant food resources on and among these plants (preliminary sampling, Fig. 4).

Approach and Scope of Work

We will evaluate characteristics of native SAV beds in the low salinity zone to assess their potential to support native fishes. In a preliminary visit to some of these beds in June 2010, we observed that direct consumption of the plants is occurring, and that the plants harbor many invertebrates, including isopods, amphipods, beetles, and spiders (Fig. 4). We also observed juvenile fish moving through the turbid water among the loose canopies of the *Stuckenia*. We hypothesize that these beds provide the physical structure to support ample food resources while discouraging predation by non-native fish species due to high turbidity in the beds, yet no dark hiding places. We are interested to know: What habitat features are present in these beds and how do they compare to open water and *Egeria* beds? What potential food resources are found in *Stuckenia* beds and how do species composition and density differ from *Egeria* beds? How is the invertebrate food web structured in these two habitats? And

Figure 4. Hypothesized trophic support of native SAV beds. Top: direct consumption of plant tissues (note feeding scars) appears to contribute to the food web. Middle three photos: isopods, amphipods, and spiders are among the abundant invertebrate fauna on these plants. Bottom: hundreds of invertebrates rinsed from a single shoot in preliminary sampling.

through preliminary sampling, to what degree are native fishes utilizing native SAV habitats?

We will address the following objectives in our study:

1) *Characterize seasonal patterns in habitat structure of native* Stuckenia *beds in comparison to non-native* Egeria *beds*

We have selected four locations in Suisun Bay and the western Delta that are likely to be our native SAV study sites (Fig. 5) based on a preliminary reconnaissance in summer of 2010. Additional *Stuckenia* distribution surveys in summer of 2011, funded by NOAA Fisheries, may lead us modify our selections due to considerations such as accessibility. During four quarterly periods, we will characterize the canopy architecture of the *Stuckenia* beds, including densities, heights, branching patterns, and biomass. Preliminary observations of several morphs of these pondweeds suggest there may be additional subdominant native SAV species in these beds, and Dr. Peter Baye will be given samples of floral parts to aid in identification of these species. Direct herbivory on native SAV species will be quantified as number of shoots encountered with grazing scars (see Fig. 4), number of leaves per shoot showing grazing, and area of tissue grazed per leaf (quantified using photography and digital imaging software). To characterize differences in physical characteristics within and outside of beds, we will measure temperature and salinity continuously using Hobo Conductivity Data Loggers and will quantify light attenuation through the water column with a LI-COR spherical PAR sensor at multiple depths on

