UNIVERSITY OF CALIFORNIA, SAN DIEGO

The regional population variability and larval connectivity of mytilid mussels: conserving the populations of Cabrillo National Monument (San Diego, California, USA)

A dissertation submitted in partial satisfaction of the
requirements for the degree of Doctor of Philosophy
in Oceanography

By

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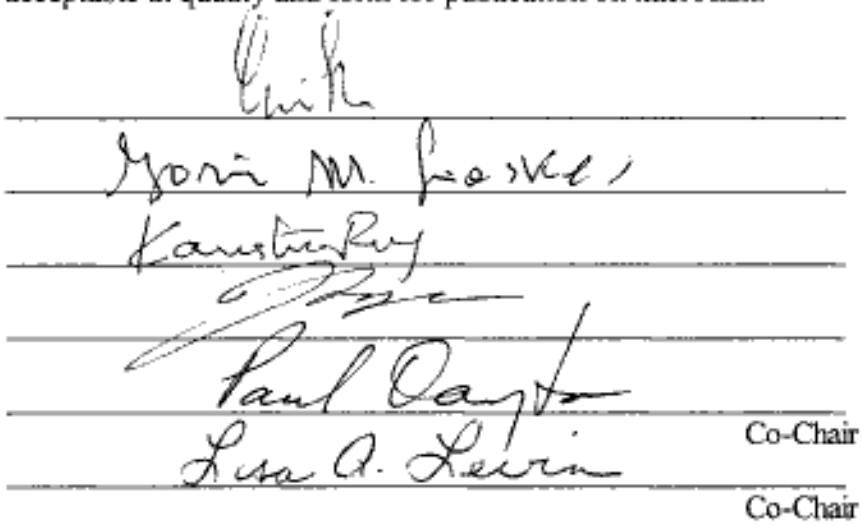

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DEDICATION

This dissertation is dedicated in loving memory to Dr. Mia J. Tegner, whose commitment to the preservation of our oceans will forever serve as an inspiration to young women ecologists.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter II

Chapter III

Chapter IV

Chapter V

LIST OF TABLES

Chapter II

Chapter III

Chapter IV

Chapter V

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Chapter 4 of this dissertation, in full, is a reprint of the material as it appears in the journal Limnology and Oceanography (Becker, B.J., Fodrie, F.J., McMillan, P., and L.A. Levin. 2005. Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: a precursor to invertebrate larval tracking. Limnology and Oceanography 50(1):48-61). The dissertation author was the primary investigator and author of this paper.

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ABSTRACT OF THE DISSERTATION

The regional population variability and larval connectivity of mytilid mussels: conserving the population of Cabrillo National Monument (San Diego, California, USA)

by

Bonnie J. Becker

Doctor of Philosophy in Oceanography

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Professor Lisa A. Levin and Professor Paul K. Datyon, Co-Chairs

To evaluate the decline of mussels (*Mytilus californianus* and *M. galloprovincialis*) in a small marine reserve (Cabrillo National Monument, CABR), the regional variability and larval population connectivity of mussels in southern California were examined. Comparisons of CABR monitoring data for mussel cover with those from 46 sites across 500 km of southern California coastline by the Multi-Agency Rocky Intertidal Network indicated that CABR mussel declines are a local phenomenon. Repeated spatial autocorrelation analysis demonstrated that regional mussel populations are structured by patchy and noisy local dynamics superimposed on occasional events much larger in temporal and spatial scale, such as large storms.

There is mounting evidence that the lack of recovery of mussels at CABR is related to low recruitment levels. The degree of larval connectivity among marine populations is a poorly understood and crucial piece of information for ecological, evolutionary, and conservation biologists. While direct determination of larval trajectories has been difficult for most marine invertebrate larvae, promise is offered by elemental fingerprinting, using geographically-unique chemical signatures in the developing hard parts of animals as a tracking tool. After validation using juvenile shell chemistry, elemental fingerprinting was used to determine natal origins of mussel juveniles collected throughout San Diego County. Mussel larvae were cultured in situ for one week at thirteen sites throughout San Diego County and their shells were analyzed. These reference chemical signatures were compared to larval shells retained on early mussel settlers from the same sites and time period, in order to predict regions of natal origin. Connectivity patterns for both species were compared to four general models of larval replenishment. Based on May 2003 analyses, most *M. californianus* originated from a single northern open coast region and *M. galloprovincialis* originated from a larger number of sources in open coast (north and south) and bay sites. Self-seeding was found to occur within natal regions (30 km extent). This work generates a number of important questions about how larval ecology interacts with circulation to drive metapopulation dynamics. This study greatly expands our understanding of local mussel population connectivity, and provides one of the first direct explorations of invertebrate connectivity.

CHAPTER I

INTRODUCTION

Over the past two decades, scientists and managers have become increasingly interested in the use of marine protected areas (MPAs) as a tool for conserving marine resources. Their goals are protecting biodiversity and representative habitats within reserves, and conserving spawning stock to supplement populations outside reserve boundaries (Lubchenco et al. 2003). Marine populations, in contrast to those in the terrestrial realm, are inherently connected by the movement of water (Carr et al. 2003). Therefore, although protecting an area is thought of as a "place-based" approach that focuses on a specific MPA or a network of MPAs, the long-term persistence and success of the reserve will depend on our ability to place it in a larger, regional context (Botsford et al. 2001, Sala et al. 2002, Allison et al. 2003). The purpose of this dissertation is to determine the larval connectivity and regional population variability of mytilid mussels (*Mytilus californianus* and *M. galloprovincialis*) in southern California. The motivation for the selection of this model system is the sustained decline of these mussels within the rocky shoreline of a small National Park, Cabrillo National Monument (CABR).

The data from long-term ecological monitoring within CABR have documented a sharp decline in mussel cover in plots from 1990 to 1995, with no recovery for an additional eight years. This monitoring program and possible causes of this decline are introduced in Chapter 2 and discussed briefly. The rest of this dissertation focuses

on specific hypotheses of this decline, and more broadly examines the regional connectivity of mussel populations in southern California.

Many of the mechanisms that lead to local changes in a given marine population are acting on a larger scale, and are therefore hard to recognize with small-scale and short-term observations (Dayton and Tegner 1984, Thrush et al. 1997, Dye 1998, Noda 2004). For example, a declining resource within a MPA could be affected by disturbances acting on scales ranging from local (e.g., trampling) to global (climate change). Without looking outside the borders of the reserve, it is difficult to identify relevant trends, causes of those trends, and appropriate management action.

In Chapter 3, local CABR monitoring data are integrated into an analysis of mussel cover in the Southern California Bight using data from a regional monitoring program, MARINe (the Multi-Agency Rocky Intertidal Monitoring Network), including 452 fixed quadrats at 49 sites. Using repeated spatial autocorrelation analyses, the spatial coherence of mussel dynamics were tracked over time (from 7 to 17 years), allowing for a synoptic examination of variability on scales ranging from meters to hundreds of kilometers. Determining the scale of variability of populations allows for the generation of appropriate hypotheses of mechanisms leading to population changes (Levin 1992, Koenig 1999). This study puts the mussel trends at CABR in a regional context, with broader implications for the management of other marine reserves (Channel Islands National Park) and mussel populations in southern California, and for our understanding of the long-term variability of bed-forming mussels worldwide.

Due to the apparent dispersive nature of the larvae of many marine species, it is important to understand the degree of larval connectivity among multiple marine reserves and populations outside of reserves. The goals of many MPAs include the export of larvae to enhance external populations, while the persistence of populations within MPAs might also depend on import of larvae from outside sources (Botsford et al. 2001, Roberts et al. 2003a, Roberts et al. 2003b). It is therefore important that the connectivity of the reserve and nearby populations is characterized.

Marine ecologists have long debated whether marine populations were demographically "open" (highly connected, so that the majority of new recruits in a population originated elsewhere) or "closed" (less connected, so that the majority of new recruits originated locally or were "self-recruited"). Due to the logical assumption that larvae that are in the plankton for days to months would be transported great distances by average currents, most marine populations have long been considered to be open (Caley et al. 1996). The recognition of the importance of larval behavior and physical variability in limiting dispersal distances has recently led to a focus on processes and features that could lead to a higher level of larval retention (reviewed in Swearer et al. 2002, Sponaugle et al. 2002, Levin submitted). This theoretical question clearly has important implications for marine reserve design and management (Warner et al. 2000).

Despite the importance of defining and quantifying larval connectivity, it has rarely been accomplished directly due to the difficulty of tracking microscopic larvae for long periods of time through a highly complicated physical environment (Levin

1990, Thorrold et al. 2002). Elemental fingerprinting, the determination of geographically-unique chemical signatures in the hard parts of marine animals, has been successfully applied to fish species to address several issues related to juvenile and adult movements (Campana 1999, Campana and Thorrold 2001). A more limited number of studies have applied this method to determine larval trajectories of fish (Thorrold, et al. 2002, Levin submitted). Although the use of chemical signatures of invertebrate larval hard parts (e.g., shells and statoliths) as a larval tracking tool shows great promise (DiBacco and Levin 2000, Dibacco and Chadwick 2001, Zacherl et al. 2003a, Zacherl et al. 2003b, Becker et al. 2005), no studies have used elemental fingerprinting to determine natal origins of invertebrates.

In this dissertation, I use elemental fingerprinting to determine the larval connectivity among populations of mytilid mussels in San Diego, California and to test the applicability of four general models of larval replenishment. In Chapter 4, the shell chemistry of juvenile mussel shells from eight sites is analyzed to verify the existence of location-specific elemental fingerprints. These signatures are examined at seasonal and weekly intervals to determine their stability. In Chapter 5, I apply this method to determine the natal origins of two species of mytilid mussels (*M. californianus* and *M. galloprovincialis*) from thirteen sites that were divided into four "natal regions". In situ larval culturing was used at the thirteen sites to generate reference elemental fingerprints. These fingerprints were then compared to those of the larval shells of field-collected mussel settlers in order to predict their natal origins and describe connectivity patterns.

The implications for this study are many-fold. Characterizing the degree of recruitment and sources of new mussel production within CABR will help determine the best management practices to address this decline. Self-seeding on a mostly linear coast (without isolated populations or obvious retention mechanisms) was documented in this study and the connectivity patterns in this region were resolved. This study is one of the first to directly determine the natal origins and larval connectivity in a marine invertebrate. The use of in situ larval culturing expands the application of elemental fingerprinting techniques to invertebrate species with wholly planktonic larval phases. This approach shows promise to characterize the connectivity patterns of a broad number of species in systems worldwide.

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CHAPTER II

Possible causes of the decline of mytilid mussel populations within Cabrillo National Monument (San Diego, California)

ABSTRACT

A long-term ecological monitoring program has revealed that mussel populations have declined within a small marine reserve under the administration of Cabrillo National Monument, a unit of the National Park Service. Mussel cover in plots in areas receiving medium or low levels of visitation declined from approximately 50% in 1990 to less than 5% by 1995. No recovery has occurred for at least the following eight years. In contrast, an increase in mussel cover has occurred in the high-use area. Possible mechanisms causing this pattern, including overharvesting, shoreline alteration, visitation effects, pollution, food limitation, predation, natural variation, regional declines, climate change, disease, and recruitment failure, are discussed and evaluated.

INTRODUCTION

> "…shall promote and regulate the use of the…national parks, monuments, and reservations…which purpose is to conserve the scenery and the natural and historic objects and the wildlife therein and to provide for the enjoyment of the same in such a manner and by such means as will leave them unimpaired for the enjoyment of future generations."
> -The NPS Mission Statement, from the National Park Service Organic Act, 16 U.S.C. §1.

The mission statement of the National Park Service (NPS) clearly states that it is the responsibility of park managers to protect the natural resources within their jurisdiction. In practice, it is difficult to achieve this goal without a clear definition of the word "unimpaired". Ecosystems, even without human influences, are dynamic systems. The balance of nature that we see, with a mosaic of coexisting organisms, is the sum of numerous natural disturbances, successional events, and random chance. If the changes in community structure we witness are due to human impacts as well as natural processes, how can managers determine what "unimpaired" is in order to fulfill the NPS mission and protect their parks from anthropogenic disturbances?

The purpose of long-term ecological monitoring is to evaluate the amount of natural variation an area experiences, and to have an early warning when abnormal changes occur. The design of a monitoring program will be intimately linked to its goals, which are usually formed before the results of the monitoring are known. Once such a program allows managers to recognize that unusual change is occurring, it is sometimes possible to identify probable causes using correlation to known environmental or biological variation. But beyond this correlative speculation, most monitoring programs are not designed to identify actual agents of change, especially unpredictable changes that occur after the original design takes place. Supplemental studies will often be needed to more fully explore causation.

Since 1990, the tidepools of Cabrillo National Monument (CABR, a unit of the National Park System in San Diego) have been monitored for long-term changes in the populations of 13 "key" taxa. From this monitoring program, it has been determined

that over the past fifteen years, the shoreline within CABR has been experiencing a dramatic decline in its mytilid mussel populations. The original goals of this monitoring program were rather broadly defined and did not specifically focus on mussels, since at its inception in 1990 mussels were not in decline. Therefore, the monitoring program, although crucial to the recognition of mussel losses, is not sufficient to determine causality. The cause of this decline remains unknown, and the search for the cause and management solutions provides the motivation for the research described in this dissertation. The purpose of this chapter is to describe the conditions of mussels within CABR, list possible reasons for this change, and discuss six of these possible causes. More detailed studies of five possible causes of mussel declines are described in Chapters 3, 4, and 5 of this dissertation and are introduced here.

DESCRIPTION OF THE PROGRAM

Cabrillo National Monument (CABR) is located at the end of Point Loma, a long (approximately 5.5 km) peninsula that is bordered by the Pacific to the west and San Diego Bay to the east (Figure 2.1). Although it is a small (160 acres or 6.5×10^5 m^2 of land) urban National Park by area, it receives over 1.2 million visitors per year. Within the administration of CABR is approximately 1.5 km of rocky intertidal that lines the western shore. These tidepools are a valuable and valued public resource, with 300 to 600 visitors per day in the area during appropriately low tides (T. Huff, pers. comm.).

The Cabrillo National Monument Rocky Intertidal Monitoring Program (CRIMP) was established in spring 1990 by Gary Davis, NPS and Dr. Jack Engle, University of California Santa Barbara. Davis and Engle modeled CRIMP as an extension of the prototype intertidal monitoring efforts that they began in the Channel Islands National Park (CHIS) during the 1980s. The CRIMP sites were to provide park-specific information, as well as serve as a comparison for the CHIS and other coastal Pacific sites. The goals of the program are listed in Table 2.1.

The CRIMP study area encompasses about one kilometer of shoreline, consisting mostly of flat, gently-sloping benches with scattered, hard, metavolcanic boulders at the base of soft, eroding sandstone cliffs. It was divided into three zones (I, II, and III, Figure 2.1), each about 330 m in length. The northern section of Zone I, where most of the plots for that zone are located, consists of a flat bench with numerous large boulders and narrow channels. The southern section has few boulders, and contains a short stretch (<100 m) of permanent sandy beach. Zone I is approximately 40-65 m wide on a fairly low tide. Zone II resembles the northern section of Zone I, although it is a bit wider (40-90 m). Zone III is much wider (90-120 m) and flatter, with few large boulders and many small, flat rocks. There is a single line of large boulders at the southern end of the area where the majority of the plots for that zone are located. This area is close to the mouth of San Diego Bay. The base of the cliffs in this area is artificially reinforced with granite riprap. These boulders were placed there in the 1960s and dramatically changed the natural sedimentation patterns in the zone (D. Leighton, pers. comm.).

Each zone receives a different amount of human visitation. There is a single public entry point to the tidepool area, located in the middle of Zone I; since it is the most accessible, this area receives the most visitation. In order to access Zone II, people must amble through a rugged boulder field; therefore, fewer visitors venture into this area. Zone III has traditionally received the least visitation, and has been closed to all visitors since November of 1996. During most daylight low tides in the fall, winter, and spring, there is a National Park Service volunteer (Volunteers In Parks, VIPs) stationed near the border of Zones II and III to inform and educate visitors about the closure. There is an additional access point into Zone III located on property belonging to the U.S. Coast Guard that is rarely, but occasionally, used.

Thirteen species or species groups were chosen as "key" taxa to serve as a proxy of overall ecological health (Table 2.2). A number of different techniques are used to track the populations of these species: circular plots (to determine size-frequency distributions of owl limpets, *Lottia gigantea*), photoplots (percent cover of organisms growing on boulders), line transects (percent cover of organisms growing on flat bench areas), and timed searches (presence/absence of rare organisms). All of these techniques, with the exception of timed searches, are done in fixed plots that were established at the inception of the program. In each zone there are a total of 33 plots per zone. Monitoring is conducted twice per year, in the spring and fall. In addition, shorebird and visitor censuses are conducted throughout the year. A complete description of the methodology is presented in Engle and Davis (2000c).

Due to the limited amount of park staff, CRIMP is and always has been mostly conducted by volunteers. Between its inception in 1990 and 2004, over 300 volunteers have donated over 750 sampling days (over 2500 hours) to this effort. This volunteer aspect of the program is one of its strengths, since it serves education and outreach purposes as well as scientific and management ones. Since participants often have a lack of expertise, the program was designed to be simple, so that well-trained volunteers and non-expert staff members with direction by a limited number of experienced staff could continue the effort in perpetuity.

