Bonnie J. Becker
Coastal Environmental Quality Initiative
Final Report
December 1, 2004

PROJECT TITLE

Using LA-ICP-MS elemental fingerprinting to evaluate transport and retention of mussel (*Mytilus spp*.) larvae

RESEARCH ACTIVITIES

Despite a century and a half of interest, major questions in conservation, ecology and evolutionary biology remain unanswered due to an inability to directly determine larval trajectories and population connectivity in most invertebrates with planktonic larval phases. One method to track marine larvae, elemental fingerprinting, utilizes a natural "tag" derived from the physical and chemical environment. While larvae are developing, they can incorporate non-calcium elements into the carbonate matrix of their newly-forming hard parts in relationship to the environmental conditions experienced by the individual at the time of development (Thorrold et al. 2002). If the environmental conditions are sufficiently different at the various locations in which the larvae are developing, and are sufficiently stable over time, it should be possible to determine the spatial location where the hard part was formed by analyzing its chemical composition.

Beginning in the 1980's, and increasingly since the 1990's, this technique has been applied to otoliths to determine the adult, juvenile, and larval movements of numerous fish species (reviewed by Campana 1999, Campana and Thorrold 2001, Thorrold et al. 2002). Although this technique shows great promise for application to invertebrate larvae, very few studies have explored this possibility. In this project, I have been developing this technique to determine natal origins of settled mollusks with a wholly planktonic larval phase, *Mytilus californianus* and *M. galloprovincialis*.

I have approached this work in two stages. Initially, I used recently settled mussels to determine if chemical differences in shells collected at various sites around San Diego County would be sufficient to discriminate between sites. Dissoconch (post-larval shell) was analyzed for nine minor and trace elements using Laser-Ablation Inductively-Coupled-Mass Spectrometry (LA-ICPMS). The resulting chemical ratios were analyzed using Discriminant Function Analysis (DFA). This important first step provided a necessary validation that location-specific chemical signatures existed within mussels and could be detected at a relevant spatial scale (20km). These chemical signatures were examined on a seasonal and weekly time scale to determine signal stability. Water chemistry and temperature time series were compared to the mussel shell chemistry for consistency.

One limitation of this method was the lack of a reference prodissoconch (larval shell) that could be used to characterize reference signals (i.e. "known" signals) for comparison with the prodissoconchs of settlers (i.e. "unknown" signals). Unfortunately, the dissoconch of Mytilus mussels has a different mineral structure from the prodissoconch, making chemical comparisons of the settler shell (formed at a known location) to the larval shell (formed at an unknown location) invalid. In some invertebrate species, early larval development occurs near the site of fertilization (in benthic egg capsules, for example), allowing researchers to collect these larvae of known origin to create a reference signal. Since many invertebrates, including mytilid mussels, are free spawners (fertilization and all larval development occurs planktonically), another approach will be needed to create reference larval material.

The second stage of this project was to develop a method to raise larvae in traps *in situ*, therefore forming prodissoconch in a known location. Larvae were placed at sites around San Diego County and allowed to grow for a week. The resulting samples were then analyzed in a similar manner to the juveniles. Bay vs. open coast differences were found, as well as regional differences on the open coast. Using this method, I have been able to demonstrate that the larvae have location-specific elemental fingerprints incorporated into their shells. This larval outplanting method will broaden the use of elemental fingerprinting as a larval tracking tool to taxa with a wholly planktonic larval phase.

## ACCOMPLISHMENTS and PRINCIPAL FINDINGS

During the first stage of this study, I analyzed the shell chemistry of early mussel settlers that were smaller than 2mm in length. I developed a molecular assay to determine the species of the settlers, since visual determination is very difficult in small samples. Both *M. californianus* and *M. galloprovincialis* were present in these samples. Initial chemical analyses showed no notable differences between the chemistry of the two species. They were therefore lumped together to increase statistical power.

The settlers were collected in December 2001 from 8 sites around San Diego that spanned 45 km of shoreline and included a site in each of two major bays (Figure 1). In addition, mussels were collected from a single site in May 2001, September 2001, and February 2002. In February, samples were collected every week for 5 weeks. I discriminated among mussels collected in two bays and the open coast using Mn, Pb, and Ba shell concentrations (Figure 2). Shell concentrations of Pb and Sr were sufficiently different to discriminate between mussels from the northern and southern regions of the open coast, each representing approximately 20 km of coastline (Figure 3).