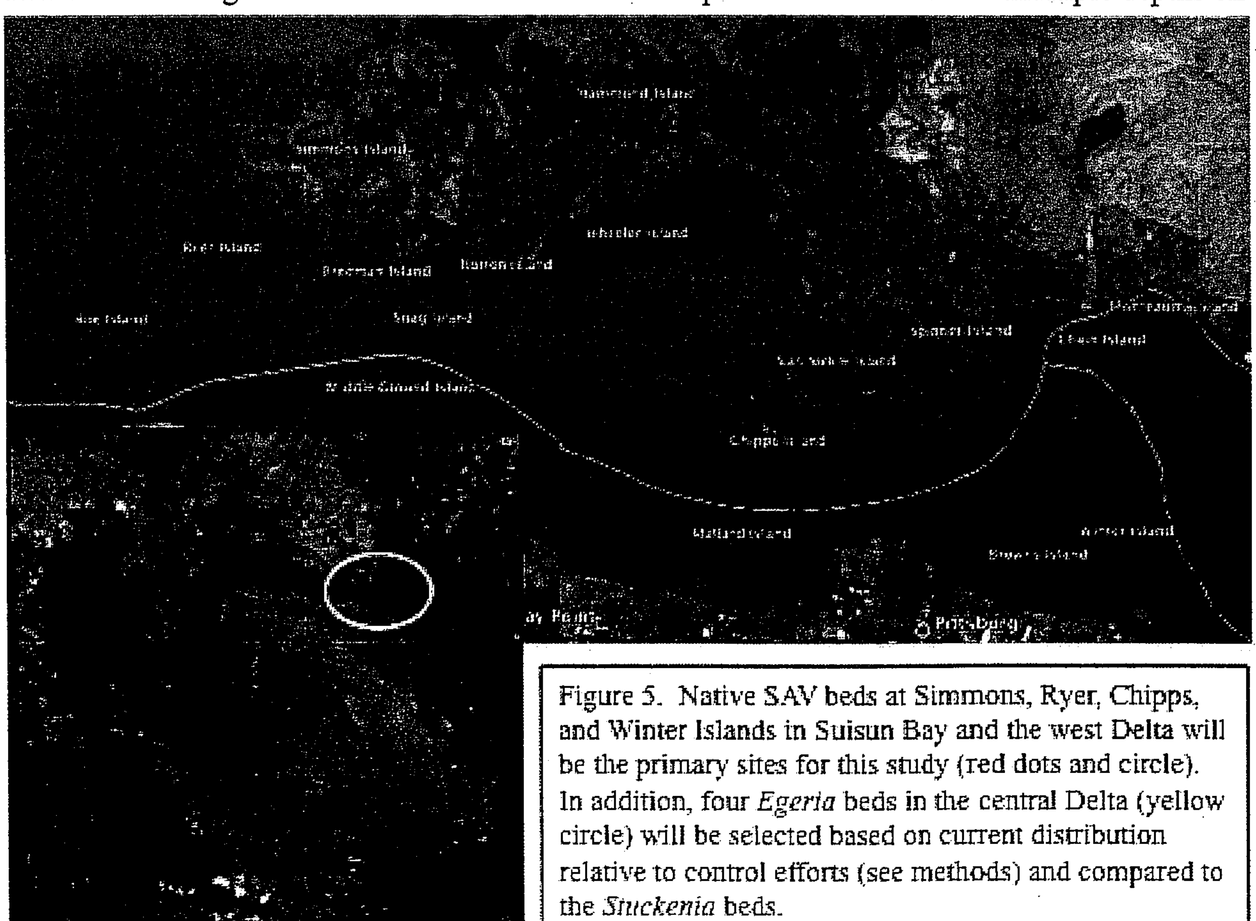

Figure 5. Native SAV beds at Simmons, Ryer, Chipps, and Winter Islands in Suisun Bay and the west Delta will be the primary sites for this study (red dots and circle). In addition, four *Egeria* beds in the central Delta (yellow circle) will be selected based on current distribution relative to control efforts (see methods) and compared to the *Stuckenia* beds.

each quarterly sampling date.

During the same sampling periods, we will repeat these measures inside and outside of selected *Egeria* beds in the west and central Delta so that characteristics of *Stuckenia* beds can be compared to the better-known *Egeria* beds within the context of the same climatic and seasonal setting.

During the summer sampling period, productivity of *Stuckenia* beds will be determined using the oxygen evolution method and equations of Littler and Littler (1985). Replicate SAV samples will be collected and brought back to the lab for incubation under temperature and light conditions simulating field conditions at the time of collection. Tissue will be rinsed to remove epiphytes, invertebrates and debris and spun in a lettuce spinner for 1 min to remove excess water. One-gram wet-weight samples will be placed into each 300 ml BOD bottle with water from the collection site. Four light and two dark bottles will be incubated in a flow-through water table under appropriate light conditions. After one hour, dissolved oxygen will be measured using a WTW 197i meter with self-stirring probe. Oxygen evolution will then be converted to carbon fixed using the equations of Littler and Littler (1985). This same assay will be conducted for *Egeria* samples during the summer sampling period to allow for comparisons of productivity rates in non-native and native dominated SAV beds.

In addition, our stable isotope analysis (see below) will result in total nitrogen and carbon content of plant tissues, permitting us the opportunity to assess C:N ratios, an indication of relative food quality for invertebrates or fishes that may directly consume SAV tissues.

We will analyze plant structural (heights, densities, etc.) and functional (productivity rates) data using ANOVA designs to compare sites and to compare characteristics of *Stuckenia* and *Egeria* beds. We will use non-metric multidimensional scaling to assess patterns in plant characteristics relative to abiotic conditions.