After the first five years of monitoring, Engle and Davis (2000b) produced a report stating that seven of the thirteen key species were shown to have either declined or disappeared entirely from the area (Table 2.2). After the release of this report in 1996, CABR staff made a number of management decisions to try to reverse this trend. The monitoring program became part of normal park operations, and a long-term commitment was made to continue it. One third of the area under park administration was closed to all visitors; this small, no-use section of the reserve commonly referred to as "Zone III" is still closed as of 2005. The purpose of this closure is to allow the area to recover from the pressures of high visitation and serve as a control area for research. In addition, the presence of volunteers was increased dramatically, so that a uniformed person is in the areas open to the public during most daytime low tides and weekends to educate the public and enforce park policies. The creation of a marine biologist position in the park was a direct result of this effort, and a commitment was made to conduct research to determine the best management approach to protecting the tidepools.

In 1995, the U.S. Navy contracted Jack Engle to begin an effort similar to CRIMP north of CABR on Point Loma (Engle and Davis 2000a). A number of other organizations had established comparable programs between 1990 and 1997 modeled on the original CHIS program. In 1997, the Multi-Agency Rocky Intertidal Network (MARINe) was established in order to foster communication between the various governmental and academic bodies that were monitoring the rocky intertidal in central and southern California (www.marine.gov). This network, under the administration of the Minerals Management Service, provides for a rare opportunity for scientists to standardize their protocols so that larger-scale interpretations of results are possible. Through involvement with MARINe, CRIMP has been greatly expanded to include a larger group of core taxa and to ensure consistency with the other programs. The MARINe groups have recently completed the creation of a large central database including all past and future monitoring data from over seventy sites in a single format, which allows for a regional perspective that the individual programs cannot provide. Chapter 3 of this dissertation focuses specifically on the regional trends in mussel cover as compiled in the MARINe database.

Additional information about CRIMP can be found in Becker (2003, in preparation).

MUSSEL MONITORING IN CABR

Mussel percent cover has been tracked in five fixed photoplots per zone. Plots are photographed using a camera affixed on a PVC quadrapod of standard dimensions (image area measured 50 cm x 75 cm or 0.375 m^2). Resulting images are scored using a non-random point contact method. A grid of 100 evenly-spaced points is projected on the images, and the type of cover that falls under each point is identified and recorded. Summing each point in this procedure yields percent coverage data. The level of detail of the type of cover has varied over time (e.g., identifying species of algae vs. lumping all non-targeted algae into "other algae" category). Cover of mussels was always determined.

This method does not discriminate between the two species of bed-forming mussel within CABR, *Mytilus californianus* and *M. galloprovincialis*. The majority of the intertidal within CABR, especially in Zones I and II, is exposed coastline that is dominated by the larger and robust *M. californianus* (California or sea mussels), although some of the smaller, more bay-tolerant *M. galloprovincialis* (bay or blue mussels) can be found in the bay-influenced Zone III. A third species of *Mytilus*, *M. trossulus*, has been found in San Diego Bay (Suchanek et al. 1997) and is difficult to distinguish from *M. galloprovincialis*; it is possible that this species is present in the park as well. The small and solitary mussel, *Septifer bifurcatus*, is commonly found in CABR but was not usually found or scored in the photoplots. Mussel results presented here are not divided by species, although the vast majority of the mussels were *M. californianus*.

Mussels declined during the study period throughout all three zones of the park from a cover of 40% ± 23% (mean ± 1 SD) in spring 1990 to 12% ± 19% in fall 2003. This decline had a very clear geographic pattern, with mussel cover in Zones II and III, the medium- and low-use areas, declined quite rapidly in the first few years of the study (Figure 2.2). Mussel cover within plots in Zone II averaged 55% ± 24% in spring 1990, less than 1% ± 2% in spring 1994 and has not increased above 3% as of fall 2003. In Zone III, average mussel cover within plots steadily declined from 47% ± 12% in spring 1990 to 2% ± 2% in spring 1995, without increasing above 3% as of fall 2003. On the other hand, average mussel cover remained fairly constant in plots in Zone I, the high-use area, from 16% ± 5% in spring 1990 to 16% ± 12% in spring 1994. Since 1995, average mussel cover in Zone I increased somewhat to 32% ± 23% in fall 2003.

Reports of mussel abundance prior to 1990 are relatively rare. In 1976, Zedler described healthy mussel beds in Zones I and II. She mentioned that mussels were "very common in large colonies, attached to large mid-tide rocks; smaller colonies found at base of cliff face". Today a few isolated individuals can be found within goose barnacle patches at the bottoms of the cliffs, but are mostly isolated to small patches on mid-intertidal boulders. She reports finding 230 to 610 individuals / m^2 on what she called "*Mytilus* boulders" and 13-127 individuals / m^2 on "*Pelvetia* boulders" (now *Silvetia* or rockweed). Similar densities have not been determined in recent studies, although very few mussels are found on rocks containing *Silvetia*. A photograph from around 1962 (Figure 2.3a) depicts a dense mussel bed on a boulder in Zone II; that same area did not

contain mussels as of 2005 (Figure 2.3b).

In sum, mussels have declined precipitously between 1990 and 1995 within the tidepools of CABR. This decline has been particularly severe in Zone III, the low-use and more bay-influenced part of the park, with plots in the northern part of the park experiencing an increase in cover. Through qualitative observations of mussels outside of plots, it appears as if there are some remaining beds of larger individual mussels in Zone II, while Zone III and areas further around Point Loma have only occasional, very large individuals, with few small beds throughout the area.

POSSIBLE CAUSES OF MUSSEL DECLINE

The CRIMP data document the sustained decline of mussels within the tidepools of CABR, but the cause of this trend remains elusive. There are a number of possible causes of this decline, none of which are mutually exclusive:

1. Overharvesting/Poaching
2. Shoreline alteration
3. Visitation effects
4. Pollution or poor water quality
5. Food limitation
6. Predation
7. Natural variation
8. Regional mussel declines

9. Climate change
10. Disease
11. Recruitment failure

Hypotheses one through six will be discussed briefly here. The other five hypotheses will be addressed in more detail in Chapters 3 through 5 of this dissertation and will be introduced below.

Overharvesting/Poaching

Although legal and illegal collecting of marine organisms is an important source of anthropogenic disturbance in the rocky intertidal of high population areas like southern California (Murray et al. 1999, Thompson et al. 2002), this activity is uncommon at CABR. It is illegal to harvest any invertebrates (except lobsters and crabs in traps offshore) from the park, and enforcement of this rule is extremely strict. Uniformed rangers and volunteers patrol the park during the day, and during the night access is limited since most of Point Loma is a naval base that is closed to the public. The prohibition of human collection with effective enforcement at CABR has resulted in larger-sized gastropods than are found in the rest of southern California (Roy et al. 2003). It is highly unlikely that overharvesting of mussels is occurring in the park, especially compared to less protected areas in San Diego.

Shoreline alteration

Habitat loss through shoreline alteration is another major source of anthropogenic disturbance that threatens rocky intertidal habitats (Thompson et al. 2002). Although there have been structures in these tidepools in the past (e.g. a dolphin training facility, artificial "riprap" erosion control), development of the CABR coastline has not occurred in the past two decades and is unlikely to have led to the observed mussel declines over this time period. The park affords long-term protection of this habitat from development, and therefore future development in this area is also unlikely. The effects of shoreline alteration outside of the park boundary on mussel populations within CABR are not known.

Visitation effects

A large number of people consistently visiting the rocky intertidal over time can have chronic ecological effects (Thompson et al. 2002) through trampling, rock turning, poking, and other non-harvesting disturbances. Mussels in the park are usually found on large, immovable boulders, which are less vulnerable to trampling and rock-flipping. Although it is likely that an occasional visitor will pull on or otherwise harass mussels in the park, this would likely lead to declines in the high use area (Zone I) and healthy populations in the closed area (Zone III). In reality, the pattern is exactly reversed. For this reason, this agent of change is unlikely to be directly responsible for the mussel trends. Indirect effects of visitation, such as human influence of predator distribution, cannot be ruled out.

Water quality

The geographical patterns of the mussel trends within the park, with the more bay-influenced area declining and the more northerly area somewhat increasing, could potentially be caused by differences in exposure to pollution from San Diego Bay, a major industrial and military port known to be enriched in a number of contaminants (e.g., Flegal and Sañudo-Wilhelmy 1993, Goldberg and Bertine 2000, Esser and Volpe 2002). Most models and observations of local currents indicate that during ebb tide a plume of Bay water is transported to the south of the channel, with small-scale recirculating eddies transporting less water to the north. It is likely that, on average, marine organisms growing on the southern part of the point are exposed to more contaminants than those to the north. This exposure could affect populations through various mechanisms at different life stages, including sublethal effects and lower competence of settling larvae.

Beginning in 1986, a regional group associated with the National Oceanic and Atmospheric Administration (NOAA) and the California State Water Resources Control Board used the soft tissue of mussels and other bivalves as a way to monitor pollution. The program, called "Mussel Watch", gave scientists more information than water samples alone (Goldberg et al. 1978, Goldberg and Bertine 2000). A single water sample reveals information on the pollutants in the water at the moment when that sample was taken. Pollutants can vary significantly within a small area, or over short time scales; therefore, concentrations found in seawater samples are not

necessarily representative of the long-term influences of pollutants on the organisms. By using tissue samples of animals that are constantly filtering seawater, the longer-term exposure of the animals can be determined. In addition, mussels can concentrate chemicals that are dilute in seawater to levels that can be more easily measured.

One of the sites used in the original Mussel Watch program was the U.S. Coast Guard Point Loma Lighthouse (i.e., Cabrillo Zone III), and the results were quite startling. Cabrillo was on a list of 21 sites with "high and increasing concentrations" of mercury and nickel in 1993. The State efforts included several points in proximity to Cabrillo (including the Coast Guard Station and the Sewage Treatment Plant Outfall) and unusually high concentrations of copper and silver and occasionally zinc were reported (Engle and Davis 2000b). A status report stated that "the Point Loma shore has silver levels in mussels among the highest levels measured in the State" (SWRCD 1989). By 1993, Mussel Watch stopped using Cabrillo as a site due to the lack of mussel samples; in other words, there were not sufficient mussels to continue monitoring without affecting the remaining population.

Since August 2004, the Mussel Watch concept is being used to examine water quality and Bay influence within the park. In order to avoid collecting from declining mussel populations within CABR, mussels are acquired from offsite, outplanted at sites around Point Loma, allowed to grow for three months, and then collected for chemical analysis. This approach has many benefits – local mussels are not killed, a standard starting concentration, free from bay influence, for all of the sites occurs, and growth parameters for the animals throughout the experiment can be measured. This

process will be repeated four times, once each season. As of April 2005, two sets of mussels have been outplanted for three months each, and a third set is still in the field.

M. galloprovincialis are raised on set lines off of the Scripps Institution of Oceanography (SIO) Pier in La Jolla for a year prior to this study. Bay mussels are used because bay sites are included in this study, and in contrast to *M. californianus*, this species can thrive in both protected and exposed conditions. Mussels are harvested from the lines, cleaned, and sorted into approximate size classes (1 mm intervals). Mussels are selected according to size in order to evenly distribute the different classes in each cage, trying to focus on individuals between 4 and 6 cm, but occasionally using slightly smaller or larger individuals as needed. Each cage contains 16 mussels, which are later combined to serve as a single sample for chemical analyses. Each individual is engraved with a number, and its weight, length, width, and height are recorded, so that the survival and growth of each mussel can be tracked separately. Mussels are then placed in cages made of electrical conduit and PVC that were designed to withstand great wave energy.

Three cages are outplanted at each of seven sites (Figure 2.4). One site ("Inner Bay") is located within San Diego Bay, at the Scripps Nimitz Marine Facility (MARFAC), on a pier used for large research vessels. The "Outer Bay" site is on the southeastern corner of Point Loma, on property administered by the U.S. Navy, on scattered boulders. The "CABR I", "CABR II", and "CABR III" sites are located within each management zone of the park, all on scattered boulders. The "Ocean Side" site is on the ocean-facing side of Point Loma, on the U.S. Navy Space and

Naval Warfare System Center property. Cages are installed on artificial riprap on one of the only accessible intertidal benches on this part of Point Loma. The last set of cages are suspended by rope on SIO Pier in La Jolla ("SIO" site), and are considered to be free of San Diego Bay influence due to its distance from the area. Within each site, cages are located approximately 10 meters apart. A laser leveler was used to locate them all at approximately the same tidal height (approximately 0.5 to 0.6 m above mean lower low water, MLLW). At pier sites, a transect line and local tidal predictions were used to ensure consistency in tidal height. In addition, a set of mussels are collected directly off of the SIO pier pilings on the same day that the SIO cages are retrieved in order to control for cage effects in flesh chemistry. The growth and mortality of these control mussels are not determined.

After three months, the cages are retrieved. Each mussel is checked for survival and then frozen in a ziplock bag (mussels from a single cage are combined in one bag). Starting from the second deployment, mussels are weighed prior to freezing; in the first case mussels were weighed after freezing. The following day, the samples are taken to the City of San Diego Alvarado Laboratory for further processing. The shell is once again weighed and measured, opened with a carbon-steel blade, and the flesh scraped into a clean beaker. All of the flesh from a single cage is homogenized, split into two separate containers (one for organic analysis and the other for metals analysis), and re-frozen. Shells are retained for a separate paleontological study to be conducted by Dr. Stephen Schellenberg (San Diego State University).

All further analyses are carried out by the City of San Diego Wastewater

Chemistry Laboratory. Mussel soft parts are analyzed for polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organotin, and a variety of metals. Contaminants for analysis were chosen from a few different sources, including a similar pilot study conducted in the Point Loma Kelp Forest (offshore of CABR) by Dr. Ed Parnell of the Scripps Institution of Oceanography.

The first quarter mussels were outplanted on August 27 and retrieved on November 26, 2004. There was an unusual amount of rain during October, which led to a massive sewage spill at the City of San Diego Point Loma Wastewater Treatment Plant in between the Cabrillo sites and the Ocean Side site. One of the Ocean Side cages was lost, as well as a number of individual mussels that were too small to stay in the mesh. There was a high level of mortality at the Outer Bay sites, possibly related to rockweed that was piled around the cages that could have suffocated the mussels. Eight dead mussels from various sites had evidence of predation (small drill holes). In the second quarter (December 10, 2004-March 10, 2005), some of these problems were fixed. The Outer Bay sites were cleared of rockweed, and survivorship was similar to the other sites. No mussels small enough to slip out were added to the cages (none were missing). Inner bundles were suspended by attaching them to the top of the cages, which helped exclude predators from reaching the mussels (no evidence of predation was found). Unfortunately, due to the large swell during this period, four cages were lost (one at Outer Bay, one at CABRI, and two at Ocean Side).

During both outplantings, mussels outplanted on Point Loma did not grow as much as those outplanted at the Inner Bay or SIO sites (Figure 2.5a). In the second

quarter, the same pattern was seen in weight change, with SIO gaining the most weight, followed by the Inner Bay site, and the Point Loma sites gaining a lot less weight (Figure 2.5b). Since the weights before and after outplanting the first quarter are not comparable, they will not be presented here.

Although all of the chemical analyses have not been completed yet, the organic compounds for mussels outplanted in the first quarter have been analyzed. These preliminary results indicate that PCBs are highest in the Inner Bay site, intermediate at the Point Loma sites, and lowest at SIO (Figure 2.6); therefore, PCBs are considered to be a tracer of Bay influence. However, it is possible that the distributions and bioavailability of other contaminants, especially certain metals, are complicated due to speciation and scavenging or other interactions with suspended and benthic sediment (Libes 1992, Deheyn and Latz 2005). No compounds were higher in mussels outplanted at Point Loma than elsewhere in the first quarter, but analyses are ongoing.

In sum, it appears as if there is a demonstrable influence of polluted San Diego Bay water on Point Loma, but PCB body burdens in mussels not correlate with the geographic patterns in mussel health. Further analyses, especially of inorganic contaminants, are being conducted to further refine our understanding of water quality within the park specifically.

Food limitation

A number of studies have found a correlation between poor mussel health or growth rates and low primary productivity in the water adjacent to the mussels (e.g., Dahlhoff and Menge 1996, Jasprica et al. 1997). It is possible that these low growth

rates are related to small-scale differences in food availability for the filter-feeding mussels.

At the time of mussel cage deployment, seawater samples were taken at all of the sites; chlorophyll was not measured, so turbidity (which would include biotic and abiotic particles) served as a very rough proxy for productivity. There were no apparent differences in turbidity between the sites in these samples. However, there is some indication that the primary productivity patterns in this area are quite complex. Studies have documented localized upwelling due to coastally trapped waves (Pringle and Riser 2003) and current separation at the headland of Point Loma (Roughan et al. in press). In the latter study, an area of increased primary productivity was found in the lee of Point Loma. Additionally, Esser and Volpe (2002) found elevated chlorophyll A levels in the mouth of San Diego Bay. Although these studies indicate that a lack of food is unlikely in this area, the details and variability of the mussel food sources in this area, and how they have changed through time, should be more thoroughly studied.

