

# CALFED SCIENCE FELLOWS PROGRAM



In cooperation with the

California Sea Grant College Program

APPLICANT TYPE	Postdoctoral Res	earcher	Ph D. Graduate Student
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	in the San Francisco Estuary		·
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		2 years	
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# Effects of Freshwater Flow and Population Connectivity on Benthic Community Dynamics in the San Francisco Estuary

#### Introduction

Anthropogenic climate change is predicted to have wide-ranging impacts on northern California climate regimes, including the precipitation patterns (Knowles 2007) which directly influence salinity in the San Francisco Estuary. Meanwhile, a significant portion of the San Francisco Estuary's normal freshwater supply is diverted for agriculture and drinking water use, causing well-documented increases in salinity throughout the bay (Peterson et al. 1995). These climate- and water diversion-driven changes in salinity are likely to have a wide range of impacts on important components of the San Francisco Estuary including the distribution of threatened/endangered species, the success of habitat restoration and the management of invasive species.

While nearly all research examining ecological consequences of water diversions in the San Francisco Estuary has focused on the upstream portion of the Estuary, I propose to focus on the equally important downstream, mesohaline areas of the Estuary where changes to the salinity regime driven by water diversions and winter floods also have important ecological effects. In my dissertation work, I have studied the ecological effects of salinity changes on mesohaline fouling communities, whose filter-feeding organisms make up a significant portion of the Estuary's food web. My work shows that increases in salinity throughout the Estuary during periods of drought, enhanced by water diversions, promote the establishment and maintenance of fouling communities dominated by non-native species in the mesohaline portions of the Estuary. Meanwhile, I have found that winter storm-driven salinity decreases in the Estuary cause large-scale mortality in fouling communities and significant decreases in non-native species abundance. Fouling communities that are overwhelmingly dominated by non-native species before the large winter storms shift to a more equal mix of native and non-native species for some time afterwards (Chang et al. unpublished).

My work suggests that the positive effects of wet winters in terms of increasing native species representation in fouling communities are not simply due to the removal of non-natives. Following wet winters, my data show a significant increase in the biomass and prevalence of native species, including the mussel *Mytilus trossulus* and the native oyster *Ostrea conchaphila*. Several times in the past eight years, I have observed the mass recruitment of key benthic species following winter storm-driven dieoffs, including mussels *Mytilus* spp., the native oyster *O. conchaphila*, and barnacles *Balanus* spp.

Are the mass recruitment events I have observed a direct consequence of decreases in salinity during wet winters? Do these wet winters trigger species such as *Mytilus*, and *Ostrea* to shift into a

"reproductive overdrive"? And do increases in salinity linked to water diversions interfere with these periodic mass recruitments, which might otherwise play a significant role in maintaining populations?

Organisms exposed to low salinity stress may increase their allocation of resources to reproduction, leading to increased production of larvae and recruitment. Such changes in offspring provisioning by parents have been observed in several benthic invertebrate species in response to a variety of stressors (e.g. Marshall and Keough 2006). While some of heavy recruitment I have observed might be due to greater availability of bare space for settlement following the concomitant flood-induced die-offs, my removal experiments during dry years (when there is no mass die-off) mimicking this availability of bare space of similar heavy recruitment (Chang unpublished data). Thus, the heavy recruitment events are more likely related to greater larval abundances, which suggests increased reproductive output or changes in larval movement throughout the Estuary.

My proposal centers around the hypothesis that low salinity exposure during wet years can stimulate high levels of reproduction, and thus lead to mass recruitment events; conversely, recruitment will be relatively lower during dry years. The overall goals of this research are to examine this hypothesis by answering two important, closely-linked questions: (1) How does low salinity exposure affect the fitness and reproductive output of three key species, the mussels *Mytilus galloprovincialis* and *Mytilus trossulus*, and the native Olympia oyster *Ostrea conchaphila*; and (2) How are local populations of these species in San Francisco Bay connected to each other?