These signals were very stable during most months studied, although they varied during February (Figure 4). Weekly samples showed very little variability (Figure 5). The stability of the signal will dictate when and how often reference material will need to be collected in order to use shell chemistry as a larval tracking tool for future studies. Based on these analyses, I inferred that it would be most useful to collect reference samples in the same month that larvae of the unknown mussels are in the plankton, unless the seasonal-scale variability can be well characterized beforehand. One would need to collect frequent samples throughout multiple

years, rather than in one or two different seasons in order to determine the seasonal stability within and among years. The finding that shells collected weekly from a single site in a single month could not be differentiated based on their elemental fingerprints indicates that the elemental signals of mussels from SIO were quite stable on small temporal scales. Collection of samples from numerous sites simultaneously can be logistically difficult. Samples collected weeks apart would still be comparable due to low temporal variability in shell chemistry.

In the second stage of the study, I successfully raised larvae of both *M. californianus* and *M. galloprovincialis* in larval "homes" in May of 2003. Larval homes were mounted on buoys (Figure 6) at 15 sites around San Diego, spanning over 75 km of shoreline (Figure 7). Six sites were within three enclosed bays. Embryos were outplanted within seven to ten hours of fertilization, so that all shell formation occurred in the field. The larvae were allowed to grow *in situ* for seven days and were then harvested. Survivorship was relatively high (*M. californianus* 2.3%, *M. galloprovincialis* 0.3%), given the harshness of the conditions the larvae were exposed to. Harvested larval shells were approximately 120μm in diameter, all of which was formed in the field. Three weeks after the outplanting, mussel juveniles were collected for the third stage of the project.

The resulting larvae were analyzed using LA-ICPMS. Comparison with larvae raised in the lab within and without larval homes indicated that there was a chemical difference between them, but the differences were minor when compared with field-raised larvae (Figure 8). All of the lab-raised individuals contained very low concentrations of most elements, while those raised in the field had higher and variable concentrations, despite the fact that all the larvae came from the same original batch of embryos (Figure 8). Lead was the one element that showed the opposite pattern, probably due to a local contamination problem in the laboratory. This result indicated that the field-raised larvae had incorporated local signals into their shells independent of maternal or lab-derived influences.

Using larval shell chemistry, I was able to discriminate between larvae raised in two out of the three bays and those raised on the open coast (Figure 9). Similarly to the juveniles, the open coast larvae could be discriminated into a northern and southern region (Figure 10). These analyses are ongoing, and will be summarized in a peer-reviewed paper early next year. The next step will be to analyze the prodissoconchs of the early settlers collected three weeks after the outplanting. If possible, the shell chemistry of these individuals will be mapped on to the DFA's created during the larval outplanting experiment.

This study is the first to assess the viability of using bivalve shell elemental fingerprinting as a larval tracking tool. These results indicate that this method will have practical applications for larval ecology of two southern Californian mussel species that are vital components of intertidal systems worldwide. The implications of this work are much broader. Exploration of the use of a diversity of invertebrate hard parts, including exoskeletons (DiBacco and Levin 2000), statoliths (Zacherl 2003a) and shells (this study, Zacherl 2003b), are likely to yield signals that can be used for larval tracking and connectivity studies in many invertebrate species around the world.

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Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology-Progress Series **188**: 263-297.

Campana, S.E. and S.R. Thorrold 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences **58**: 30-38.

DiBacco, C and L.A. Levin 2000. Development and application of elemental fingerprinting to track the dispersal of marine invertebrate larvae. Limnology and Oceanography **45**(4):871-880.

Thorrold, S.R., G.P. Jones, M.E. Hellberg, R.S. Burton, S.E. Swearer, J.E. Neigel, S.G. Morgan, and R.R. Warner 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. Bulletin of Marine Science **70**: 291-308.

Zacherl, D.C., P.H. Manriquez, G. Paradis, R.W. Day, J.C. Castilla, R.R. Warner, D.W. Lea, and S.D. Gaines 2003. Trace elemental fingerprinting of gastropod statoliths to study larval dispersal trajectories. Marine Ecology-Progress Series **248**: 297-303.

PARTICIPANTS

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Graduate advisor: Dr. Lisa Levin (Scripps Institution of Oceanography)

Collaborators: F. Joel Fodrie, Pat McMillan (Scripps Institution of Oceanography)

PUBLICATIONS and PRESENTATIONS

Becker, Bonnie J., F. Joel Fodrie, Pat A. McMillan, and Lisa A. Levin. 2005. Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: A precursor to invertebrate larval tracking. Limnol. Oceanogr. 50: 48-61.