Predation

The classic "keystone predator" of rocky intertidal systems (e.g., Paine 1974, Robles et al. 1995), *Pisaster ochraceus* (ochre seastar), is virtually absent from CABR, despite being documented in small numbers in 1976 (Zedler) and thriving in nearby sites, including Ocean Beach Pier, only 9 km from the park. There are a handful of other seastar species (e.g., *Asterina miniata*, *Pisaster giganteus*, and

Astrometis sertulifera) that are found in low numbers in the park, but it is doubtful that these taxa serve as important predators of mussels in CABR.

Another source of predation on CABR mussels are Mexican unicorn snails, *Mexacanthina lugubris*. It is believed that this species has experienced a recent northern expansion from its historical range further south in Mexico. After being absent from the area for forty or fifty years, this species was found "in large numbers" on Point Loma in 1974 (Radwin 1974). During a species inventory in 1976, they were found to be "common on lower cliff faces and on *Mytilus* rocks" (Zedler 1976), and their numbers and tidal range have been observed to increase greatly over the last decade. They appear to be extending their range slowly northward, and were first reported reappearing in other parts of San Diego in 1994 (Hertz 1995). There are no studies of this snail in CABR, although it appears to be a mussel predator and increased in numbers at the appropriate time to lead to a decline in adult mussel populations. The large size of the remaining mussels in the southern part of the park might indicate that these individuals "escaped in size" from this predation pressure. However, it is not clear why the snail would affect Zones II and III populations and not those in Zone I. This potential agent of mussel change needs further study.

A number of shorebird species are known to feed on mussels (e.g., Hilgerloh 1997). Birds have been repeatedly censused as part of CRIMP since 1990. There is a negative relationship between the number of people and number of birds in a given census (Figure 2.7a). Within Zone III, which has always received little visitation and serves as a resting place for a very large number of seagulls and terns, there are more

birds than in Zone I; Zone II tends to be intermediate between them (Figure 2.7b). This large difference in avian predation pressure could have an effect on mussel populations.

There are a number of predators on mussels that are present in large numbers within the park (e.g., lobsters, *Panulirus interruptus* and octopus, *Octopus bimaculoides*) that have not been well-studied. Although more lobsters are observed in Zone III than elsewhere in the park, there have been no studies on the distribution, abundance, or trends in either of these species.

Natural variation, Regional mussel declines, Climate change, Disease

It is possible that the sustained decline of mussels in CABR is simply part of a natural successional cycle, and not enough time has passed for recovery of populations. Additionally this trend could be part of a larger-scale decline with a regional or global cause, such as climate change or an emerging disease.

In Chapter 3, I examined mussel percent cover trends at MARINe sites across the Southern California Bight. From this analysis, there is no evidence of a widespread decline in mussel abundance in the region. Mechanisms that are likely to occur on larger scales, like climate change and disease, do not appear to be the causes of local changes at CABR. In addition, by comparing the increases in mussel cover after a disturbance in sites further north, it appears as if recovery can occur in as little as two years. Comparison of CABR with other sites using similar methods suggests that the changes in mussel cover at the park have local causes. The lack of mussel

recovery at CABR over a period of almost ten years also indicates that pre-recruitment processes might play an important role in the CABR mussel trends.

Recruitment failure

There is quite a bit of speculative and quantitative evidence that mussel recruitment is limited within CABR. As mentioned above, the lack of recovery of mussel populations after the decline in the early 1990s and the anecdotal observation that there are few small mussels in mussel beds, indicate that either mussel larvae are not being transported to the park, pediveligers (late larvae) are not settling into the park, or plantigrades (recent settlers) are not surviving.

From July 2001 to May 2003, the numbers of mussel settlers in the three zones of the park and two sites to the north (La Jolla Dike Rock and Cardiff Reef) were monitored six times (B.J. Becker and L. Fajardo Mellor, unpubl. data). Three fist-sized samples of turf-forming red algae were collected from close proximity to adult mussels at each site and frozen in separate ziplock bags. At a later date, approximately 15 g of algae and 5 g of sand (wet weight) were separated from the sample for sorting. If there was less than 5 g of sand in the sample, extra algae was retained to keep the total amount as close to 20 g as possible. The retained algae and sand were sorted under a dissecting microscope and all mytilid mussels smaller than 2 mm were removed and then dried and weighed. Settlement was quantified as the number of mussels found per dry gram of substrate. As of May of 2005, 60 out of the 90 collected samples have been sorted, and preliminary data are shown in Figure 2.8.

From these collections, it appears as if recent recruitment of mytilid mussels into the smallest size class (<2 mm) into CABR was consistently much lower than into sites further north (Figure 2.8). This low level of recruitment might have contributed to the decline of mussel populations and probably is related to the lack of recovery in this area. Mussel settlers were not identified to species, since it is difficult to visually distinguish between very small *M. californianus* and *M. galloprovincialis*.

When focusing on the recovery of mussel populations in CABR, it will be important to understand the degree of larval connectivity between this population and more healthy ones outside of the park. If the waning CABR populations are poorly connected to others and are mostly self-seeding, then it is possible that the existing spawning stock will not be able to sustain itself. In this case, it would probably be beneficial to try to artificially restore adult populations, since the resulting larvae would be retained in the local area. If populations are replenished from outside of the park and most of the local production is exported outside of the area, then this type of restoration will not increase the supply of larvae to the park.

Unfortunately, it is often very difficult to determine larval connectivity, since for most species larvae are small and are in the plankton for a relatively long time (more than a day to many months), where they are transported in complicated trajectories. In Chapters 4 and 5 of this dissertation, I describe a method to track larvae and determine larval connectivity in mussel populations in San Diego County using in situ larval culturing and the relatively new technique of elemental fingerprinting. From these results, it appears as if *M. californianus* recruits in the park arrive as larvae transported

from the northern part of San Diego County, and *M. galloprovincialis* are coming from a number of sources in the north and south.

CONCLUSION

From this combination of anecdotal and quantitative evidence, there are a number of explanations for the sustained decline of mussel populations in CABR. It is important to note that multiple causal mechanisms could interact (e.g., Marsh 1986, Menge et al. 1997) and the dominant processes could change over time. Based on large-scale, long-term monitoring, it seems that the cause is most likely to be found in a local process rather than a larger-scale regional one. It is possible that changes in predatory patterns could have led to the decline or recovery failure in CABR mussels, although in low-recruitment mussel habitats the effects of predation have been shown to be dampened (Robles 1997). There is some indication that water quality is somewhat compromised in the park; however San Diego Bay influence in and of itself does not appear to limit adult mussel growth. A more complicated water quality scenario, such as differences in specific available pollutants in different regions of the Bay or during different seasons, is possible. In addition, current adult mussel condition within the park is quite poor compared to mussels in San Diego Bay and on Scripps Pier. Larval recruitment is considerably lower in the park than elsewhere in the county, likely limiting the recovery of these populations after the initial population decline.

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Table 2.1: Goals of the Cabrillo National Monument Rocky Intertidal Monitoring Program (CRIMP).

- To collect long-term, baseline information on the "ecological health" of the rocky intertidal area, and to determine normal limits of variation.
- To be conducted in perpetuity.
    - *In order to maintain the program in the long-term, all techniques should be doable by volunteers with limited training and basic supervision (by a non-expert) with oversight by a limited number of experienced staff. In addition, the program should be low-cost.*
- To determine differences between the three zones, which experience very different amounts of visitation, and to determine the effects of the closure of Zone III.
- To be comparable and compatible with existing data and similar programs in southern California (e.g., Channel Islands National Park and the Multi-Agency Rocky Intertidal Network).
    - *Large changes in existing protocols can only be made after consultation with these other programs. Measurements for additional components that are unique to CABR are acceptable.*
- To detect large changes in community structure reasonably quickly.
    - *Correlation of these temporal data with other factors (environmental, anthropogenic) should guide further research to determine causation of trends of concern.*
- To provide for baseline data in case of an acute disturbance (e.g., oil spill, sewage spill, riprap), and to serve as an opportunity for public education and outreach.

Table 2.2: Key species, monitoring techniques, and the resulting types of data for the Cabrillo National Monument Rocky Intertidal Monitoring Program. Taxa in brackets are not targeted in a specific plot-type but are considered "key" taxa. Species marked with an asterisk (*) were not included in the original 13 "key" taxa, but have been consistently counted during monitoring. Species marked with a plus ($^{+}$) were found to have declined or disappeared from the park between 1990 and 1995 (Engle and Davis 2000b).

Technique/Taxa	Dimensions of Plot	Number per Zone	Type of Data
Circular Plots:	3.14 m^2 (circle)	6	Size Frequency
Owl Limpets (*Lottia gigantea*)$^{+}$			
Line Transects:	10 m (line)	6	% Cover
Red Algal Turf (*Corallina* spp. *et. al.*)			
Surf Grass (*Phyllospadix* spp.)			
Boa Kelp (*Egregia menziesii*)$^{+}$			
[Aggregating Anemone (*Anthopleura elegantissima*)]			
[Sargassum Weed (*Sargassum muticum*)]			
Photoplots:	50 x 75 cm (rectangle)	21	% Cover
Acorn Barnacles (*Chthamalus* spp., *Balanus glandula*)			
Thatched Barnacles (*Tetraclita rubescens*)$^{+}$			
Rockweed (*Silvetia compressa*)			
California Mussels (*Mytilus* spp.)$^{+}$			
Goose Barnacles (*Pollicipes polymerus*)$^{+}$			
Timed Search:	30 person-minutes	1	Presence/Absence
Black Abalone (*Haliotis cracherodii*)$^{+}$			
Green Abalone (*Haliotis fulgens*)*$^{+}$			
Ochre Sea Star (*Pisaster ochraceus*)$^{+}$			

[Taxa in brackets are not targeted in a single plot-type but are considered "key" taxa.]

* Not included in the original 13 "key" taxa, but has been consistently counted

$^{+}$ Declined or disappeared from the park between 1990 and 1995

Figure 2.1: Map of Cabrillo National Monument, located in San Diego, California. Three management and study zones have been delineated. Zone I receives the highest amount of visitation, Zone II receives and intermediate amount and Zone III has traditionally been a low-use area and has been closed to all visitors since 1996. Park boundary is shown in pink in the right panel.

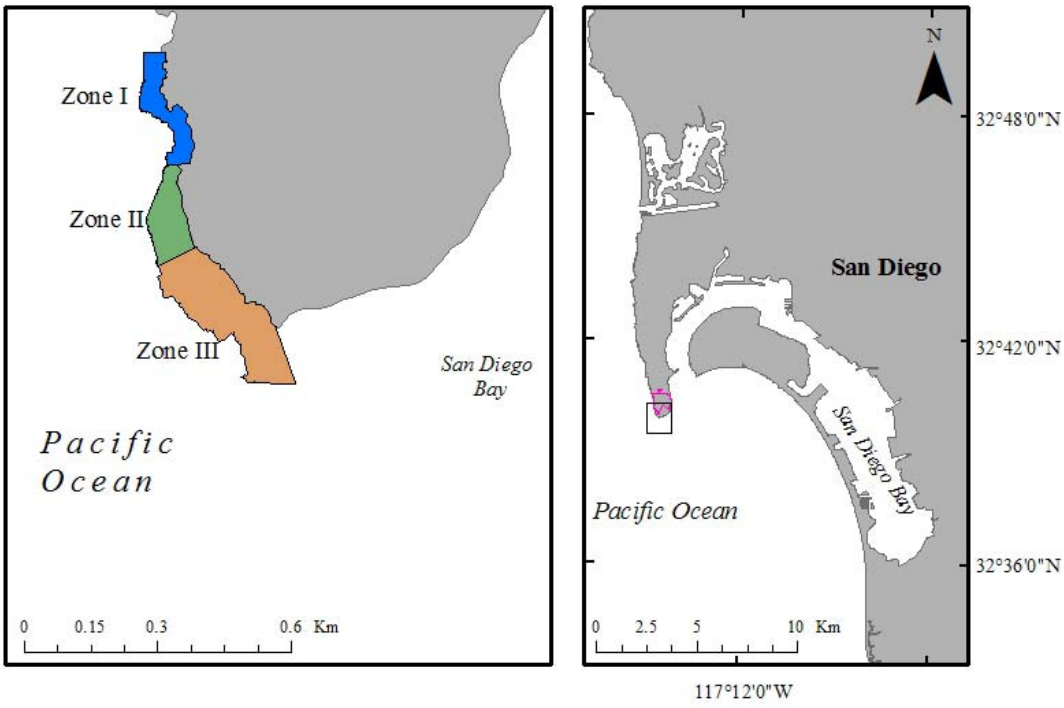


Figure 2.2: Change in mussel percent at Cabrillo National Monument cover through 14 years, measured every spring (SP) and fall during long-term ecological monitoring. CAB1= Zone I, a high use area; CAB2 = Zone II, an intermediate-use area; CAB3=Zone III, a low-use area that has been closed to visitors since 1996. Each line represents one of five photoplots in each zone.

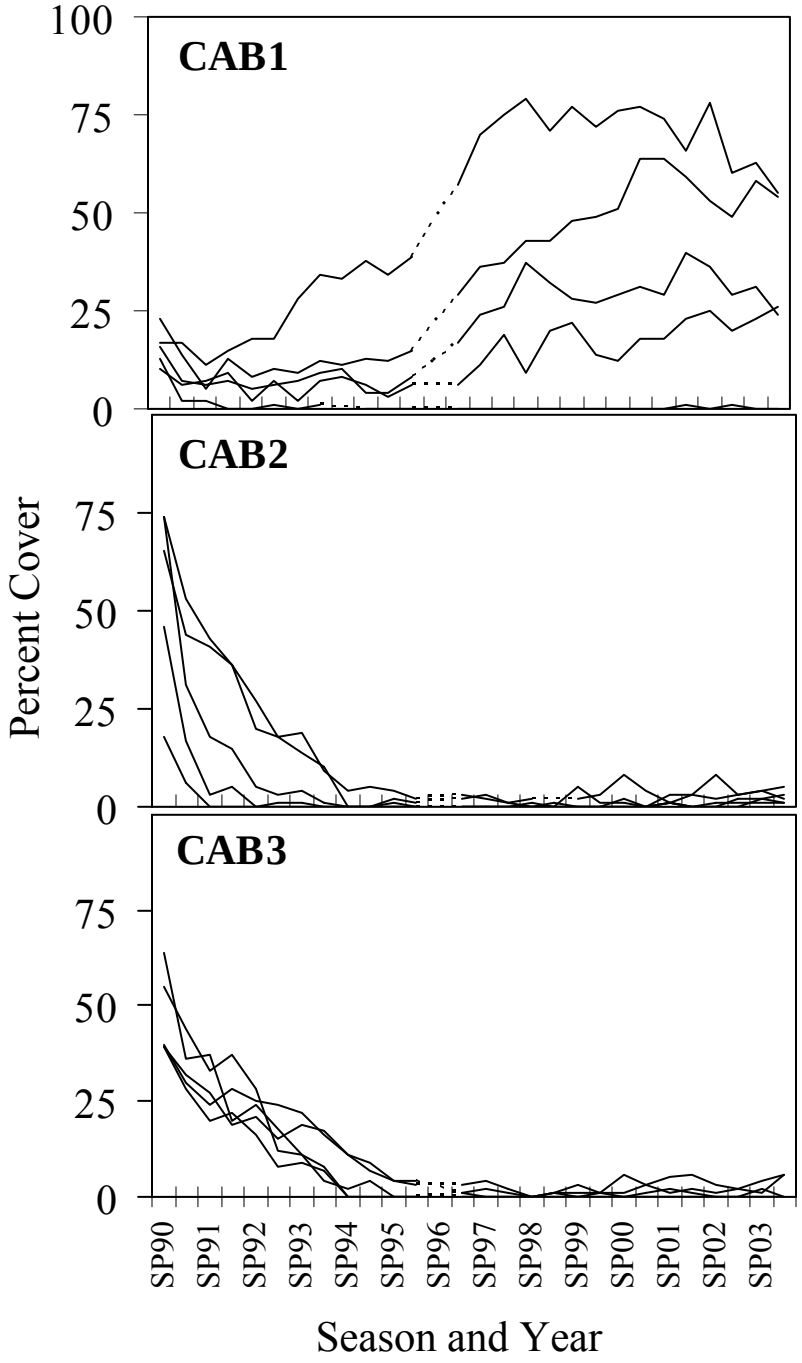


Figure 2.3: Photographs of the same area in Cabrillo National Monument Zone II rocky intertidal taken in (A) around 1962 (courtesy of Gary Davis, NPS) and (B) 2005.

A

B

Figure 2.4: Map of seven sites used in "Mussel Watch"-style water-quality monitoring program conducted by Cabrillo National Monument beginning in August 2004. At each site, three cages (locations represented by open circles), each containing 16 mussels, are being outplanted for three-month periods over one year. CAB1= Zone I, a high use area; CAB2 = Zone II, an intermediate-use area; CAB3=Zone III, a low-use area that has been closed to visitors since 1996.