2) *Document seasonal changes in epifaunal invertebrate community composition and abundance in* Stuckenia *versus* Egeria *beds*

We will sample epifaunal invertebrates (those invertebrates present on the plants) at each *Stuckenia* and *Egeria* bed during each quarterly sampling period. This will likely entail a combination of methods, and we will conduct a series of comparisons during summer 2011 to determine the most effective and efficient techniques to collect invertebrates without damage to plants. One possibility is the use of a small net or suction sampler to remove insects and spiders on the plants that are at the surface of the water. In addition, we are likely to remove whole shoots and place them in a bucket, shaking vigorously, followed by sieving the contents of the bucket as Grimaldo et al. (2009). Finally, we are likely to scrape or hand pick individuals still attached to the plants. We will determine the number of samples (shoots) to collect in the selected manner at each bed based on the variability observed in preliminary trials in summer 2011. These samples will be spaced at distances across the bed to capture the spatial extent, with no less than 10 m between samples. Samples will be placed into jars of ethanol and returned to the Romberg Tiburon Center. Invertebrates will be sorted, counted, and identified. Voucher samples will be archived.

We will use cluster analysis and non-metric multi-dimensional scaling to evaluate differences in invertebrate community composition and densities among beds and seasons. Environmental variables of light attenuation, salinity, temperature, plant density, biomass, and heights will be included in the analyses to assess their importance in invertebrate community composition.

3) *Utilize stable isotope analyses to evaluate food web relationships within and among the beds*

We will evaluate isotopic signatures of species using *Stuckenia* and *Egeria* beds to assess food web relationships. At each of the eight beds in three of the quarterly sampling periods (spring, summer, and fall), we will collect samples of each abundant species using the methods above. We will collect at least 5 tissue samples from different individuals of each species as available, expecting to analyze at least 10 producer or consumer species at each of the 8 beds (total of 400 samples for each of the three periods). Plants will be scraped of epiphytic algae and epizoa (hydroids were common in preliminary sampling of *Stuckenia*) to isolate these potential food sources. We will use standard methods for phytoplankton collection to include this group in our analysis (Cloern et al. 2002, Grimaldo et al. 2009). Invertebrates will be allowed to depurate in vials for several hours. Samples will be kept on ice until frozen back at the lab, then dried at 60°C, ground if necessary to homogenize larger samples, and measured into tin cups for analysis of isotopic composition of nitrogen (ratios of heavy to light nitrogen isotopes corrected to a standard, reported as $\delta^{15}N$), carbon ($\delta^{13}C$), and sulfur ($\delta^{34}S$). Isotope analysis will be conducted at the UC Davis Stable Isotope Facility using isotope ratio mass spectrometry. Our research team is currently working on a similar project to evaluate food web relationships within eelgrass beds in San Francisco Bay and has used natural abundance and enriched isotopes in past studies to trace flows of N through aquatic ecosystems (e.g., Boyer and Fong 2005, Huntington and Boyer 2008). In depicting food web relationships, species with unique isotopic signatures can be detected in the diets of other species at higher trophic levels. With N, C, and S isotope data, we will be able to develop a 3-way mixing model, a graphical depiction of food web relationships based on the isotopic signatures of all sampled species. While it can be difficult to distinguish isotopic signatures of different producers due to similar signatures among producers or variation when sampled over large spatial scales and seasons (Cloern et al. 2002, Grimaldo et al. 2009), adding sulfur isotopes to the more common N and C isotopes should aid in distinction of patterns (Howe and Simenstad 2011).

4) *Begin preliminary assessment of fish use of* Stuckenia *beds*

During the summer sampling period, we will conduct preliminary sampling of fish use both inside and outside of *Stuckenia* beds. The relatively open canopies should make beach seining possible within beds (Nobriga et al. 2005), but block net enclosures with repeated hauling will be used if the vegetation impedes seining (Grimaldo et al. 2009). We will defer to our colleagues in Peter Moyle's lab at UC Davis for the best methods; they have graciously agreed to collaborate with us on this preliminary sampling. Collected fish will be identified in the field and measured to the nearest 1mm total length or fork length (if the tail is forked). Seine sweep areas will be quantified (length, width, depth) to permit estimates of volume swept. If possible, we will take tissue samples of fishes to add to the isotopic food web analysis described above, and will conduct gastic lavage or gut content analysis on a subsample of fish to begin to assess their use of food resources within the *Stuckenia* beds.

We will utilize non-metric multidimensional scaling to compare patterns in fish species composition inside and outside of *Stuckenia* beds. Environmental variables of light attenuation, salinity, temperature, plant density, biomass, and heights will be included in the analyses to assess their importance in fish species composition.