Becker, Bonnie J., F. Joel Fodrie, Pat A. McMillan, and Lisa A. Levin. 2005. Using *in situ* larval culturing to determine the spatial variation in trace elemental fingerprints in mytilid mussel larval shells. In preparation.

Becker, Bonnie J. Determining natal origins of mytilid mussels using *in situ* larval culturing and elemental fingerprinting.

- Centro de Investigación Científica y de Educación Superior de Ensenada, Mexico (Invited seminar). June, 2004.
- Larval Biology VI Meeting, Hong Kong (Winner: Best Student Presentation). June 2004.

Becker, Bonnie J. Using trace element concentrations in mytilid mussel shells to determine larval sources.

- Scripps Institution of Oceanography Ecology Luncheon Seminar (invited presentation). February, 2004
- Western Society of Malacologists Meeting, Los Angeles (invited presentation). June 2003.

Becker, Bonnie J. Mussels, lasers, and marine reserves: Why we care about the chemistry of larval mussel shells.

- San Diego Shell Club (invited presentation, open to public). February, 2004.

Figure 1. Map of *Mytilus* mussel Stage 1 collection sites in San Diego County, California USA. Northern region (filled circles): CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Institution of Oceanography Pier; Southern region (open circles): PB= Pacific Beach (Crystal) Pier, OB=Ocean Beach, CABR=Cabrillo National Monument; Bay sites (open squares): CPMS=Crown Point Mitigation Site (Mission Bay), HI=Harbor Island (San Diego Bay). San Diego coastline data were provided by National Oceanic and Atmospheric Administration Medium Resolution Digital Vector Shoreline Database. To be published in Limnology and Oceanography.

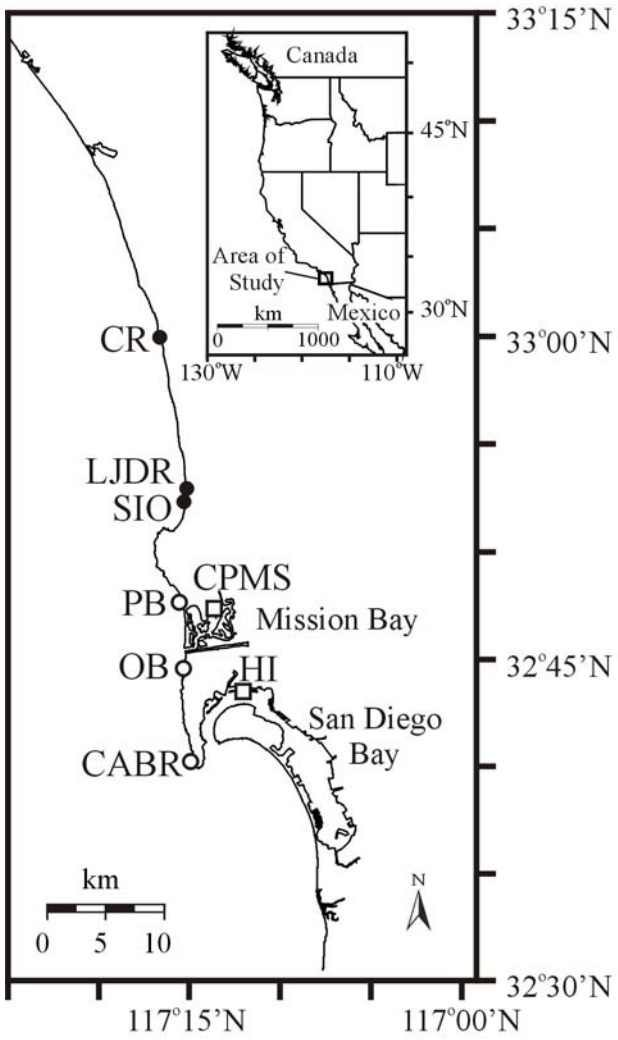

Figure 2. Discriminant scores of element (Mn, Ba, Pb) ratios to Ca in shells of *Mytilus* mussel recruits collected between 26 December 2001 and 9 January 2002 at sites in San Diego County, grouped as Mission Bay, San Diego Bay, and Open Coast sites (Cardiff Reef, La Jolla Dike Rock, Scripps Institution of Oceanography Pier, Crystal (Pacific Beach) Pier, Ocean Beach Pier, and Cabrillo National Monument). **(A)** Scatterplot of DFA scores; **(B)** Same data as A plotted as averages with ± 95% confidence intervals; **(C)** Discriminant functions, standardized by within variances, for the element ratios used to create the DFA. Vectors represent the relative contribution of each element ratio to the resulting scores. To be published in Limnology and Oceanography.