While recent advances have improved our understanding of linkages between source regions of Delta Smelt (Hobbs et al. 2007), we have a very limited knowledge of how populations of other species in the Estuary are connected to each other, hindering restoration efforts of native species such as *Ostrea conchaphila* and management of invasive species such as *Mytilus galloprovincialis.* 

*O. conchaphila* has suffered large population declines since European settlement began in the Gold Rush era, and efforts to restore oyster populations in the Bay are just beginning (Grosholz et al. 2007). Understanding how salinity regimes in the Estuary affect recruitment and connectivity of *O. conchaphila* will greatly aid restoration efforts. For example, if populations within the Estuary are triggered to shift into reproductive overdrive by low salinity, then we might expect to see local populations that experienced salinity decreases driving Estuary-wide recruitment. Given limited resources, managers could choose to protect those areas rather than focusing on areas that produce few larvae.

The mussels *Mytilus* spp., are conspicuous and dominant members of the fouling and intertidal communities. Previous work has shown that *Mytilus* spp. in the Bay are composed of the native species *M. trossulus*, the invader *M. galloprovincialis*, and hybrids of the two, and that the balance between the two has shifted over time (Suchanek et al. 1997, Braby and Somero 2006b). Our ability to predict the

movement and spread of invasive species throughout the Estuary would greatly benefit from increased knowledge of the movement of larvae and connections between populations. A secondary reason for studying *Mytilus* in addition to *O. conchaphila* is that *O. conchaphila* is a relatively rare species with somewhat erratic recruitment patterns, whereas *Mytilus* is a much more reliable recruiter (A. Chang pers. obs.). Hence, should *O. conchaphila* fail to recruit during the proposed study, I will still be able to work with *Mytilus*, and the resulting information on *Mytilus* larval movement can be used as a proxy for understanding possible larval movements of *O. conchaphila*.

Since adults of my proposed study species are sessile and benthically attached, their dispersal potential is entirely dependent on mobile larval stages that spend only weeks in the water column. Hobbs and colleagues (2007) found significant population structure within the San Francisco Estuary system for fishes, a relatively mobile group of organisms, so it is plausible that similar structure exists for *O. conchaphila* and *Mytilus.* 

#### **General Approach**

I will focus my research on key taxa in the benthic fouling and intertidal communities occurring throughout the San Francisco Estuary. One is a native species that is currently the target of restoration efforts, and the other two are a widespread, dominant invasive species and a native congener with which it hybridizes. I will first establish long-term survey sites to track the abundance of these focal species in the Bay, then use trace elemental fingerprinting methods to determine the natal origin and fate of larvae of these taxa, and also conduct salinity stress tests to determine low salinity tolerances and examine effects of low salinity stress on reproductive output.

#### **Field surveys**

Twice each year, once in late fall before the rainy season starts and once in early spring after the rainy season ends, I will conduct field surveys of the assessing the relative abundance of the focal species throughout the Bay. These surveys will determine possible source populations for larvae and also allow for comparisons between sites of any impacts of low salinity events during the winter rainy season. Based on previous surveys (Grosholz et al. 2007, Miller et al. unpublished data), I will choose sites of known mussel and oyster abundance. I will select four sites in each of three regions in the mesohaline portion of the Estuary (north, central, and south, for a total of 12 sites), representing a gradient of freshwater inflow (and thus salinity regimes) as well as different regional circulation patterns. At least two sites in each region will be located near marinas so that I can investigate subtidal floating dock populations in addition to adjacent intertidal populations.