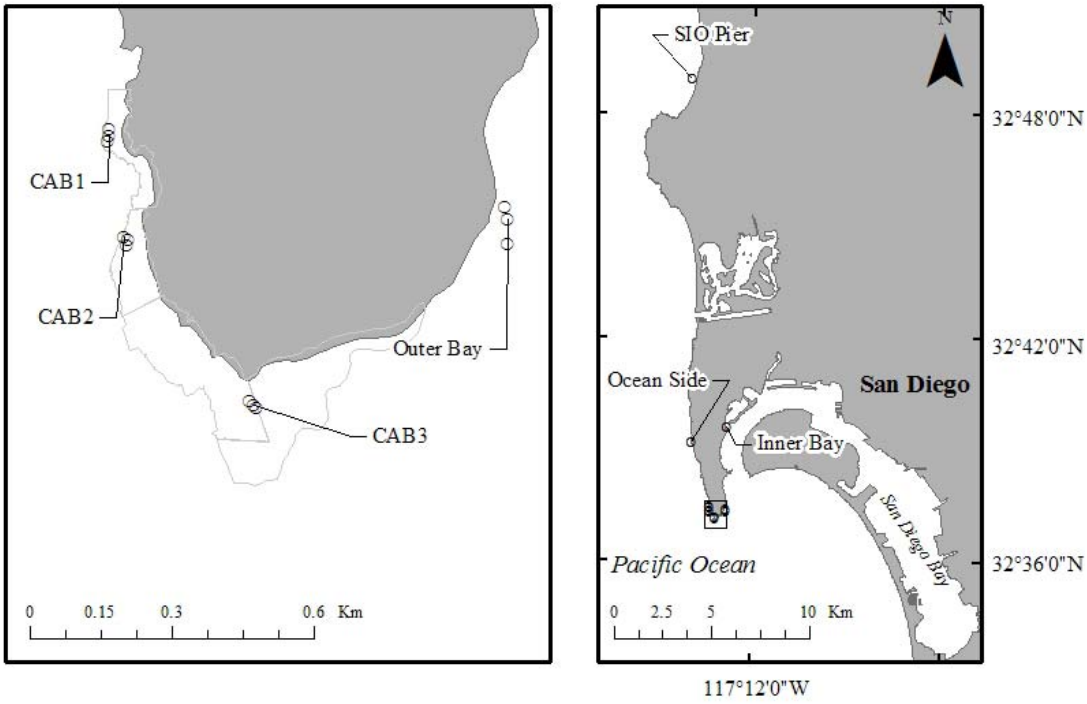


Figure 2.5: Change in length and weight of mussels outplanted for three months at sites on Point Loma and La Jolla as part of a "Mussel Watch"-style water-quality monitoring program conducted by Cabrillo National Monument beginning in August 2004. Mussels were weighed and measured before and after outplanting. The first quarter was from August 27 through November 26, 2004; the second quarter was from December 10, 2004 through March 10, 2005. Locations of sites are shown in Figure 2.4. (A) Percent change in maximum length of mussels for both quarters. (B) Percent change in weight of mussels for the second quarter (was not determined for first quarter). Error bars represent ± 1 SE. CAB1= Zone I, a high use area; CAB2 = Zone II, an intermediate-use area; CAB3=Zone III, a low-use area that has been closed to visitors since 1996.

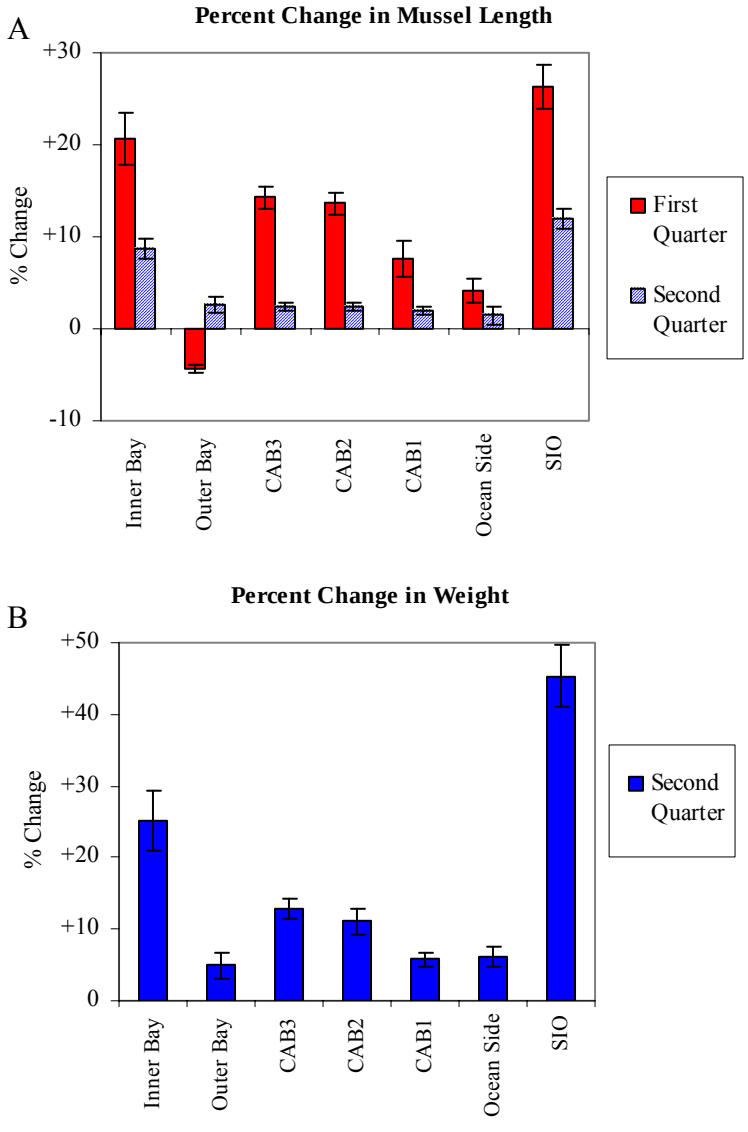


Figure 2.6: Preliminary determination of the amount of PCBs of mussels outplanted for three months (August 27-November 26, 2004) at sites on Point Loma and La Jolla as part of a "Mussel Watch"-style water-quality monitoring program conducted by Cabrillo National Monument beginning in August 2004. Locations of sites are shown in Figure 2.4. The control samples were taken directly from SIO Pier and were never placed in mussel cages. Error bars represent ± 1 SE. CAB1= Zone I, a high use area; CAB2 = Zone II, an intermediate-use area; CAB3=Zone III, a low-use area that has been closed to visitors since 1996.

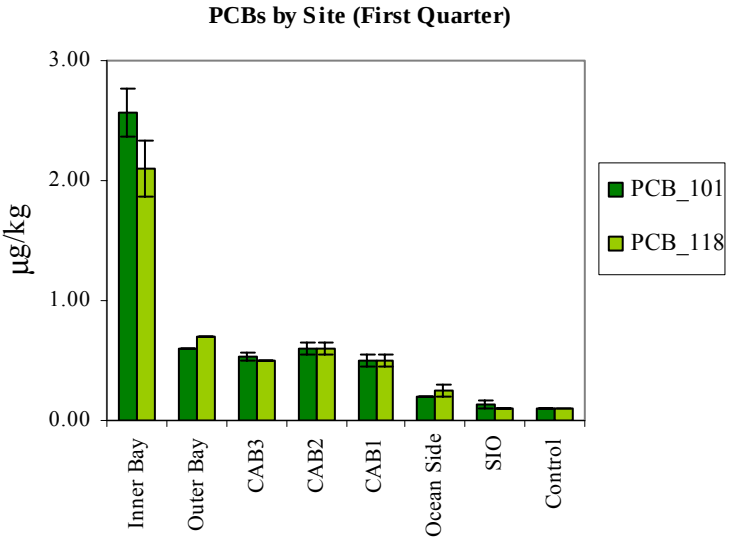
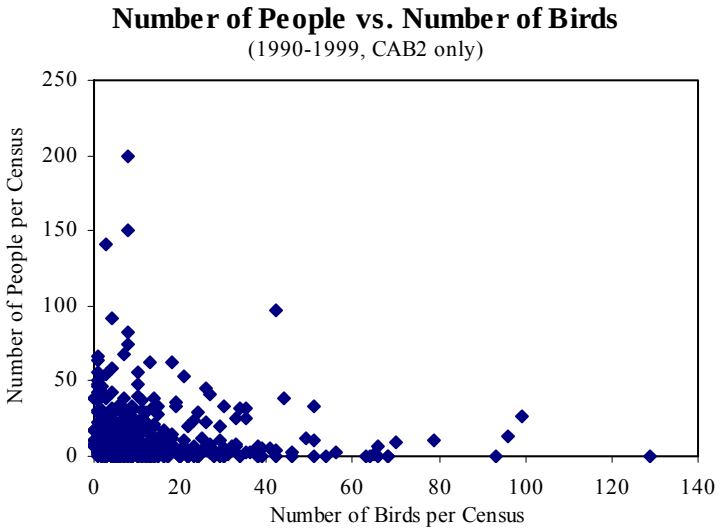


Figure 2.7: Average number of shorebirds and visitors counted during 456 one-hour censuses taken during low tides between 1990 and 1999 in Cabrillo National Monument. (A) Relationship between the number of visitors and the number of birds in CAB2. (B) Average number of people and birds found in each zone of the park. Error bars represent ± 1 SE. CAB1= Zone I, a high use area; CAB2 = Zone II, an intermediate-use area; CAB3=Zone III, a low-use area that has been closed to visitors since 1996.

A



B

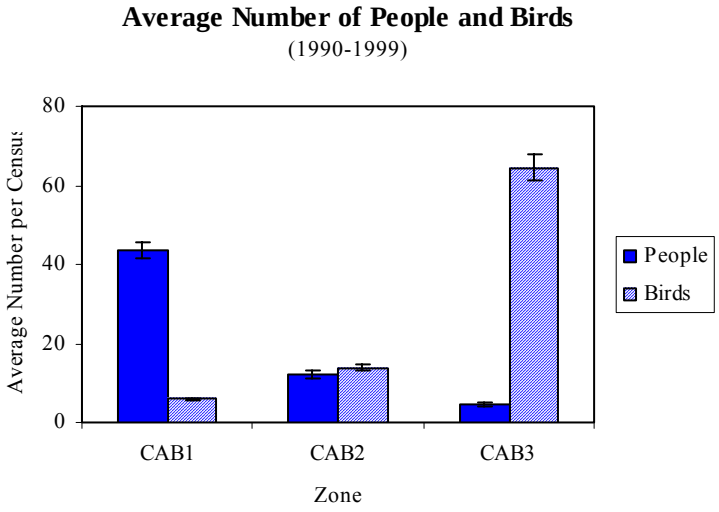
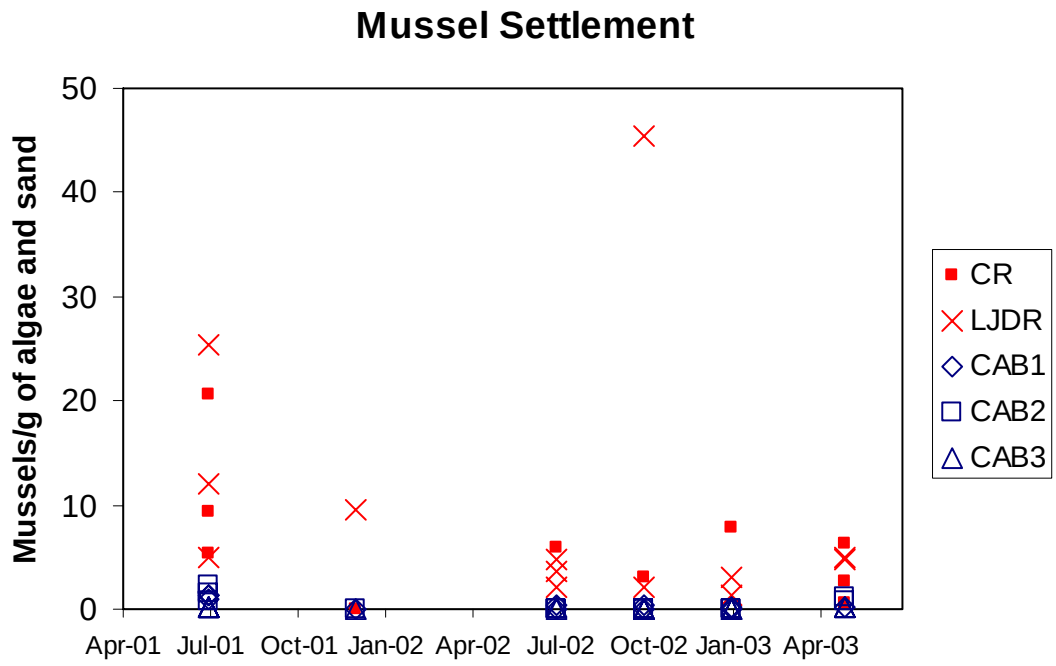


Figure 2.8: Number of mussel settlers (<2 mm) found per gram of algae and sand collected from five sites in San Diego County, California. Each symbol represents a single replicate for a site and time; up to three replicates are shown per site. CR=Cardiff Reef, LJDR=La Jolla Dike Rock, CAB1=Cabrillo National Monument Zone I, CAB2=Cabrillo National Monument Zone II, CAB3=Cabrillo National Monument Zone III.



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CHAPTER III

Spatial and temporal scales of California intertidal mussel dynamics: the value of long term monitoring.

ABSTRACT

The Multi-Agency Rocky Intertidal Network (MARINe) is a collaborative effort to conduct long-term (decades), regional-scale (500 km) ecological monitoring in central and southern California. As part of this effort, the cover of mytilid mussels was monitored semiannually in 452 fixed quadrats at 49 sites on the mainland and islands of the region. Some island sites have been monitored for 20 years, although most mainland sites were established over the past 10 years. Correlation analysis and mapping was used to determine the spatial coherence of mussel dynamics. These analyses demonstrate that intertidal mussel dynamics are highly variable and extremely complex. In most seasons, quadrats within a site are most correlated to each other, with little larger-scale structure. There are a number of years where both declines and increases in mussel cover within quadrats are independent of quadrats less than one kilometer away. However, during some notable seasons, a strong regional pattern is evident. For example, during spring and fall 1997, declines in mussel cover were consistent across a large scale (100-300 km); these declines are likely related to damage to mussel beds from large storms in the area at that time. Between spring 2003 and fall 2003, mussel cover increased across 150 km stretches of

shoreline. These increases were likely due to large-scale recruitment events and/or widespread conditions favorable to recruitment and growth of mussels in the region. These results demonstrate the importance of interannual variability and rare events in understanding the mechanisms structuring intertidal populations.

INTRODUCTION

Understanding how populations vary over different scales of space and time is a fundamental step in identifying the mechanisms that structure them (Levin 1992). Once pattern is identified, cross-correlation with suspected environmental and biological factors can be used to focus hypothesis testing and experimentation on appropriate spatial and temporal scales (Levin 1992, Koenig 1999). In addition, effective resource management strategies, especially spatially-based ones such as the design of marine reserves, should target populations and communities of interest at the appropriate scale. Moreover, assessments of acute human disturbances, such as oil spills, benefit from an understanding of natural variability at many potential spill sites.

The Multi-Agency Rocky Intertidal Network (MARINe, www.marine.gov) is a monitoring program that has compiled a large-scale (over 500 km), long-term (some sites since 1982) dataset depicting mussel cover dynamics at multiple sites in central and southern California. This collaborative program includes government, academic, and private institutions, which allows for many sites to be monitored concurrently. This continuous dataset provides an opportunity for analyses of regional mussel dynamics across an unprecedented spatial and temporal scale.

Although the dynamics of mytilid mussel beds through space and time have been the subject of many ecological studies for decades (e.g., Coe 1956, Dayton 1971, Paine 1974), research has usually been limited to small spatial scales (meters to tens of meters) for short durations (a year to three years). Only a few studies have examined distribution, trends, and scaling in mytilid mussel populations over long (>7 years) time scales (Paine and Levin 1981, 16 km for 10 years) or over a larger (>16 km) spatial gradients (Paine and Levin 1981, Aguilar Rosas et al. 1988, McKindsey and Bourget 2000, Kostylev and Erlandsson 2001). In addition to species of *Mytilus*, researchers have monitored brown mussel (*Perna perna*) populations in South Africa to determine spatiotemporal patterns in recruitment (Harris et al. 1998), complexity (Lawrie and McQuaid 2001, 10 cm up to 25 km for 5 months), and abundance dynamics (Dye 1998a, 1998b). These studies of both *Mytilus* spp. and brown mussels have demonstrated a high degree of patchiness and variability in mussel dynamics over scales ranging from centimeters to kilometers and months to years. The MARINe program has amassed a rare dataset of mytilid mussel cover that concurrently incorporates many (49) sites over an unprecedented spatial scale (>500 km) and over a continuous long time period (7 to 17 years).

The population structure of marine animals can be influenced by multiple factors including oceanic variability, which can affect recruitment success over large areas. Therefore, shorter studies that fail to incorporate critical events such as recruitment pulses can generate conclusions that are trivial in terms of the mechanisms that structure populations over time, reveal dynamics that are highly context-

dependent, difficult to generalize from, and which provide little predictive ability (Noda 2004). It is often challenging to study populations over large spatial scales and for long time periods, because of limited resources and pre-determined grant and graduate student cycles (Schoch and Dethier 1996, Dye 1998c). Therefore, where data are available, it is worthwhile to examine systems with a wider observational lens in order to develop hypotheses that can be tested using targeted and manipulative studies (Dye 1998b).