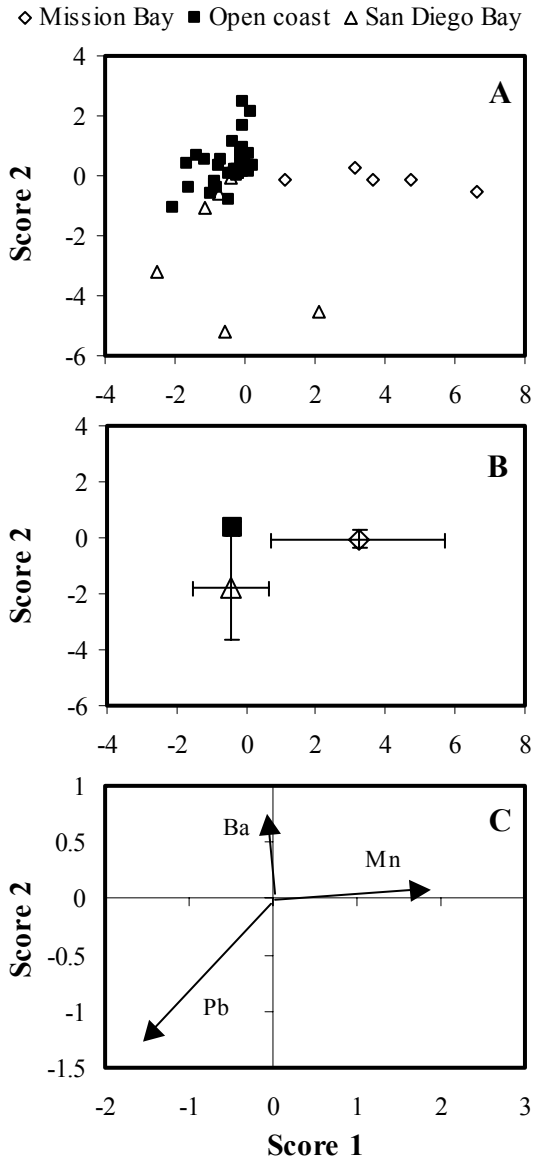

Figure 3. Discriminant scores of element (Pb, Sr) ratios to Ca in shells of *Mytilus* mussel recruits collected between 26 December 2001 and 9 January 2002 at open coast sites in San Diego County. Sites are listed from North to South. The northern region is represented by filled shapes; the southern region is represented by open shapes. **(A)** Scatterplot of DFA scores; **(B)** Same data as A plotted as averages with ± 95% confidence intervals; **(C)** Discriminant functions, standardized by within variances, for the element ratios used to create the DFA. Vectors represent the relative contribution of each element ratio to the resulting scores. Northern region: CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Pier; Southern region: PB=Crystal (Pacific Beach) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument. To be published in Limnology and Oceanography.