At each site, I will establish long-term transects to be revisited over the course of this study. Intertidal populations will be quantified using 30m-long transects at a low and a high tidal height within the zone of occurrence of each species. For Ostrea conchaphila, I will photograph, measure (maximum length), and count the number of each focal species within ten 0.25 m<sup>2</sup> guadrats randomly placed within each transect. A separate protocol will be used for Mytilus spp., as counting and measuring mussels occurring in aggregated clumps is difficult without destructive sampling. I will therefore use a combination of destructive and non-destructive sampling techniques: ten permanent, randomly-placed  $0.25m^2$  photo quadrats will be established in each transect, while ten randomly-placed  $0.25m^2$  quadrats per transect will be destructively sampled and all Mytilus spp. collected and transported to the lab for later counting and measurements of length. I estimate that this sampling process will take approximately 1 hour per site, plus up to 4 hours to process mussel samples in the lab at densely-settled sites. At marina sites (2 per region in the Bay), I will also survey populations of both species on the sides of floating docks using 10 0.25m<sup>2</sup> quadrats randomly-placed along a 30m transect along the dock. Quadrats will be placed just below the water surface on the vertical surface of the dock. One floating dock transect will be used to assess both species at each marina site, as they tend to occur in the same vertical zone on floating docks (A. Chang pers. obs.)

Temperature loggers (Onset Computer Inc., Hobo TidbiT v2 model) will be placed at each site to record temperature at 1h intervals, and salinity will be measured during each visit using a hand-held refractometer or YSI meter. Additional information on salinity conditions at each site will be gathered using continuous monitors set up as part of an unrelated long-term study (Chang et al. Unpublished) and supplemented by long-term monitoring stations throughout San Francisco Bay run by USGS and the CiCORE monitoring program (Center for Integrative Coastal Observation, Research, and Education (California State University)).

#### **Trace elemental fingerprinting**

The second component of the proposed work is to develop a picture of the connections between populations in the San Francisco Estuary. I propose to do this using trace elemental fingerprinting, a method recently developed and proven to be useful for determining natal regions of bivalve larvae as well as fishes (e.g. Becker et al. 2005, 2008, Hobbs et al. 2007). Previous work on Delta Smelt in the Estuary has shown significant population structure (Hobbs et al. 2007); as invertebrates are relatively less mobile, this method is likely to show similarly strong evidence of regional differences in *Mytilus* spp. and *Ostrea conchaphila*. In this application, trace elemental fingerprinting takes advantage of the fact that trace elemental metals are frequently incorporated into the calcareous shells (prodissoconchs) of developing pelagic larvae of marine bivalves, and that the amounts of various elements incorporated differ

depending on environmental concentrations. Inductively-coupled plasma mass spectrometry can be used to determine the ratios of these elements present in the larval shells, which are retained in juvenile bivalves. The various ratios of several elements, when considered together, can then be used as distinctive fingerprints that distinguish juveniles that developed in one region from those originating elsewhere. Becker et al. (2005, 2008) showed that trace elemental fingerprinting could be used to effectively distinguish between larvae of the mussels *Mytilus californianus* and *M. galloprovincialis* originating in four contiguous regions within a 75km stretch of shoreline in southern California, including two regions along exposed coast and two regions inside embayments. The two regions inside embayments were further separated into an "outer bay" region near the mouth of the embayment and an "inner bay" region near the back of the embayment, and juveniles originating in each part were distinguishable from each other. The regions in San Francisco Bay (north, central, and south) that I propose to use are 10-20km in length, comparable in size to the distances between sites used by Hobbs et al. (2007) in determining natal regions of Delta Smelt, and similar to those used for *Mytilus* spp. by Becker et al. (2005, 2008).