In this study, I examined the spatial coherence of changes in mussel cover by comparing the autocorrelation of sites separated by distances varying from meters to over 500 kilometers. This spatial structure was examined over many years to determine how it varies through time. These analyses were used to address the following questions: Are the dynamics of individual quadrats or individual sites independent of those near them? How do the dynamics of mussel cover vary over time? Are there large-scale patterns or events that lead to large, coherent changes in cover across the region? Are there particular sections of regions, such as islands, northern mainland, or southern mainland sites, which behave similarly over time? Can hypotheses be formed about the underlying dynamics of mussel populations using the determined spatial scales of coherence?

METHODS

The Marine Monitoring Protocol

MARINe is a collaborative monitoring program that was formed from existing separate but similar intertidal monitoring programs in 1997. Sites are monitored by multiple groups using techniques that have been rigorously standardized and the resulting data are contributed to a centralized database. Currently, 23 governmental, academic, and private organizations participate in MARINe, with six groups monitoring 57 California sites distributed from San Luis Obispo County to San Diego County, including most of the eight offshore southern California islands.

Although MARINe monitors many taxa using several techniques, this study focused on a single species and a single technique: mussels in photoplots. These analyses included 49 sites, 26 on the mainland and 23 on the southern California Channel Islands (Figure 3.1 and Table 3.1), spanning over 500 kilometers of shoreline. The sites were originally established at different times (as early as spring 1983 and as recently as fall 1999) and continue to be monitored by investigators at six different institutions: the National Park Service (Channel Islands National Park and Cabrillo National Monument), California State University at Fullerton, the University of California Los Angeles, the University of California Santa Barbara, and the University of California Santa Cruz. All sites are exposed or semi-exposed rocky benches, with varying amounts of boulders. Table 3.1 lists all of the MARINe sites and their assigned abbreviations to be used in this paper.

Within each site, five fixed quadrats were selected to target bed-forming mussel populations. At three sites, a different number of quadrats were used (see Table 3.1), and a total of 452 quadrats were included in these analyses. Quadrats (25 cm x 75 cm) were marked with either Z-Spar epoxy or stainless steel bolts to ensure accurate relocation during field assessments. Each quadrat was photographed twice per year, in spring and fall.

The resulting photographs were analyzed by projecting 100 evenly spaced points across the image and recording the organism or substrate under each point, resulting in percent cover data. Throughout the study period all monitoring groups scored mussel cover, regardless of whether another organism was overlying them. Since most mussel beds in this region consist of only one or two layers (i.e., most mussels were attached directly to the substratum), this two dimensional approach was not confounded by multiple layers of mussels.

In most cases, groups did not differentiate between different species of mytilid mussels. In this region, *Mytilus californianus* and *M. galloprovincialis* coexist, although the former is much more abundant in exposed habitats such as the sites studied here. Therefore, the majority of mussels found in study plots were *M. californianus*.

Statistical Analysis

The raw trends in mussel percent cover for each site were plotted on the same scale using the mussel percent cover data for each quadrat. In those cases where data

were missing, values were filled by linear interpolation through the two values adjacent to the missing one. When the missing value was at the last season of the time series it was left blank.

Since the quadrats were fixed in the same place over time, and started with different but non-random initial cover values, the rates of change, rather than the actual cover of mussels was compared in this analysis. Rates of change were calculated by first standardizing each quadrat to its maximum value:

$$x_i^{'} = \frac{x_i}{x_{\max}}$$

where x_i is the percent cover of mussels in a quadrat during season i, $x_{\max}$ is the maximum percent cover of mussels in the quadrat at any point in the time series, and $x_i^{'}$ is the standardized percent cover, which is bound between 0 and 1. The rate of change was defined as the slope between adjacent seasons:

$$r_i = \frac{x_{i+1}^{'} - x_i^{'}}{t}$$

where r_i is the rate of change of mussel cover in a quadrat between season i and i+1 and $t = 1$ (each semiannual monitoring season was considered to be 1 unit of time).

In most cases, sites were established by haphazard selection from the known mussel habitat, which was defined as having an existing significant cover of mussels at the time of establishment. Therefore, most sites were established in high mussel cover for the region (69.6% $\pm$ 24.7%, mean $\pm$ 1 SD; 0.85 $\pm$ 0.19 with cover standardized to the maximum value). Since the starting values were artificially high and are percentage values, there was a bias towards sites showing declines in mussel cover over time. For example, a site starting at 70% cover could only increase by 30%, but could decline by as much as 70%. This resulting bias is not eliminated by normalizing the data, and should be considered while interpreting them.

The degree of spatial coherence of the rates of change was examined using spatial correlation analysis. Because sites had different starting dates, there were varying numbers of sites monitored during any given season. In order to make the correlograms comparable and maximize the number of sites included in the analysis, the dataset was divided into two time series: a "short" (fall 1996 to fall 2003) and a "long" (fall 1986 to fall 2003) series. Sites that were not established before the defined time periods were excluded from the analysis (Table 3.1). The longer time period only includes sites on islands.

A spatial correlogram was constructed separately, following the technique described in Legendre and Legendre (1998), for each sampling event. One series of correlograms was created for each of the long and short time series. Using latitude and longitude for quadrats (or sites when exact quadrat positions were unavailable), a matrix of distances between every possible pair of quadrats was created. These

distances were lumped into classes. The appropriate number of distance classes for comparisons was determined using Sturge's Rule (Legendre and Legendre 1998):

$$C = 1 + 3.3 \log_{10}(m)$$

where C is the number of classes and m is the number of paired sites used in the analysis. For the shorter time series, C=16 distance classes. In order to maximize the comparability between the two time series, the same distance classes were used for the longer time series, which had a smaller maximum distance between pairs, resulting in 8 distance classes.

Moran's I, a measure of the degree of autocorrelation (Legendre and Legendre 1998), was calculated for each distance class and plotted against distance class to create a correlogram. The significance for the correlogram was determined using the approach of Cliff and Ord (as described in Legendre and Legendre 1998). A p value and $\pm$95% confidence intervals were calculated for I for each distance class, which was evaluated using a Bonferroni-corrected critical value. If any value of I was found to be significant, the whole correlogram was considered significant. Individual significance values were then evaluated.

Since correlograms should be compared to spatial representations of the data for accurate interpretation (Legendre and Legendre 1998), the rates of change of mussel cover, averaged by site, also were graphed on a contour plot using Surfer Software.

The interpolation was done by kriging. For selected seasons, maps were made to demonstrate the spatial relationships among the sites using ArcMap (ESRI).

RESULTS

Mussel rates of change over time

The patterns of mussel percent cover at the sites examined over time reveal a dynamic and complicated system with much site- and quadrat-level variation (Figure 3.2 and Figure 3.3). A number of quadrats experienced sharp declines, where mussel cover dropped by 50% or more in a single season (e.g., WHPT, Figure 3.2). Other quadrats experienced a more gradual decline (e.g., ALEG, Figure 3.2). At some sites, the recovery of a quadrat after a decline occurred very quickly, within 2 years (e.g., OCC, Figure 3.2), but at others recovery was delayed (e.g., ARHO, Figure 3.2) or non-existent (CAB3, Figure 3.2). On the islands, where there is a much longer time series, a number of sites experienced multiple oscillations in mussel declines and recoveries (e.g., ANME, Figure 3.3).

The degree of coherence among quadrats also varied among sites. At several mainland sites, individual quadrats appeared to follow their own independent trajectories (ALEG, Figure 3.2), but at most sites, the observed trajectories were quite similar (TRIS, Figure 3.2). At other sites, the degree of coherence among the quadrats changed with time; sometimes the quadrats behaved similarly and at other times individual quadrats experienced declines unique to the site (ARHO, Figure 3.2). Similarly, some neighboring sites appeared quite similar (PSN, CAY, HAZ, 65 km

maximum distance, Figure 3.2) whereas others behaved completely different (CRCO and SHCO, less than 5 km apart, Figure 3.2). On the islands, there appeared to be some coherence among sites located on the same island (e.g., SRNW and SREP, Figure 3.3), although there were cases where this did not occur (e.g., SRFP, Figure 3.3).

Taken in total, there appeared to be complex spatial pattern in the mussel cover trends, with a high level of context-specific structure at the site scale, but a discernable pattern on a larger scale. Most notably, there appeared to be seasons or time periods when multiple sites experienced a decline, especially in 1997, and other periods when multiple sites experienced an increase, such as in 2003 (Figures 3.2 and 3.3).

Spatial correlograms over time

This highly variable system can be generalized for each season using a spatial correlogram of the rates of change of mussel percent cover (Figure 3.4 and Figure 3.5). The sites in the short time series had a diversity of spatial structures depending on the season of correlogram comparison (Figure 3.4). In one season (fall 1998 and spring 1999), there was no discernable spatial structure at any scale and the correlogram was not significantly different from 0 (at the $p<0.003$ level, the Bonferroni-corrected significance level for this dataset). This was a period of general increase of mussel cover across the region, with 23 out of 48 sites showing increasing mussel cover from spring 1998 to fall 1998 and fall 1998 to spring 1999 and only 13 and 7 respectively showing declines. In most years, rates of change in mussel cover

within quadrats at the same site (<1 km apart) varied together and were significantly autocorrelated, although in spring and fall 1998, there was no relationship in the dynamics of adjacent quadrats. In many seasons, this autocorrelation at the very smallest spatial scale was the most important level of spatial coherence, since little or no coherence in mussel dynamics were observed between quadrats in larger distance classes.

Certain seasons exhibited notably more spatial structure than others. Between spring and fall 1997, mussel populations at the smaller-scale (<10 km) and larger-scale (200-300 km) stretches of coastline appeared to change in a similar manner. During both spring 1997 to fall 1997 and fall 1997 to spring 1998, many sites experienced a dramatic decline in mussel cover over a short time period. In the first period, 30 out of 49 sites exhibited declines in mussel abundance, while only 4 sites increased; in the second period, mussel cover declines at 29 out of 49 sites whereas 7 increased. The resulting spatial correlogram resembles a dampened sine wave, suggesting that patches approximately 200-300 km apart are separated by areas in between that show different dynamics (Legendre and Legendre 1998). These patches likely correspond to areas of declining cover centered at 33.2^{o}N and 35.2^{o}N, which were separated and flanked by areas of little change or increasing mussel cover (Figure 3.6). The sites on the edge of the spatial range of this study (500 km apart) mostly showed increased in mussel cover, leading to a high degree of correlation at this largest scale. Similarly, between fall 1997 and spring 1998, patches of declining rates of mussel cover existed, but these were smaller in spatial scale (<100 km apart)

than in the previous season (Figure 3.4). These patches of decline during that period were centered at 33.5°N and 34.5°N (Figure 3.6).

Much like this period of widespread and rapid decline associated with increased spatial structure, a period of increasing mussel cover occurred between spring 2003 and fall 2003 which also was a period of more large-scale spatial structure. During this period, 20 out of 35 sites showed increases in mussel cover, whereas 9 declined. In this times period, patches approximately 150 km apart, centered at 33.5°N and 35.25°N, experienced increased mussel cover. In other seasons during the short time series, weaker but significant spatial autocorrelation occurred during several years, usually in a characteristic damped sine pattern which corresponds to patches on the contour plot (Figure 3.4).

The longer time series, which included only island sites, also had high season to season variability in spatial structure (Figure 3.5). In 11 of 33 seasons, there was no significant spatial structure at any spatial scale ($p<0.006$, the Bonferroni-corrected significance level for this dataset) and all correlograms were not significant. In the majority of seasons, there was significant autocorrelation among quadrats within a site. However, in 14 of 33 seasons in the series, quadrats within a site varied independently of each other and there was no significant autocorrelation at the smallest spatial scale (<1 km).

As in the short time series, during a few seasons rates of mussel change across the area indicated larger-scale spatial structure. For example, from spring 1991 to fall 1991, patches at 125 km appeared to vary together (Figure 3.5). During this period,

mussel cover on Anacapa and San Miguel Islands are generally declining, while cover on Santa Rosa and Catalina Island are generally increasing, leading to alternating patches of similar size (Figure 3.7 and Figure 3.8). From fall 1991 to spring 1992, sites less than 50 km apart and greater than 125 km were highly autocorrelated (Figure 3.5). During this period, most sites were increasing, not changing or declining slightly, but sites on Anacapa Island were declining more sharply (Figure 3.8). Since Anacapa Island is near the middle of the study region, this led to a dip in correlation at the 75 km scale, the approximate distance between Anacapa and most other sites.

Rates of change in mussel cover during two seasons (fall 1992 to spring 1993 and fall 1995 to spring 1996) demonstrated a different type of spatial pattern. Instead of alternating patches of similar and different values, a gradient pattern was evident; at the smaller spatial scales, autocorrelation was highest, and then declined through the higher spatial scales. Both of these time periods were characterized by increasing mussel cover at the southeastern sites, and generally unchanging cover in the northwestern sites (Figure 3.7).

DISCUSSION

MARINe is a successful model in using collaboration to monitor a large area for a long period of time. By working together, the monitoring groups have been able to increase the value of their individual, smaller datasets and place their specific areas into a regional context. It would be very difficult for a single agency or lab group to support enough personnel to regularly monitor all of the sites encompassed in this

study. MARINe and similar monitoring programs allow us to determine appropriate scales for more focused and hypothesis-driven studies to determine the mechanisms that drive the abundance and distribution patterns of mussels and other coastal marine species in these complex systems.

Variability of mussel dynamics in space and time

Mussel dynamics in central and southern California vary among seasons on every spatial scale. Although quadrats were often autocorrelated at the site scale (<1 km), there were years when there was no coherence in mussel dynamics at this smallest spatial scale. Likewise, during some seasons neighboring sites were autocorrelated, but most of the time they were not. During periods when large-scale patches were detected, there were no consistent areas in the study area where mussel dynamics varied together. An exception was the Channel Islands, where sites on individual islands tended to have similar dynamics.

The changes in the spatial structure of central and southern California mussel dynamics with time could complicate interpretations of shorter studies of this system. Therefore, if the extent of a study was only a single year the resulting conclusions concerning mussel dynamics would defer depending on the particular year of the investigation. Dye (1998c) came to a similar conclusion when he divided 15 years of trend data for rocky intertidal organisms into 3-year segments; fewer than half of these segments yielded the trend determined by the analysis of the longer dataset.

Using scale to form hypotheses about mechanisms structuring mussel dynamics

Between fall 1997 and fall 1998, the same set of quadrats behaved similarly on less than 25-km and 100-km scales and six months later were essentially independent of each other at all spatial scales. This implies that in the first six months of that period, mechanisms determining mussel cover acted on larger spatial scales, while in the last six months local mechanisms were responsible for changes in mussel populations. Examining these differences between periods of large spatial structure and those of practically no spatial structure allows us to form hypotheses about the mechanisms driving the dynamics of mussel cover. Many of these hypotheses could be explored using correlative environmental data, but direct causation might be difficult to test experimentally for events that occurred in the past and may not continue in the present.

Simple predictions can be made of the patterns one would expect if different large-scale mechanisms structure mussel dynamics in the region. Examples of these large-scale mechanisms include large-scale temperature change, disease, variability in nutrients or productivity, storms, and regional recruitment events. Many of these proximal agents of change can ultimately be related to more complicated global climate change or ENSO cycles. If large-scale ocean warming manifested in a gradual and sustained trend in ocean temperature (at least on a decadal time scale) and was a main cause of changes in mussel cover, one would expect a more gradual and sustained change in mussel population dynamics. In contrast, a widespread mussel disease could lead to declines in mussel cover that spread to adjacent populations as

the disease vector works its way through the region. Other variations in oceanographic conditions, which could lead to changes in food and larval availability, are more likely to lead to less sharp changes in mussel populations. There is little evidence of any of these patterns in this dataset.

Major storms would likely hit large swaths of coastline at once, and resulting large waves could rip out parts of or whole mussel beds (Paine and Levin 1981), or the substrates to which the mussels are attached. These large sections of the coast would likely have a similar geographic orientation and level of protection from offshore islands, so that a storm coming from a specific direction would hit those areas. The resulting pattern would be sharp declines in mussel cover across large areas, with possible "shadows" where the coastline changes angle or is protected by the offshore islands. The source of declines would be relatively acute, and recovery would follow at various rates depending on local conditions. However, there are a number of reasons why mussel quadrats would not recover after a storm, such as lack of recruitment or vulnerability of the remaining exposed mussels.

The spatial structure in mussel dynamics during the period from spring 1997 to spring 1998 appears to be consistent with at least two large wave events in the region. Between spring 1997 and fall 1997 mussel cover in the area between PTFM and OLDS, in the lee of the northern Channel Islands, appeared to either remain the same or increase (Figure 3.8). This spatial pattern could have been formed by a storm or a series of storms coming from the west and hitting the coast at approximately 33.5^{o}N to 35^{o}N. The following season, the patches of mussel decline were closer together and

the area of decline shifted generally to the south. This could correspond to another large storm system, or alternatively could reflect increased vulnerability of the remaining mussel beds or differential recovery across sites.