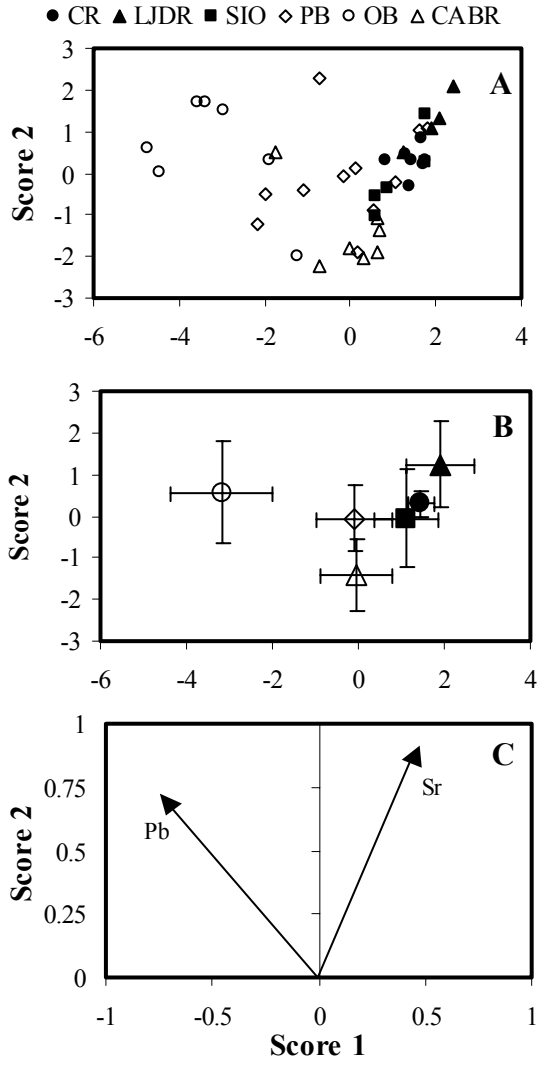

Figure 4. Discriminant scores of element (Pb, Sr) ratios to Ca in shells of *Mytilus* mussel recruits collected from Scripps Institution of Oceanography Pier in May 2001, September 2001, and February 2002 compared to those collected at various sites between 26 December 2001 and 9 January 2002 in San Diego County. Scores were calculated for shells collected at SIO during various seasons using the same DFAs depicted in Figures 2 and 3. All are plotted as averages with ± 95% confidence intervals. The standardized discriminant functions are given in Figures 2C and 3C. M=SIO Pier (1 May 2001), S=SIO Pier (8 September 2001), F=SIO Pier (5 weeks from 26 January through 21 February 2002), MB=Mission Bay, SDB=San Diego Bay, OC=Open Coast, CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Pier, PB=Crystal (Pacific Beach) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument. **(A)** Averages of DFA scores from the different seasons at SIO compared to Mission Bay, San Diego Bay, and Open Coast sites. The standardized discriminant functions are given in Figures 3C; **(B)** Averages of DFA scores of the various seasons compared to other open coast sites only. The northern region is represented by filled shapes; the southern region is represented by open shapes; the different seasons from SIO are depicted as open boxes. To be published in Limnology and Oceanography.

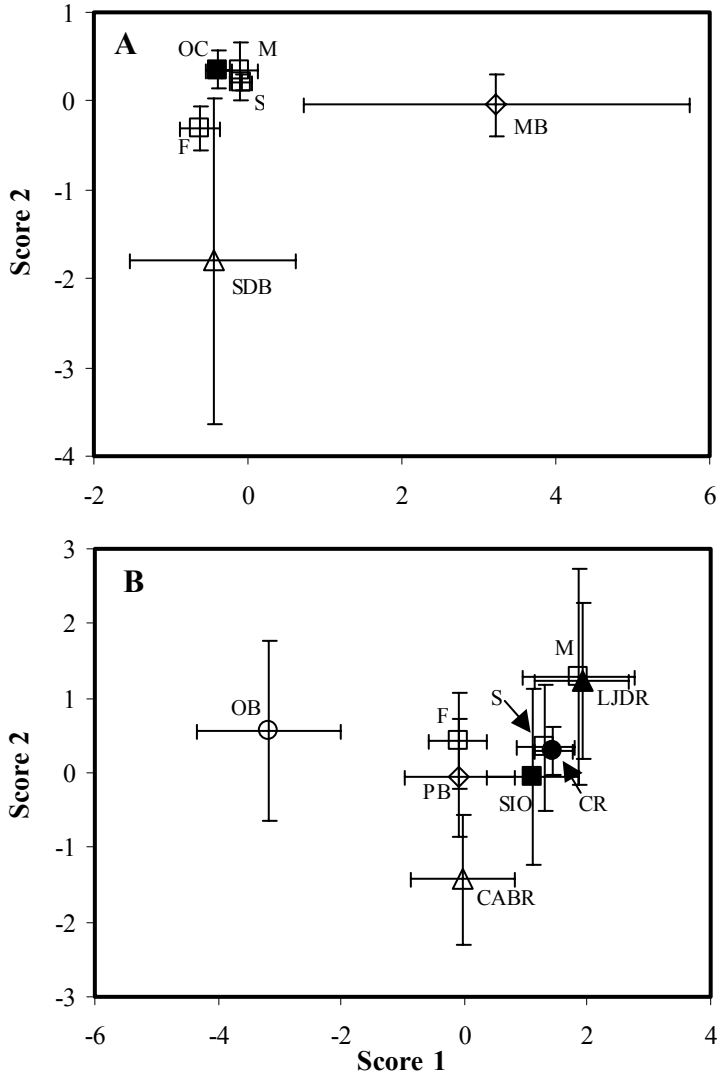

Figure 5. Discriminant scores of element (Pb, Sr) ratios to Ca in shells of mussel recruits collected once per week between 26 January and 21 February 2002 at Scripps Pier, grouped as weeks. **(A)** DFA scores, plotted as averages with ± 95% confidence intervals; **(B)** Discriminant functions, standardized by within variances, for the element ratios used to create the DFA. Vectors represent the relative contribution of each element ratio to the resulting scores. To be published in Limnology and Oceanography.