Determination of natal regions requires two steps: (1) creation of a "map" describing the trace elemental signatures that characterize various sites, and (2) collection of newly settled juveniles of the target species and comparison of their shell chemistry to the map in order to assign likely natal regions. To create the map of trace elemental signatures, I will spawn and culture larvae of *Mytilus* spp. at the 12 sites surveyed above, then determine the chemical signature of the larval shells. Work for both species will be performed during their breeding seasons (summer), but will be performed during separate blocks of time to minimize strain on resources. I will collect adult *Mytilus* spp. and spawn them using agitation and heat shock methods. The two *Mytilus* species I focus on here are closely related enough that they likely incorporate elements into their larval shells in the same way, thus obviating the need to outplant separate groups of larvae for each species (Becker et al. 2008) All tools and containers used in trace elemental fingerprinting work will be acid-washed to minimize contamination. Larvae will then be outplanted at all 12 sites in "homes" consisting of a 14-cm long, 3.8cm diameter PVC pipe with an open cap consisting of 35µm nitex mesh on either end. Homes will be leached of contaminants before use. Approximately 100,000 larvae will be placed in each home to compensate for high expected mortality rates (Becker et al. 2008). At select sites, moorings consisting of a concrete anchor attached via a nylon braided line to subsurface and surface floats will be deployed directly offshore of the intertidal survey sites in approximately 10m deep water. Larval homes will be attached to the subsurface float at 2m below MLLW so that they will remain at approximately 1-3m below the water surface (the same depth at which bivalve larvae are found in the water column, Becker 2005) but at least 1-2m above the bottom. At marina sites, I will deploy larval homes at 1m depth from ropes attached to floating docks near the mouth of each marina's inlet. Sites will be chosen so that homes will remain at least 1m below the water surface and 2m above the bottom.

Larvae of *Mytilus* spp. will be allowed to grow in situ for one week, then I will retrieve them and analyze their shell chemistry using laser ablation inductively-coupled plasma mass spectrometry (LA ICP-MS). Unlike *Mytilus* spp., *Ostrea conchaphila* brood their larvae, so there is no need to outplant larvae. Instead, I will collect brooded veliger larvae from reproductive female *O. conchaphila* which will then be processed and analyzed in the same fashion as mussel larvae. Upon retrieval, larvae will be stored in acid-washed containers at -20°C until processing. Larvae will be prepared for spectrometry using quartz-distilled Milli-Q water and trace metal-free reagents. The relative ratios of trace elements present in larval prodissoconchs will then be determined using the laser ablation inductively-collected plasma mass spectrometer at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry.

To determine natal origins of new settlers, I will then collect newly settled juveniles of each species at each site 14-21 days after the outplanted larvae are retrieved, matching the approximate development times of each species in the water column. Larval shell chemistry of collected juveniles will then be determined using LA ICP-MS in the same manner as for the outplanted larvae and will then be compared to elemental fingerprints characterized using larval outplanting in order to determine the collected juveniles' natal origins. While adult *Mytilus* can be readily distinguished using morphological characters, larval *Mytilus* are difficult to distinguish visually, so tissue samples from each mussel will be frozen and later genotyped to determine exact species identification. Genetic identification of new settlers will be conducted using additional funds from an unrelated project.

#### Low salinity stress responses

The third element of the proposed work is to examine the effects of salinity stress on the focal species, and to test the hypothesis that salinity stress can induce a shift into reproductive overdrive. I will test the effects of salinity stress on adult and larval survival as well as measure effects on the reproductive allocations and offspring of adults subjected to salinity stress.

To test the effects of salinity stress on adult fitness, I will collect adults of each species will be collected from each site and assess heart rate responses at a range of salinity and temperature conditions mimicking natural conditions, as well as determine lethal levels. Heart rate measurement allows a simple, quick, fine-grained quantification of the level of stress imposed on the test animal. These methods have been successfully used in the past to investigate mytilid responses to salinity and temperature stress (Braby and Somero 2006a). For both species, heart rate will be measured using impedance pneumography, in which electrodes are placed around the animal and changes in impedance between the electrodes due to the animal's cardiac cycle are generated and converted to a voltage signal

that is recorded by a computer. Copper magnet wire probes will be inserted into each valve via a small drilled hole and glued into place, with a 2 hour resting period for each animal following placement of probes. The analog impedance signal produced by this method is converted to a voltage signal (UFI, Impedance Converter model 2991) and digitally recorded using a data acquisition system (LabJack, model UE9), sampling at 100Hz and connected to a laptop computer. The data acquisition system allows simultaneous continuous monitoring and recording of data from as many as 12 animals at any one time, plus the temperature and salinity of the experimental chamber.