Feasibility

While work in the subtidal zone is logistically difficult, all the components of this project are within reach of our research team. Much of this project will be conducted in the field utilizing the Romberg Tiburon Center's fleet of small boats, including a 21' C-Dory (Fig. 7), a 21' Twin-Vee Powercat and a 19' Boston Whaler. Our experienced team has the tools needed to conduct the proposed fieldwork while wading, snorkeling or SCUBA diving; we all have advanced scientific diving certifications that can be utilized if needed. Field equipment that will be used in this project includes beach seines and a LI-COR spherical PAR sensor, and we will purchase and install sensors to continuously log temperature and conductivity (Hobo Conductivity Data Loggers). Laboratory sorting, identification, and enumeration of invertebrate species will occur at the Romberg Tiburon Center, where labs are equipped with microscopes and associated supplies suitable to the task. Stable isotope analysis will be performed at the UC Davis Stable Isotope Facility, where Dr. Boyer has sent many samples over the years due to the facility's efficiency, data quality, and low price.

Current scientific collecting permits will be amended to include our proposed sampling. We expect no take of special status fish species during the preliminary fish sampling.

This project is not dependent on other projects for successful completion. No other projects are being proposed by members of this research team in response to this current Proposal Solicitation Package. No special project management coordination is necessary for our small project team.

Relevance to the Delta Science Program

This proposal addresses key elements of two of the Priority Research topics identified in the Proposal Solicitation Package, Native Fish Ecology and Food Webs of Key Delta Species. We intend to elucidate patterns in a poorly known habitat with high potential for food web support of native fish species of special concern, to discern current relationships, and to envision relationships that could occur to a greater spatial extent with increasing salinity via multiple potential mechanisms. In keeping with priority research questions identified in the Proposal Solicitation Package, we will characterize numerous habitat attributes, including geometry, temperature, light attenuation, food quality and quantity. Further, in relation to the Food Web priority research topic, we will address the role of native SAV beds in supporting production propagating up the food web. We will assess variation seasonally in the physical structure of SAV beds that support potentially valuable food choices for native fishes.

In addition to these identified focal areas for research, it is important to note how the proposed project sits geographically within an iconic region of the estuary, the low salinity zone, yet these SAV beds and their potential support of native fishes have received almost no attention. Further, this project will integrate well with studies by the project team in the lower estuary evaluating fish use of eelgrass beds. As the up-estuary distribution of eelgrass ends in Carquinez Strait, the proposed project will help to answer the question of what food resources are available to native fishes migrating through the Estuary as they move from one native SAV species to another (eelgrass to pondweed and vice versa) after the short break in this type of habitat feature. As sea level rise leads to greater salinities at mid-estuary, the physical location of this transition among native SAV species may be important in determining the degree to which submerged vegetation can offer cover and food web support for native fishes along their migratory routes.