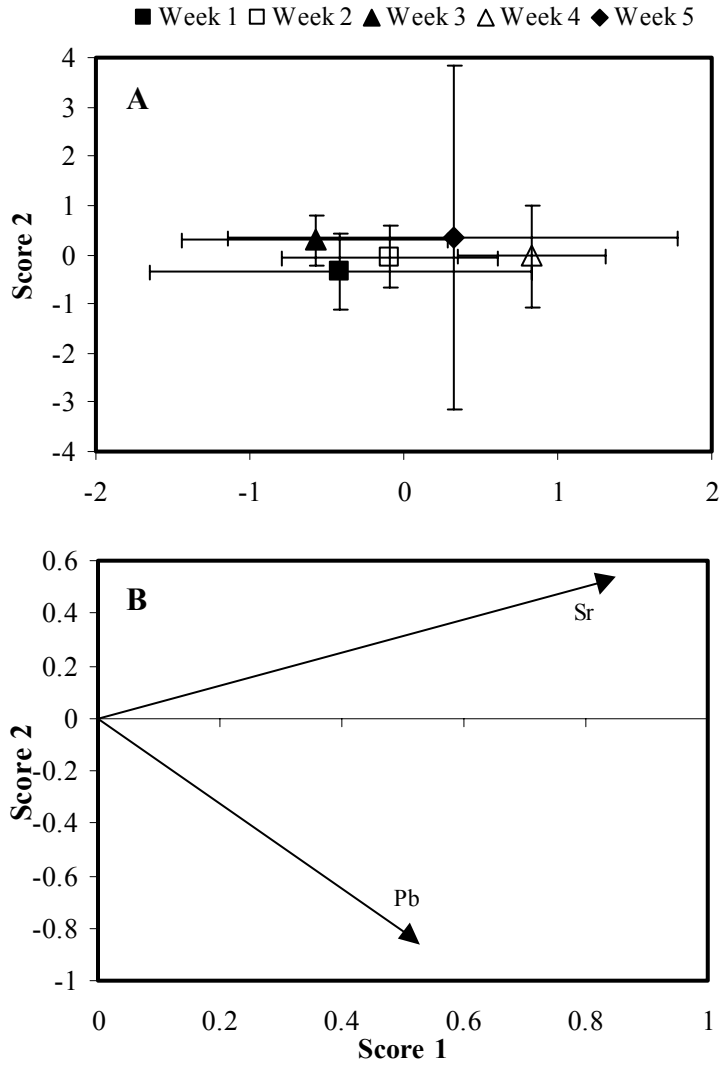

Figure 6. Diagram of buoy set up for larval home experiment conducted in May of 2003. A subsurface float was located approximately two meters below mean lower low water (MLLW), and the larval homes were attached just below it. This reduced the amount of motion the homes experienced due to swell. In addition, a quick-release knot below the homes allowed boat crews to remove the larval homes without having to hold the heavy anchor above the bottom for a long period of time. A temperature logger and settler collectors were also placed just below the homes.