I will test the responses of 10 adults of each species to salinity-temperature combinations mimicking wet and dry winter water conditions in San Francisco Bay. Organisms will be held at a starting temperature of 14°C and salinity of 33psu and then exposed to experimental conditions of 5, 10, 15, 20, 25, 30, and 33psu at 7°C, 10, and 13°C. Relatively sharp transitions over a period of 12 hours from the starting temperature and salinity to experimental conditions will be used, mimicking sudden influxes of cold low salinity water during a large winter storm. Heart rate will be monitored continuously beginning 1 hour before the start of the transition to experimental conditions and continuing up to 2 hours after salinity and temperature reach the target conditions.

Lethal low salinity limits for adult *Mytilus* spp. and *Ostrea conchaphila* will be assessed by exposing adults to experimental conditions of 5, 10, 15, 20, and 25psu at 7°, 10°, and 13°C. Animals will be held in constant salinity at 33psu and acclimated to the target temperature using 1°C changes per day with a 3 day resting period at the target temperature before being exposed to salinity changes, which will be enacted over a 12 hour period. I will count the number of mussels that gape and are unresponsive to probing (signifying death) after 1, 2, 4, 8, 12, 24, 48, and 72 hours under each target condition.

Lethal low salinity limits for larvae will be assessed to help determine the conditions under which larvae may remain viable, as mass reproduction in adults triggered by low salinity exposure would be meaningless if the larvae are unable to survive the existing conditions. Adults of each species will be spawned, and the larvae will be held in salinity and temperature combinations mimicking natural variation in the San Francisco Bay system. Larvae of each species will be held at a starting temperature of 18°C and salinity of 33psu (typical late summer / fall conditions, the usual breeding season of both species) and will then be exposed to experimental conditions of 5, 10, 15, 20, 25, 30, and 33psu at 16°C. Relatively sharp transitions from the starting temperature and salinity to experimental conditions will be used, mimicking sudden influxes of cold low salinity water during a large winter storm. 20 larvae of each species will be tested in each salinity-temperature combination, and survival will be assessed after 1, 2, 4, 8, 12, 24, 48, and 72 hours (typical winter storms in the San Francisco Estuary depress the maximum salinity for around 72 hours).

Finally, to examine possible effects of exposure to low salinity stress on reproductive allocations

and offspring, I will expose adult *Mytilus* spp. and *Ostrea conchaphila* to sublethal low salinity stress matching realistic winter conditions and assess the amount of resources invested in reproduction by measuring gonadal weight and average egg size if spawning is successful. I will collect 120 adults of each species from a central San Francisco Bay site (which would experience the least salinity stress overall) and a northern San Francisco Bay site (which would be expected to experience the most salinity stress overall). Organisms will be acclimated to typical San Francisco Bay winter temperatures of 8°C, then subjected to one of two sublethal salinity stress levels (10psu or control = ambient salinity at time of collection) for 5 days, a typical duration of salinity depression following a winter storm. Following this stress, organisms in the low salinity treatment will be re-acclimated to ambient salinities and temperatures will be raised slowly to 16°C, a typical late spring/early summer temperature in the Bay, and one at which reproduction of both O. conchaphila and Mytilus spp. is recognized to begin. Following re-acclimation, 5 oysters and mussels in each treatment will be selected for gonadal measurements biweekly. Total shell length and gonadal wet weight will be measured, and the condition of gametes assessed; if eggs held by females are mature, I will measure average egg size. An additional 5 oysters and mussels per treatment will also be selected and spawning attempted using heat shock methods. If spawning is successfully induced, gametes will be collected, eggs will be measured, and fertilization induced. Larvae will then be held in flowing seawater through settlement to look for differences in duration of larval period and to assess settlement success.