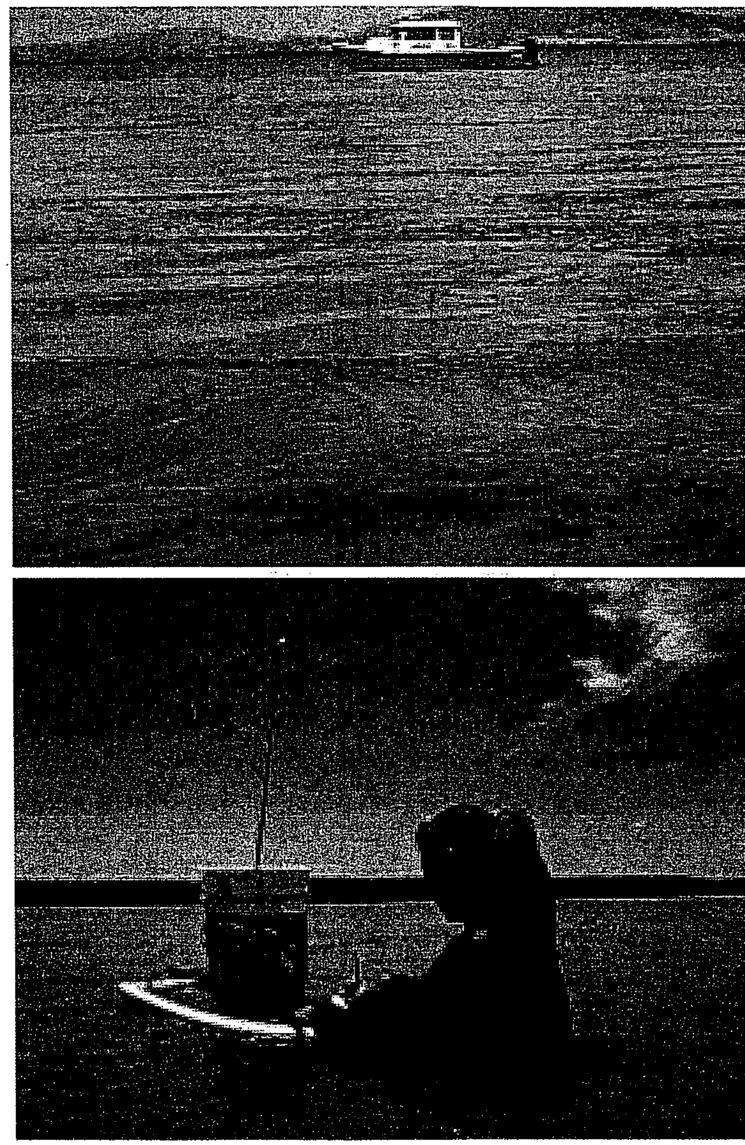

Figure 6. Our research team at the Romberg Tiburon Center has access to boats and equipment suitable for the proposed study (21' C-Dory shown here at Simmons Island), and extensive experience with the logistics of working in the subtidal zone (Research Technician and Co-PI Stephanie Kiriakopolos takes notes at Ryer Island preliminary visit).

Qualifications

Project leader Katharyn Boyer has worked extensively in estuarine habitats on the ecology of emergent and submerged communities for the past 18 years. She has worked on many aspects of the ecology and restoration of native SAV beds of San Francisco Bay (eelgrass), and recently completed as lead author a comprehensive document to guide conservation and restoration actions for eelgrass habitats for the San Francisco Bay Subtidal Habitat Goals Project (Boyer and Wyllie-Echeverria 2010). Many of the skills and methodologies she utilizes in eelgrass habitats are directly translatable to studies of SAV in the northern estuary and west Delta. Dr. Boyer has mentored a series of highly motivated and skilled graduate students on this work. She and her students publish widely in top ecology and management journals. Further, Dr. Boyer translates learning from these studies into hands-on experiences for undergraduate and graduate students in her Restoration Ecology and Wetlands Ecology courses.

In terms of organizational structure of the project, Dr. Boyer will be responsible for all project management tasks and technical assistant Stephanie Kiriakopolos will report directly to Dr. Boyer. As a contractor to the project, Dr. Peter Baye will also connect to the project through coordination with Dr. Boyer. Graduate and undergraduate students, to be determined, will work with Ms. Kiriakopolos and Dr. Boyer on day-to-day tasks.

Stephanie Kiriakopolos completed her masters thesis on abiotic and biotic constraints to eelgrass with Dr. Boyer in 2011 and now works as a Research Technician for Dr. Boyer at the Romberg Tiburon Center. Ms. Kirikopolos is highly accomplished at conducting quality ecological studies in difficult field conditions including on boats in rough conditions and on SCUBA (Fig. 6). She has become a scientific leader in her own right, as recognized by several organizations seeking her expertise in planning for conservation and restoration of subtidal habitats.

Dr. Peter Baye is a Coastal Plant Ecologist well known for his botanical expertise as well as his considerable skills in planning and evaluation of wetland restoration projects. Dr. Baye's role on this project is to conduct the difficult plant identification needed to adequately characterize submerged plant species composition in the northern reaches of the Estuary.

Our research team has the right mixture of capabilities to maximize the likelihood of an outcome that will be useful in both the short term for understanding the system, and in the long term, for predicting changes in estuarine food webs if native SAV beds expand in a saltier Delta and for considering proactive restoration measures that include native SAV to enhance native fish resources.

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Task and Budget Summary

Task #	Task Title	Start Month	End Month	Personnel Involved	Description
1	Management	1	24	Katharyn Boyer	Overall project oversight, including managing schedule and budget, environmental compliance, data handling, and quality assurance, reporting, and dissemination of the project results to scientific, management, and lay audiences.
2	Science	1	24	Katharyn Boyer, Stephanie Kiriakopolos, Peter Baye, graduate and undergraduate students	Dr. Boyer will lead and participate in all aspects of the project, help to train technicians and students, review, analyze and interpret data. Ms. Kiriakopolos will coordinate daily activity on the project, train and work with students on fieldwork and lab sample processing, and aid in analysis and interpretation of the project results. Dr. Baye will assist with identification of SAV species.