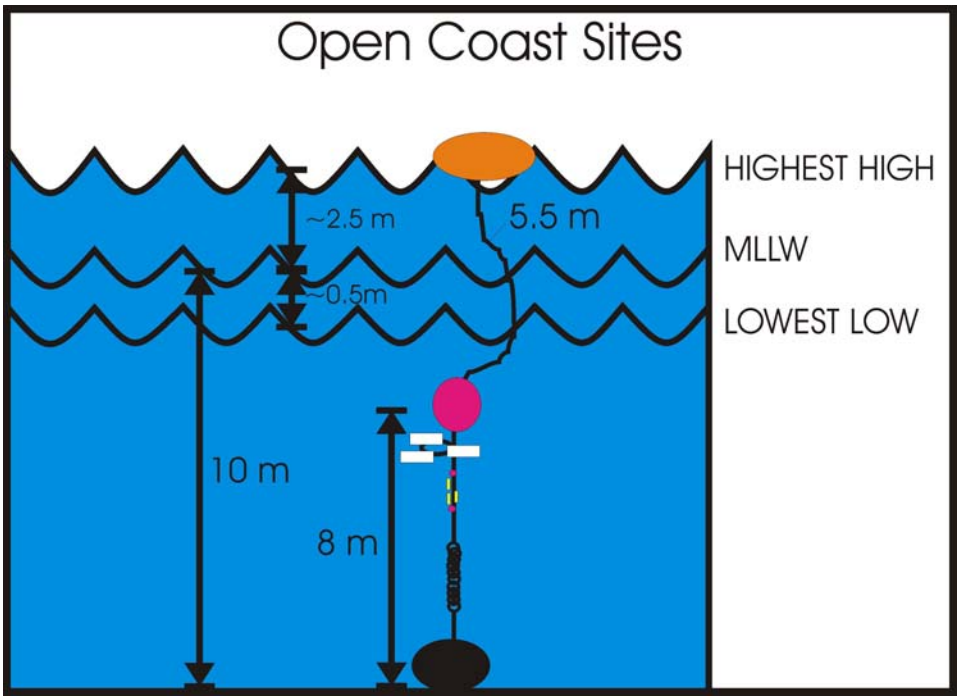

Figure 7. Map of *Mytilus* mussel Stage 2 outplanting sites in San Diego County, California USA. Northern region (filled circles): AL= Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Institution of Oceanography Pier; Southern region (open circles): PB= Pacific Beach (Crystal) Pier, OB=Ocean Beach, CABR=Cabrillo National Monument, IB=Imperial Beach Pier; Bay sites (open squares): CPMS=Crown Point Mitigation Site (Mission Bay), DL=Dana Landing (Mission Bay), SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay). San Diego coastline data were provided by National Oceanic and Atmospheric Administration Medium Resolution Digital Vector Shoreline Database.

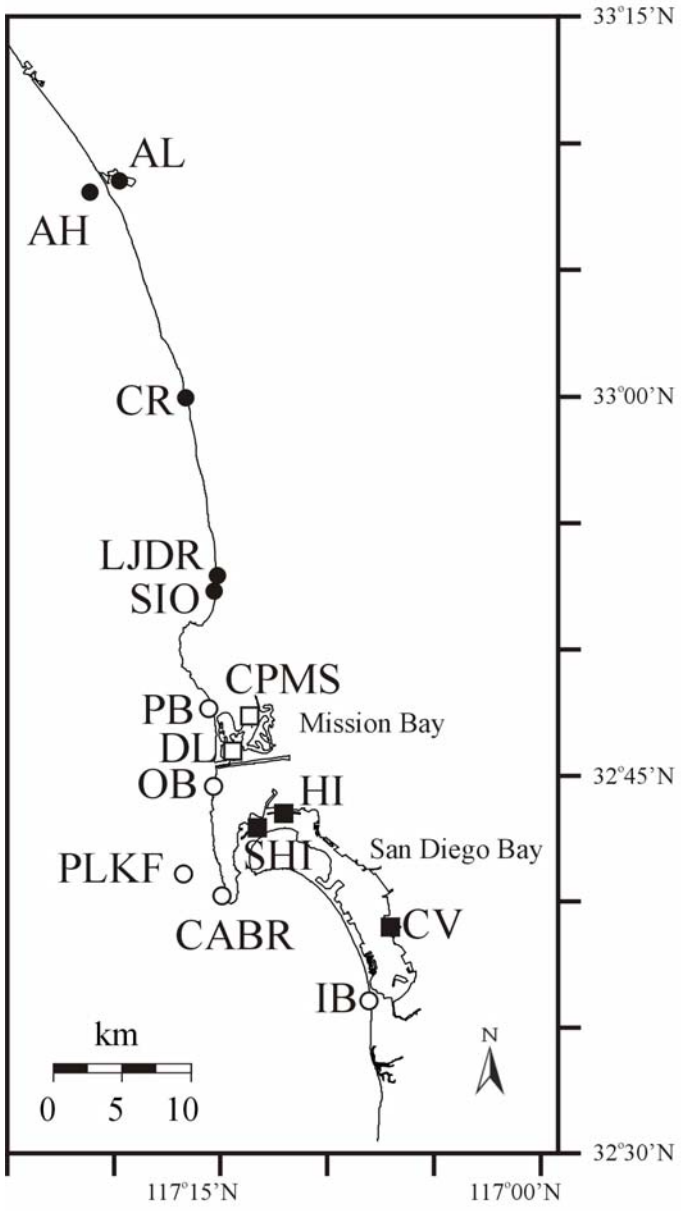

Figure 8. Preliminary DFA demonstrating the control experiment using larvae raised in the lab in larval homes ("Home"), without larval homes ("Bucket"), and in the field at three different sites (Agua Lagoon, Shelter Island, and Scripps Pier). Lab-raised larvae are illustrated using yellow and black icons, while field-raised larvae are represented by red, blue, and green icons. *M. californianus* larvae are represented by closed icons and *M. galloprovincialis* larvae are represented by open icons.