There are records of large storms in the region during 1997. Most notably, in September 1997, Hurricane Linda was one of the largest storms on record in the eastern Pacific, with gusts in excess of 290 km/h (www.usatoday.com). This was predicted to be the first tropical hurricane to make landfall on the California coast since 1939, but it remained offshore. In the meantime, however, this hurricane delivered high surf and showers to the southern California region. After this storm, many of the piers in the area were closed for repairs (www.pierfishing.com). A storm of this magnitude could surely have led to declines in mussel cover across the region. Since mussels have a primarily sessile adult phase and the migration abilities of individual animals is extremely limited, increases in mussel cover must be due to either growth of existing individuals, the spreading out of existing patches of mussels, or successful recruitment (Paine and Levin 1981). Increases in mussel cover over spans of coastline could be an indicator of regional recruitment events, with the size of the increasing patches depending on the degree of successful larval transport between populations. Alternatively, connectivity can be quite low, but good environmental conditions could lead to successful recruitment classes across a broad area. Conversely, a complete lack of population connectivity or highly spatially variable environmental conditions affecting early life stages of mussels could lead to increases in cover only on the very smallest scale. This type of analysis will allow

determination of the scale of successful recruitment, which is defined here as the growth of an individual to a size where it can be counted in a photograph. Once this scale is determined, further studies can be designed to examine the contributions of larval transport, supply, and survival of the various early life-history stages to mussel abundance dynamics.

The year following the declines of 1997 appears to be a period of increasing mussel cover, and these two seasons both had little spatial structure as determined by the spatial autocorrelation analysis. This pattern might imply that although conditions were generally good for mussel recruitment and/or growth during this period, the sites were not experiencing increases in a coherent way. Spring 2003 was a year of increasing mussel populations, but it was also a year that exhibited greater spatial structure in this analysis. The calculated 150 km patches of shoreline could represent areas of higher population connectivity or patches of environmental conditions favorable to local recruitment (Figure 3.8). The average rate of change in the period beginning in spring 2003 was 0.035, while those beginning in spring and fall 1998 were 0.016 and 0.020 respectively. Although this result does not prove that the 2003 season experienced a larger recruitment event in both degree and spatial extent than 1998, it could guide further studies of mussel population connectivity.

Mechanisms that could lead to quadrat- or site-level change without larger-scale coherence include direct human effects (such as harvesting, trampling, and pollution), small-scale recruitment events, and local ecological interactions. Causes of declines

on a quadrat or site scale, once they are identified, might not be predicted using this sort of dataset and would need to be studied on a case by case basis.

Comparison to previous studies of spatiotemporal structure in mussel populations

Yearly variability in spatial coherence has been found to be quite high in South African rocky intertidal communities (Dye 1998c) and a number of other systems (Koenig 1999). Rocky intertidal dynamics, in particular, are quite unpredictable on local scales (Levin 1992, Schoch and Dethier 1996, Dye 1998b). In their surveys of mussels in Washington over a ten-year period, Paine and Levin (1981) found abrupt declines in mussel cover related to storms, year to year variability in the synchrony of these declines on a 16-km scale, and variability among winters in terms of their effects on mussel beds. They note that synchrony was related to the aspect and exposure of a shoreline, and that even on their small scale, there was considerable noise. A number of smaller-scale studies have shown that over distances from 10s of meters to centimeters, mussel beds are patchy and heterogenous (Kostylev and Erlandsson 2001, Lawrie and McQuaid 2001, Erlandsson and McQuaid 2004). McKindsey and Bourget (2000) also found more variability in community structure within sites than among sites along a 16-km stretch of shoreline.

Harris et al. (1998) monitored the recruitment of *Perna perna* in South Africa at sites over 1000 km apart over a period of 16 months and found significant variability at regional and local scales. Erlandsson and McQuaid (2004) examined the small-scale variability (10 m) of *Perna perna* recruits at three sites and found little spatial

structure in general, but considerable structure, dependent on adult cover, when only the largest recruits were considered

Application to marine management

Understanding the spatial scales of mussel declines and increases has great value to marine resource managers. For example, three sites (CAB1, CAB2, and CAB3) are located in Cabrillo National Monument, a small National Park within the City of San Diego. Mussel populations in the southern part of the park (CAB2 and CAB3) experienced a slow, gradual decline from the beginning of monitoring in spring 1990 until 1995, when populations crashed to levels close to 0. In the following eight years, there has been no recovery. Beginning in 1996, Park managers began a rigorous Tidepool Protection, Education, and Restoration program in an attempt to facilitate mussel recovery and offset the decline of this and other species, and instituted a closure of the third site (CAB3) to all visitors. At that time it was not clear if mussel declines were part of a larger regional trend or were due to a local process.

From the present analysis, it appears that the situation at Cabrillo was driven by local processes. Although the cause of this gradual decline remains unclear, comparisons of mussel cover trends at the Cabrillo sites with those observed for other central and southern California mussel populations reveal that during this period other sites demonstrated a higher level of resilience, with populations recovering from declines within two years. Given this knowledge, managers can focus on potential local sources of change, especially those that have affected CAB2 and CAB3, but not

CAB1 which is less than 1 km away and has experienced an increase in mussel cover over the same time period. In addition, further comparisons with sites such as STA, where gradual declines in mussel cover with no recovery also have been observed, could reveal similar stressors at the two distant locations.

On a regional level, this study could help guide scientifically-informed intertidal reserve design. From this analysis, it appears that local dynamics often are very important in structuring mussel dynamics, but with some distinct periods of spatial coherence at the 100 to 200 km scale. A network of reserves that are appropriately spaced to increase the probability that they would not be hit by the same storms would be a good strategy for protecting mussel and other rocky intertidal populations on a large scale. Perhaps choosing reserves that represent different shoreline orientations would also increase the chances that not all of the reserves in the area would be affected by large storm disturbance events at the same time.

Understanding the scaling of mussel populations has benefits for interpreting disturbances that mussels might experience in the future. For example, if a disease were to cause massive mortality in mussel populations along the coast, MARINe monitoring groups would be able to detect this different spatial pattern quickly, perhaps in one or two seasons. Although many future threats and impacts to mussel populations can not be predicted in the present, this existing program can help managers make informed decisions quickly as new conditions arise.

CONCLUSION

Most ecosystems are patchy in space and highly influenced by rare events in time. This seriously compromises many efforts to understand mechanisms driving the abundance and distribution of organisms. The present analysis demonstrates that intertidal mussel populations also exhibit a combination of patchy and noisy local dynamics imbedded in occasional events much larger in temporal and spatial scope. As such, it offers a precautionary note to small scale research performed over short periods and emphasizes the importance of time-series data collected over meaningful spatial and temporal scales. Collaborative regional monitoring of the spatial and temporal coherence of a population offers a valuable tool for discerning pattern, or the lack thereof, in this complicated coastal ecosystem.

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Table 3.1: List of MARINe sites included in current analysis of mussel dynamics in central and southern California. CABR=Cabrillo National Monument (National Park Service); CINP=Channel Islands National Park (National Park Service); CSUF=California State University, Fullerton; UCLA=University of California, Los Angeles; UCSB=University of California, Santa Barbara; UCSC=University of California, Santa Cruz. L=included in longer time series; S=included in shorter time series.

Surveying Group	Site Abbreviation	Site Name	Number of Quadrats	County	Island	Initiation Date	Site Latitude (°N)	Site Longitude (°W)	Time Series
UCSC/UCLA	ALEG	Alegria	5	Santa Barbara	Mainland	Spring 1992	34.467	-120.278	S
CINP	ANCR	Cat Rock	9	Ventura	Anacapa	Fall 1981	34.010	-119.420	S,L
CINP	ANME	Middle-East	3	Ventura	Anacapa	Spring 1982	~34.006	~-119.397	S,L
CINP	ANMW	Middle-West	5	Ventura	Anacapa	Spring 1982	34.006	-119.397	S,L
CINP	ANSFC	S Frenchy's Cove	5	Ventura	Anacapa	Fall 1982	34.010	-119.410	S,L
UCSC/UCLA	ARHO	Arroyo Hondo	5	Santa Barbara	Mainland	Spring 1992	34.473	-120.145	S
UCSC	BOA	Boathouse	5	Santa Barbara	Mainland	Spring 1992	34.554	-120.611	S
CABR	CAB1	Cabrillo I	5	San Diego	Mainland	Spring 1990	32.669	-117.246	S
CABR	CAB2	Cabrillo II	5	San Diego	Mainland	Spring 1990	32.668	-117.245	S
CABR	CAB3	Cabrillo III	5	San Diego	Mainland	Spring 1990	32.664	-117.243	S
UCSB	CARE	Cardiff Reef	10	San Diego	Mainland	Fall 1997	33.000	-117.279	
UCSC	CAY	Cayucos	5	San Luis Obispo	Mainland	Fall 1995	35.448	-120.950	S
CSUF	CRCO	Crystal Cove	5	Orange	Mainland	Fall 1996	33.571	-117.838	S
UCLA	CTBR	Bird Rock	5	Los Angeles	Catalina	Fall 1994	33.452	-118.488	S
UCLA	CTLH	Little Harbor	5	Los Angeles	Catalina	Fall 1994	33.385	-118.475	S
CSUF	DAPT	Dana Point	5	Orange	Mainland	Fall 1996	33.460	-117.715	S
UCSC	GPT	Government Point	5	Santa Barbara	Mainland	Spring 1992	34.443	-120.456	S
UCSC	HAZ	Hazard's	5	San Luis Obispo	Mainland	Fall 1995	35.281	-120.888	S
UCLA	MUSH	Mussel Shoals	5	Ventura	Mainland	Fall 1994	34.356	-119.441	S
UCSB	NANO	Navy North	5	San Diego	Mainland	Spring 1995	32.694	-117.253	S
UCSB	NASO	Navy South	5	San Diego	Mainland	Spring 1995	32.683	-117.250	S

Table 3.1 Cont.

UCSC	OCC	Occulto	5	Santa Barbara	Mainland	Spring 1992	34.881	-120.639	S
UCLA	OLDS	Old Stairs	5	Ventura	Mainland	Fall 1994	34.066	-118.998	S
UCLA	PCOV	Paradise Cove	5	Los Angeles	Mainland	Fall 1994	34.012	-118.792	S
UCSC	PSN	Point Sierra Nevada	5	San Luis Obispo	Mainland	Fall 1995	35.731	-121.316	S
UCLA	PTFM	Point Fermin	5	Los Angeles	Mainland	Fall 1999	33.707	-118.285	
CINP	SBLC	Landing Cove	5	Santa Barbara	Santa Barbara	Spring 1985	33.480	-119.030	S,L
CINP	SBSL	Sea Lion Rookery	5	Santa Barbara	Santa Barbara	Spring 1986	33.472	-119.031	S,L
CINP	SCFC	Fraser Cove	5	Santa Barbara	Santa Cruz	Fall 1994	34.060	-119.920	S
CINP	SCOC	Orizaba Cove	5	Santa Barbara	Santa Cruz	Fall 1994	34.050	-119.720	S
CINP	SCPH	Prisoner's Harbor	5	Santa Barbara	Santa Cruz	Fall 1994	34.020	-119.680	S
UCSB	SCRE	Scripps Reef	5	San Diego	Mainland	Fall 1997	32.871	-117.254	
CINP	SCSR	Scorpion Rock	5	Santa Barbara	Santa Cruz	Fall 1994	34.046	-119.547	S
CINP	SCTR	Trailer	5	Santa Barbara	Santa Cruz	Fall 1994	34.050	-119.550	S
CINP	SCWA	Willows Anchorage	5	Santa Barbara	Santa Cruz	Fall 1994	33.960	-119.750	S
UCSC	SHB	Shell Beach	5	San Luis Obispo	Mainland	Fall 1995	35.169	-120.696	S
CSUF	SHCO	Shaws Cove	5	Orange	Mainland	Fall 1996	33.545	-117.800	S
CINP	SMCH	Cuyler Harbor	5	Santa Barbara	San Miguel	Spring 1985	34.049	-120.336	S,L
CINP	SMCP	Crook Point	5	Santa Barbara	San Miguel	Spring 1985	34.041	-120.409	S,L
CINP	SMHP	Harris Point	5	Santa Barbara	San Miguel	Spring 1985	34.070	-120.360	S,L
CINP	SMOH	Otter Harbor	5	Santa Barbara	San Miguel	Spring 1985	34.050	-120.410	S,L
CINP	SREP	East Point	5	Santa Barbara	Santa Rosa	Fall 1986	33.937	-119.970	S,L
CINP	SRFP	Ford Point	5	Santa Barbara	Santa Rosa	Fall 1985	33.920	-120.090	S,L
CINP	SRFR	Fossil Reef	5	Santa Barbara	Santa Rosa	Spring 1988	33.990	-120.240	S,L
CINP	SRJL	Johnson's Lee	5	Santa Barbara	Santa Rosa	Fall 1985	33.910	-120.100	S,L
CINP	SRNW	NW Talcott	5	Santa Barbara	Santa Rosa	Fall 1986	34.010	-120.220	S,L
UCSC	STA	Stairs	5	Santa Barbara	Mainland	Spring 1992	34.731	-120.615	S
CSUF	TRIS	Treasure Island	5	Orange	Mainland	Fall 1996	33.513	-117.758	S
UCLA	WHPT	White's Point	5	Los Angeles	Mainland	Fall 1994	33.715	-118.320	S

Figure 3.1: Map of MARINe sites included in current analysis of mussel dynamics in central and southern California. Site abbreviations are listed in Table 3.1.

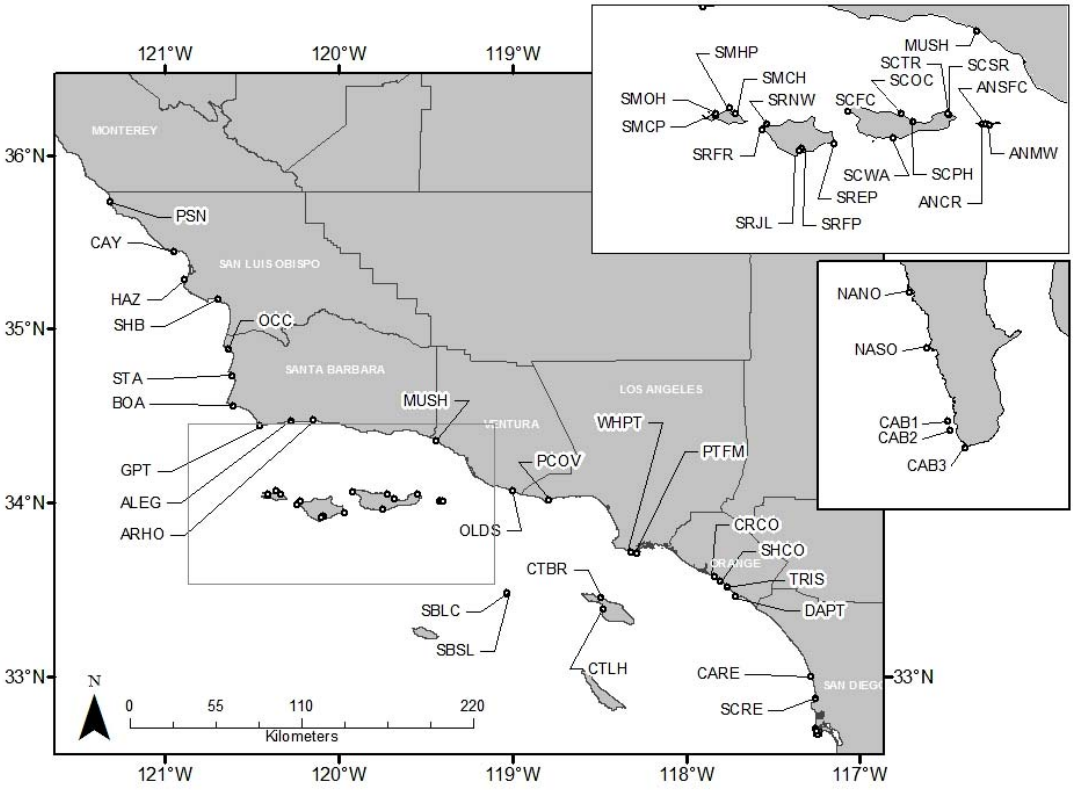


Figure 3.2: Raw trends of mussel percent cover, as determined from photographs, at mainland MARINe sites in central and southern California. Each graph represents a single site, and each line on the graphs represents a quadrat locate within that site. Sites are ordered from north to south. Missing values were filled by linear interpolation between adjacent seasons, and are represented by dotted lines. SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001). Site abbreviations are listed in Table 3.1.