# **Proposed Timeline**

YEAR 1 (2009-2010)

May-June: field surveys

June-Aug:

\* Salinity stress experiments on adults and larvae

\* Maternal effects of stress exposure experiments

Sept: Trace elemental fingerprinting: larval outplanting and collection of juveniles — initial pilot studies

Oct: data analysis of salinity stress experiments and LA ICP-MS sample processing

Nov-Dec: field surveys

Dec-Apr: LA ICP-MS sample processing and genotyping of Mytilus spp. samples

# YEAR 2 (2010-2011)

May-June: field surveys

June-Aug:

\* Further salinity stress experiments as necessary

\* Trace elemental fingerprinting: larval outplanting and collection of juveniles

Sept-Oct: LA ICP-MS sample processing and genotyping of Mytilus spp. samples

Nov-Dec: field surveys

Dec-Feb: LA ICP-MS sample processing, genotyping, and data analysis

Mar-Apr: writing

#### **Output/Anticipated Benefits**

This project will generate important information about the responses of three key benthic species in San Francisco Bay to changing salinity regimes, as well as critical information on the connections between populations of these species in different parts of the Bay. These represent two key pieces of information that are crucial for effective management and restoration of native oyster populations as well as managing non-native species and understanding the responses of benthic communities to salinity changes, an oft-overlooked, yet potentially very powerful driver of community change linked to climate change. The responses of these species to changes in the salinity and temperature regimes may be especially crucial to understanding the apparent boom-bust nature of their recruitment from year to year, which may have cascading effects throughout the ecosystem.

This research will benefit all agencies and authorities involved in native oyster restoration and invasive species management throughout San Francisco Bay. Examples include NOAA, California Department of Fish and Game, US Fish and Wildlife Service, local residents, aquaculture, NGOs, etc. Many of these groups spend significant amounts of resources on the management of non-native species and restoration of native oyster populations in the San Francisco Estuary. Our assessment of salinity and temperature responses will allow managers to predict the responses of mussel and oyster stocks in the bay to various salinity and temperature conditions, and to predict mass mortality and possibly also large recruitment events. In addition, the need for a better understanding of larval oyster transport during the spawning season has been identified as a key area in which more information is needed (Mulvey and Cosentino-Manning 2007). For example, the proposed study would generate information on which sites or regions in the Estuary are net sources or sinks of larvae, which could directly inform site selection for restoration, monitoring, and conservation efforts. Conservation groups with limited resources would be

able to use this information to select sites that are known to be important sources of larvae for oyster populations in the Estuary. Similar information on connection patterns between invasive species such as *Mytilus galloprovincialis* can be useful in a number of ways, for instance in models that aim to predict the spread of similar non-native species entering the Estuary, or to determine whether a new invader in a particular area is likely to spread outside that area; if a new invasion is found in an area from which high rates of larval transport have been shown, then immediate eradication or management action could be taken to limit the invader's spread.

Specifically, the results of this research will be useful for management and restoration efforts as follows:

YEAR 1 (2009-2010)

\* An assessment of the relative abundances and size distributions of oysters and mussels at sites throughout San Francisco Bay will supplement existing information on these species, and will provide new information for *Mytilus* demographics, as no long-term monitoring programs for *Mytilus* currently exist in the Bay

\* Describing the responses of adults and larvae to low salinity stress, which will allow managers to predict responses of stocks to various environmental conditions

\* Identification of effects on offspring of salinity and temperature stress experienced by parents

\* Pilot trace elemental fingerprinting studies may yield preliminary information on patterns of connections between Bay populations of *Mytilus* and *Ostrea* 

# YEAR 2 (2010-2011)

\* A "map" describing the connections between oyster and mussel populations in different regions of the bay will allow managers to better target restoration efforts for native species such as oysters, and eradication efforts for new invaders with pelagic larvae.

\* Description of changes in intertidal oyster and mussel populations over the course of this study, especially changes linked to salinity regime changes (including freshwater diversions and climate changedriven winter storms)

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