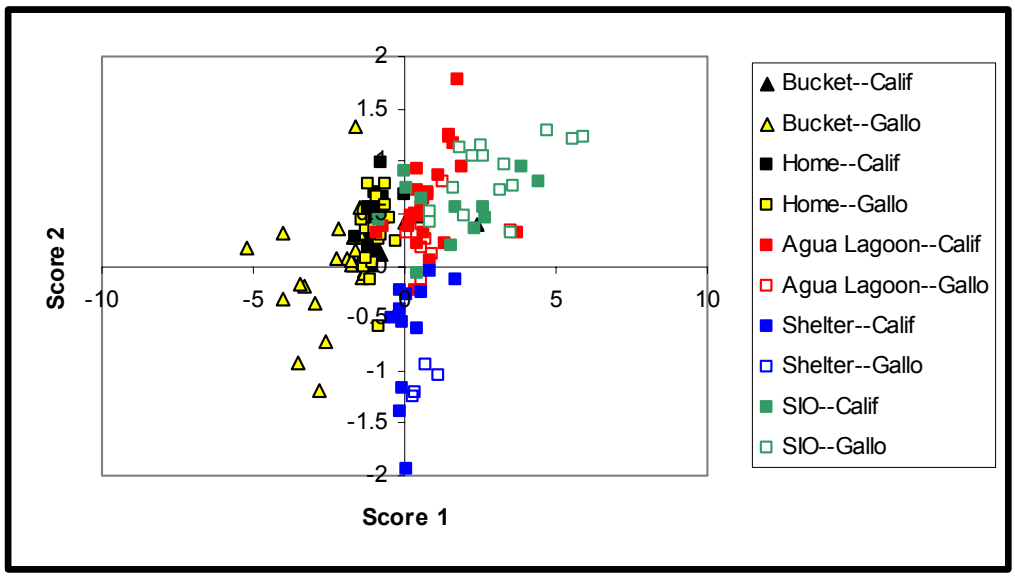

Figure 9. Preliminary DFA comparing shell chemistry of larvae raised in homes on the open coast compared to those raised in three bays in San Diego, California.

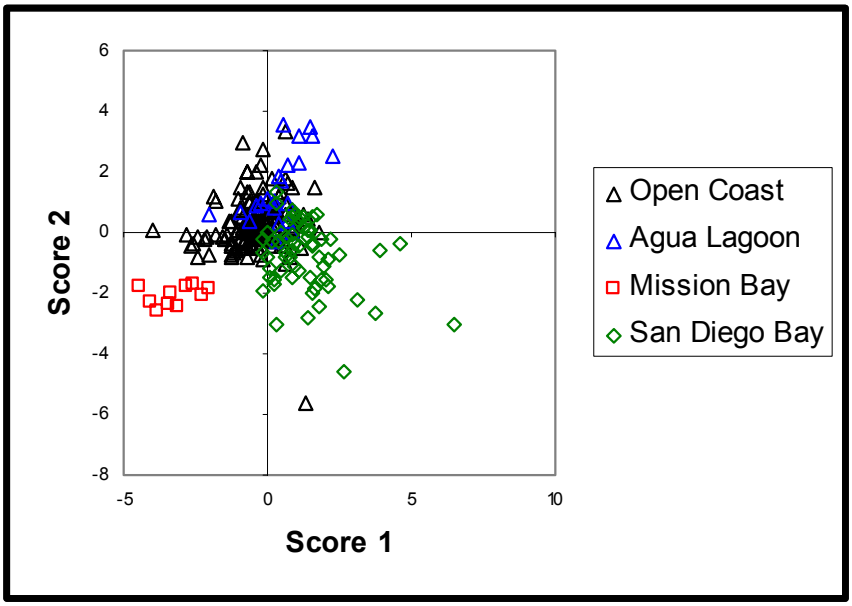

Figure 10. Preliminary DFA comparing shell chemistry of larvae raised in homes different regions on the open coast of San Diego, California. Northern sites are shown in blue while the southern sites are shown in red. AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Institution of Oceanography Pier, PB= Pacific Beach (Crystal) Pier, OB=Ocean Beach, CABR=Cabrillo National Monument, IB=Imperial Beach Pier