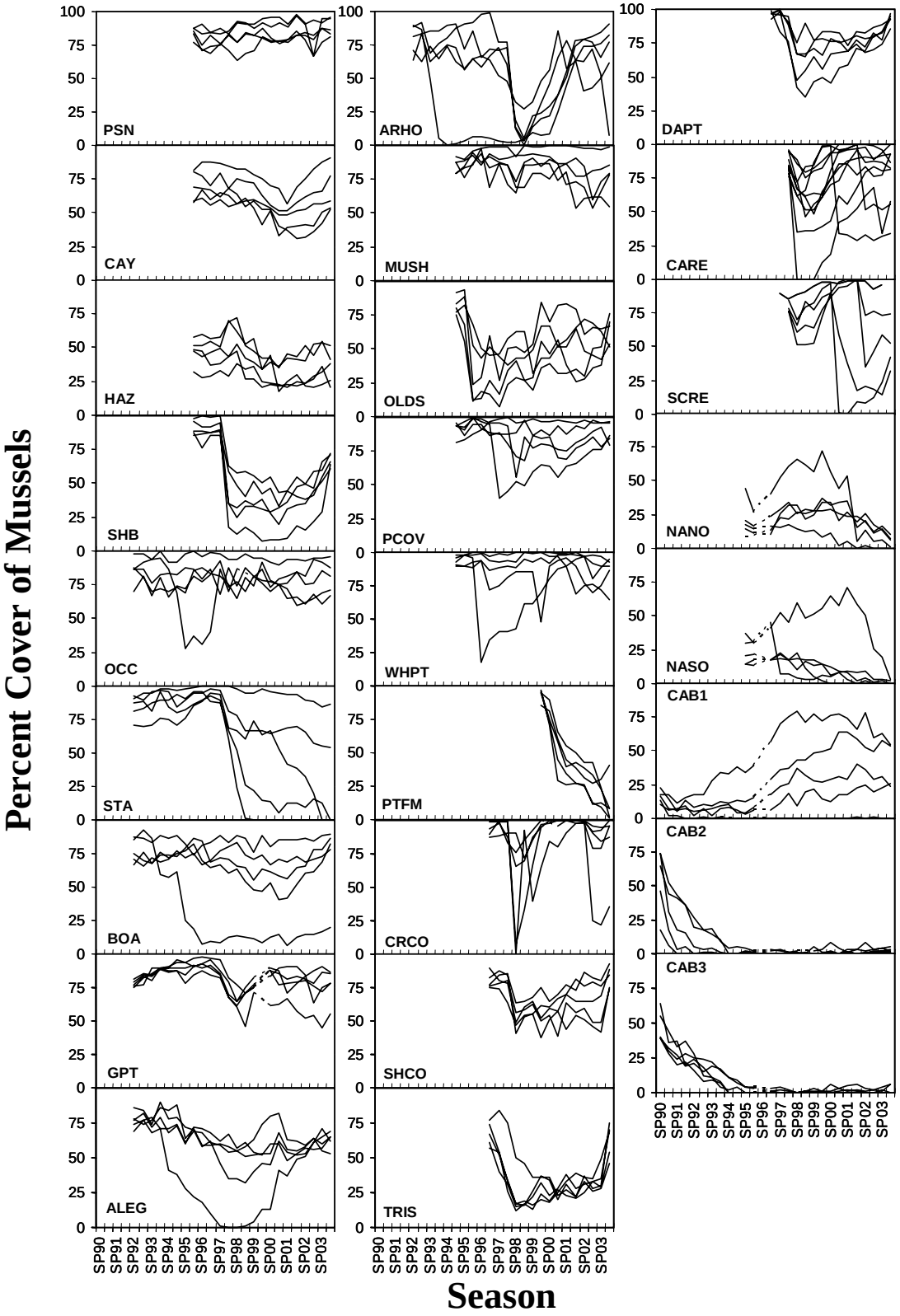

Percent Cover of Mussels
PSN
ARHO
DAPT
CAY
MUSH
CARE
HAZ
OLDS
SCRE
SHB
PCOV
NANO
OCC
WHPT
NASO
STA
PTFM
CAB1
BOA
CRCO
CAB2
GPT
SHCO
CAB3
ALEG
TRIS
0
25
50
75
100
SP90
SP91
SP92
SP93
SP94
SP95
SP96
SP97
SP98
SP99
SP00
SP01
SP02
SP03
Season

Figure 3.3: Raw trends of mussel percent cover, as determined from photographs, at island MARINe sites in central and southern California. Each graph represents a single site, and each line on the graphs represents a quadrat locate within that site. Sites are ordered from north to south. Missing values were filled by linear interpolation between adjacent seasons, and are represented by dotted lines. SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001). Site abbreviations are listed in Table 3.1.

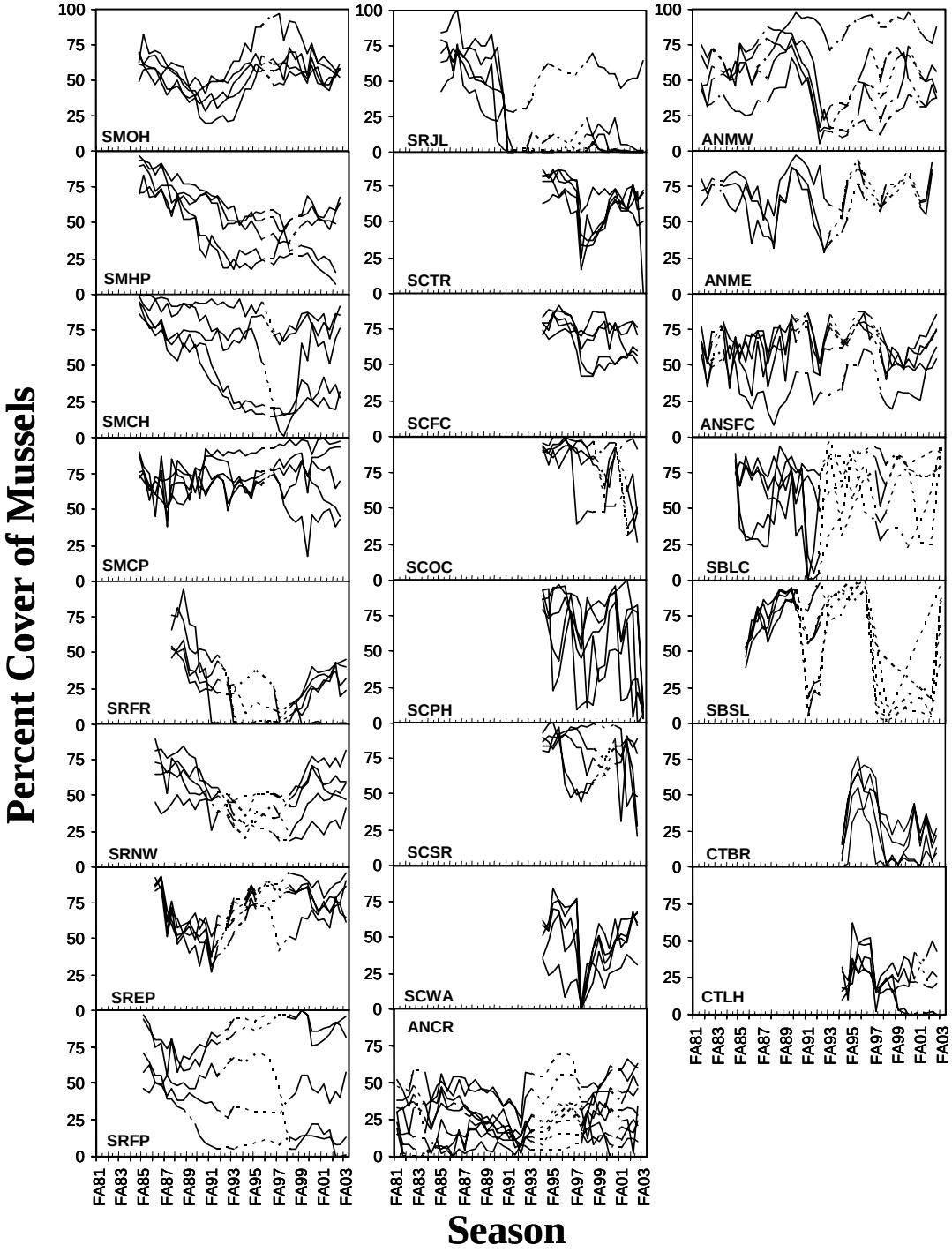

Percent Cover of Mussels
Season
SMOH
SMHP
SMCH
SMCP
SRFR
SRNW
SREP
SRFP
SRJL
SCTR
SCFC
SCOC
SCPH
SCSR
SCWA
ANCR
ANMW
ANME
ANSFC
SBLC
SBSL
CTBR
CTLH
0
25
50
75
100
FA81
FA83
FA85
FA87
FA89
FA91
FA93
FA95
FA97
FA99
FA01
FA03

Figure 3.4: Correlograms of mussel rates of change for all MARINe sites monitored during the short time series (fall 1996-fall 2003). On each graph, Moran's I (the spatial autocorrelation of rates of change of mussel cover) is plotted as a function of distance between quadrats, which was separated into 16 bins. Each graph represents the correlogram of the rates of change between the seasons listed. Bars indicate ± 95% confidence intervals. Filled in squares are significant (Bonferroni corrected, $p<0.003$). SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001).

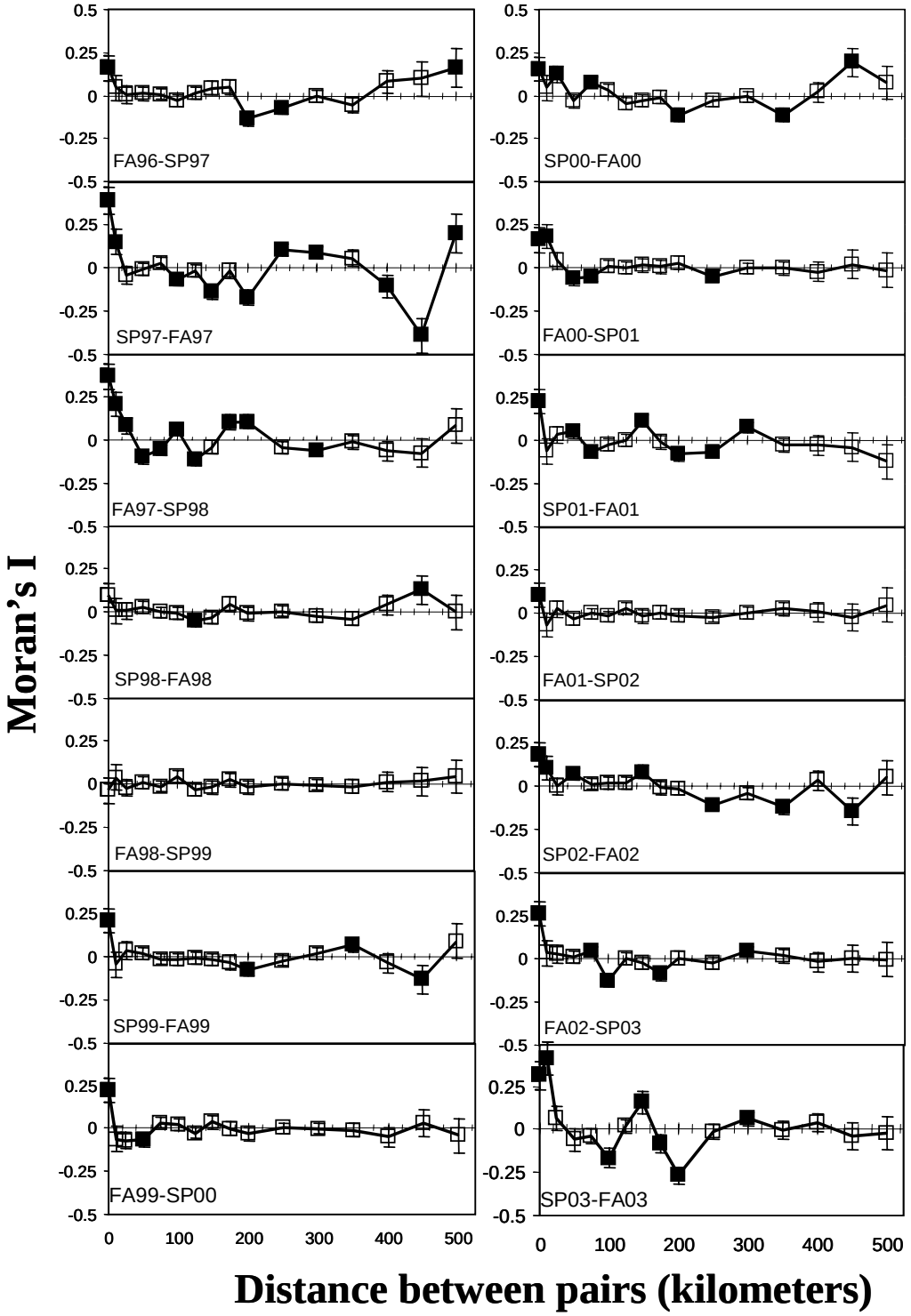
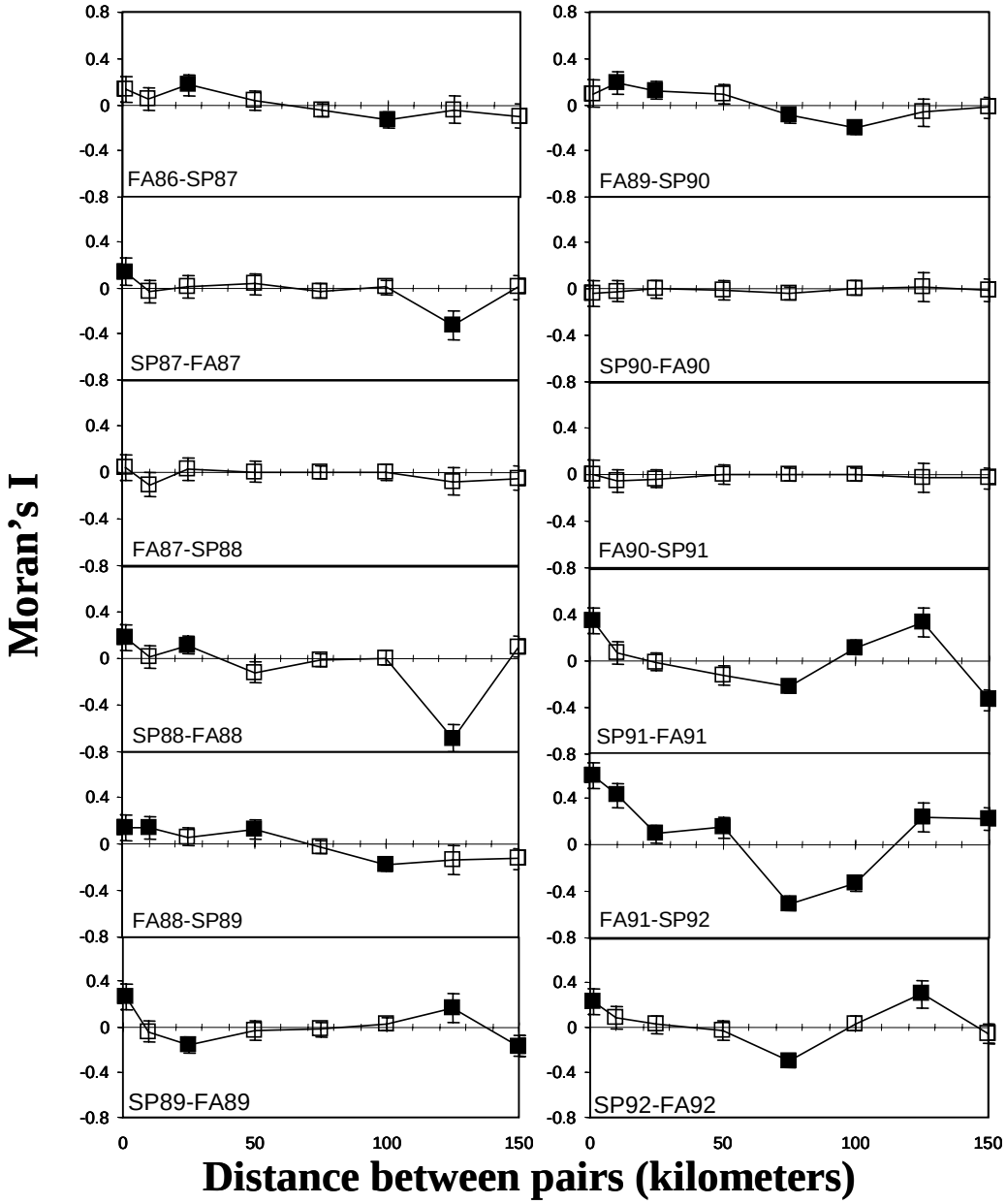

Moran's I
Distance between pairs (kilometers)
FA96-SP97
SP00-FA00
SP97-FA97
FA00-SP01
FA97-SP98
SP01-FA01
SP98-FA98
FA01-SP02
FA98-SP99
SP02-FA02
SP99-FA99
FA02-SP03
FA99-SP00
SP03-FA03
0.5
0.25
0
-0.25
-0.5
0
100
200
300
400
500

Figure 3.5. Correlograms of mussel rates of change for all MARINe sites monitored during the long time series (fall 1986-spring 1996). On each graph, Moran's I (the spatial autocorrelation of rates of change of mussel cover) is plotted as a function of distance between quadrats, which was separated into 16 bins. Each graph represents the correlogram of the rates of change between the seasons listed. Bars indicate ± 95% confidence intervals. Filled in squares are significant (Bonferroni corrected, $p<0.006$). SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001).



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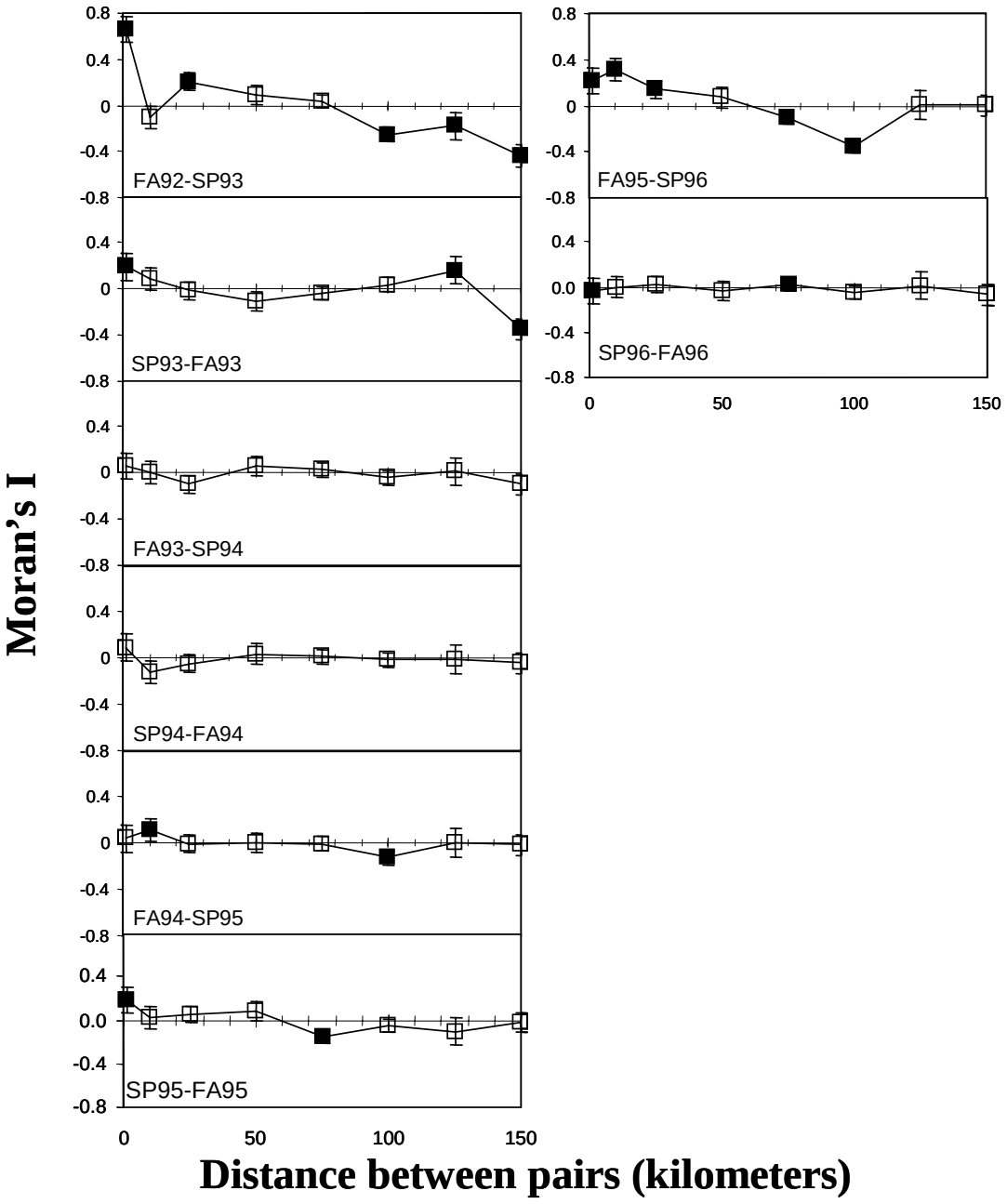


Figure 3.6: Contour plot of the rate of change of mussel cover plotted through space (latitude and longitude) and time (sampling season). Includes all MARINe sites monitored during the short time series (fall 1996-fall 2003). SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001).

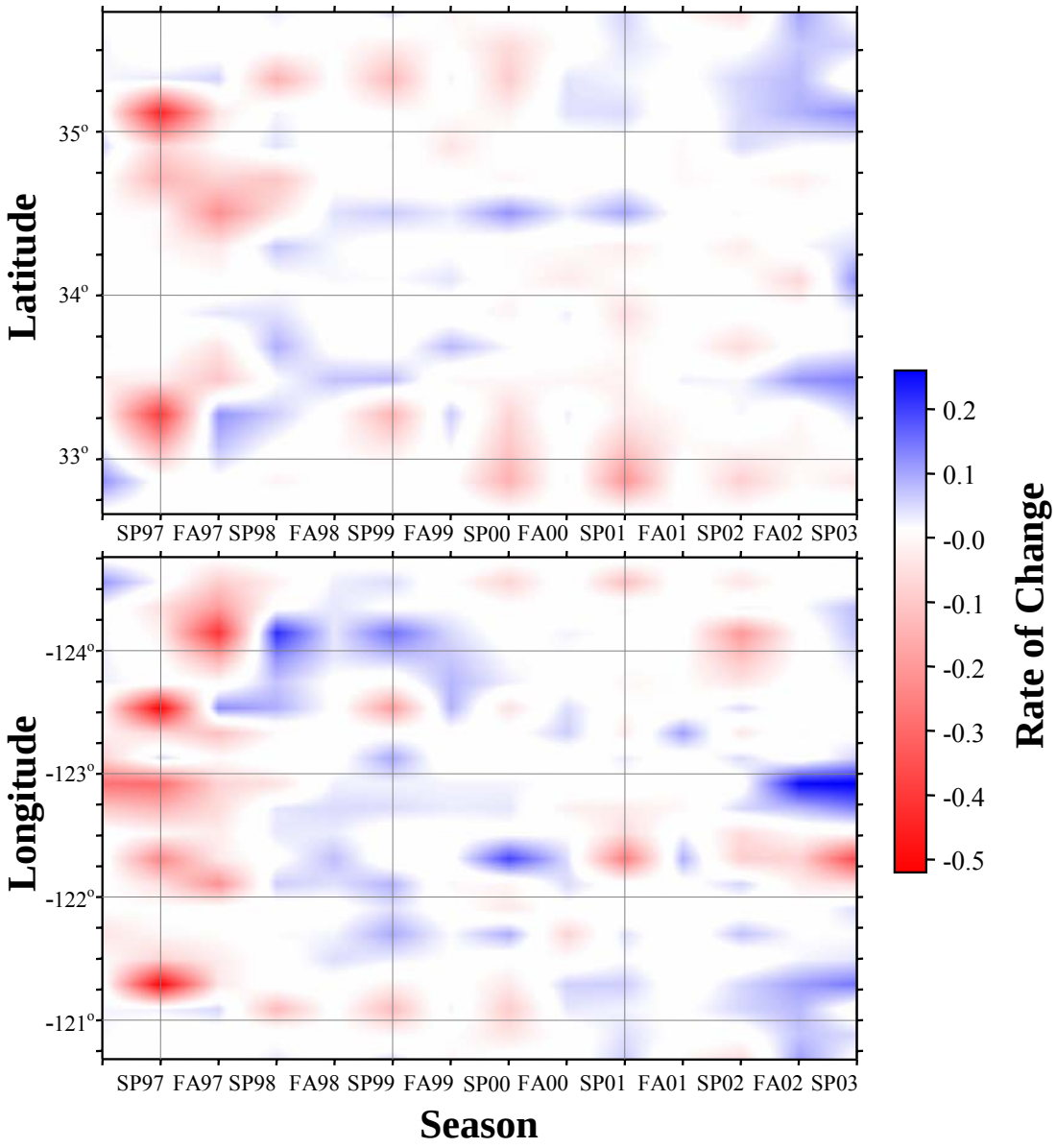


Figure 3.7: Contour plot of the rate of change of mussel cover plotted through space (latitude and longitude) and time (sampling season). Includes all MARINe sites monitored during the long time series (fall 1986-spring 2003). SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001).

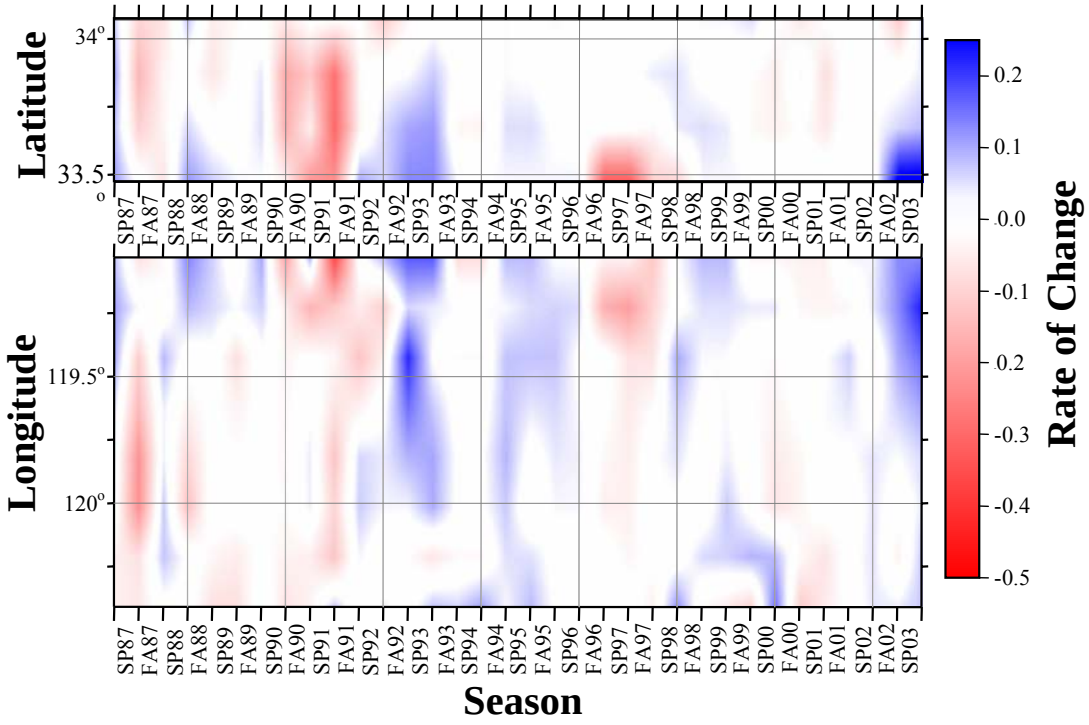
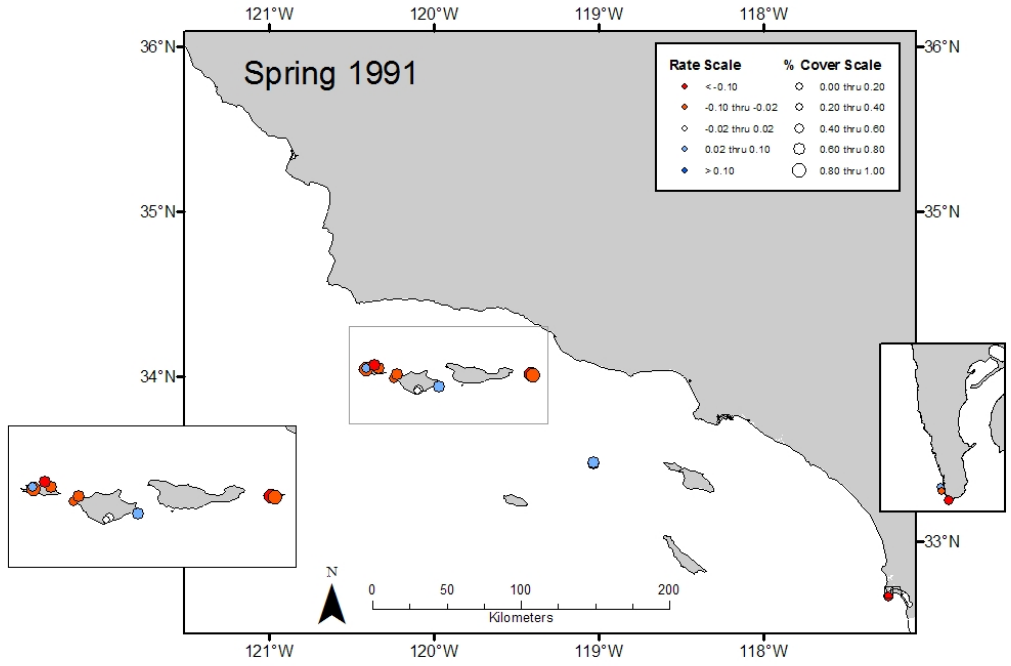
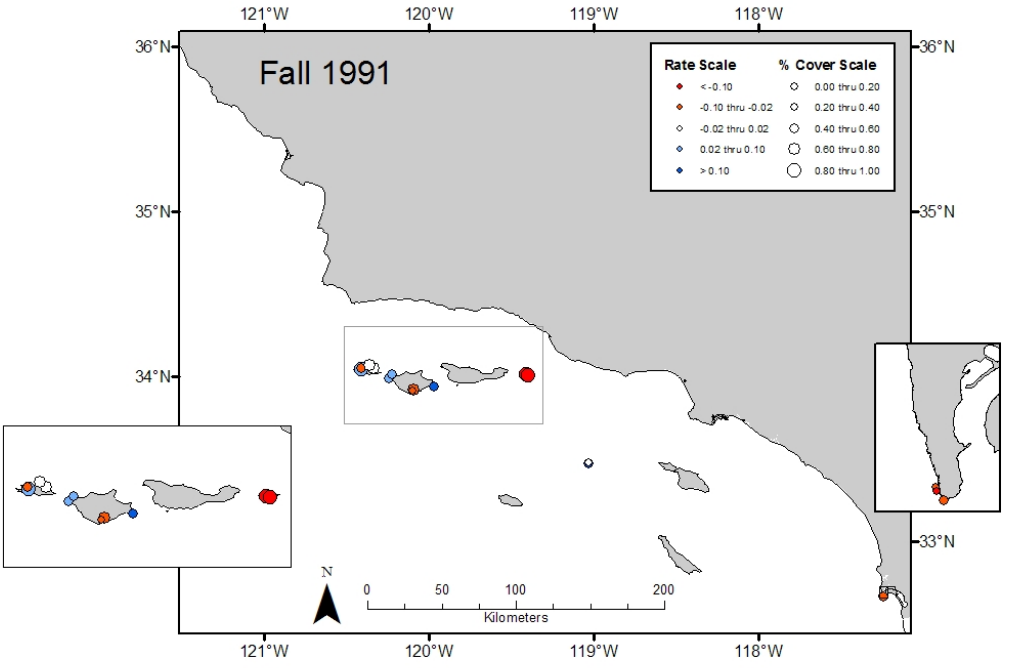


Figure 3.8: Map of mussel dynamics at all MARINe sites in central and southern California in spring and fall 1991, spring and fall 1997, and spring 2003. Each dot represents the average value for a site. The size of the dot corresponds to the starting mussel percent cover for the time period and the color represents the rate of change of mussels during the time period. All sites monitored during the time period are included and there are no interpolated missing values included. SP=spring, FA=fall, and the two digit number indicates a year (e.g., "97" is 1997 and "01" is 2001).

Figure 3.8, Cont.





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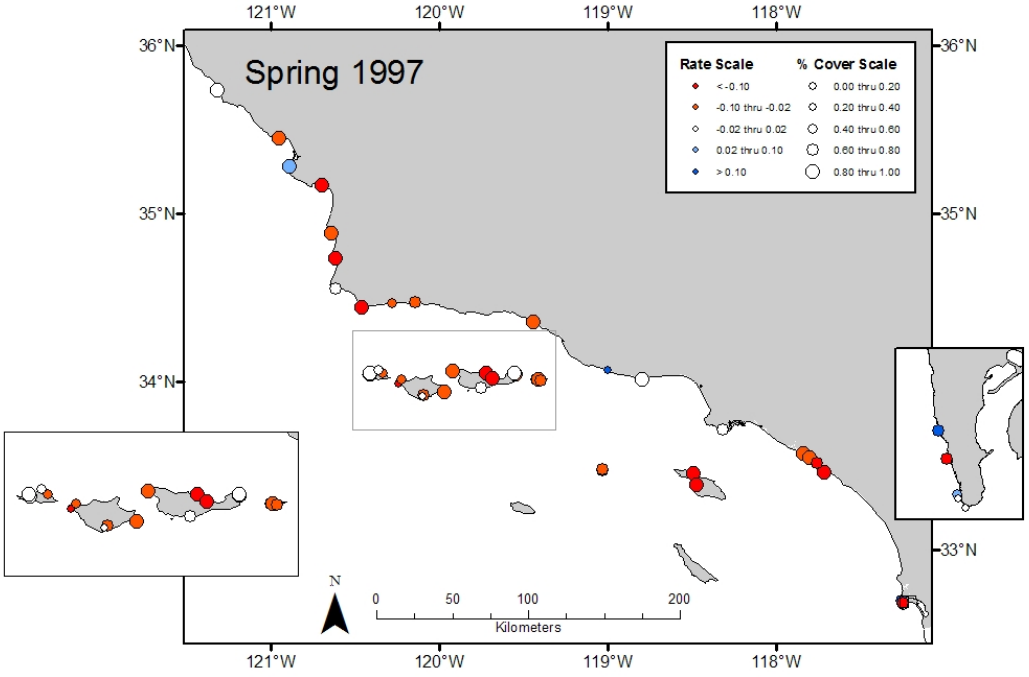

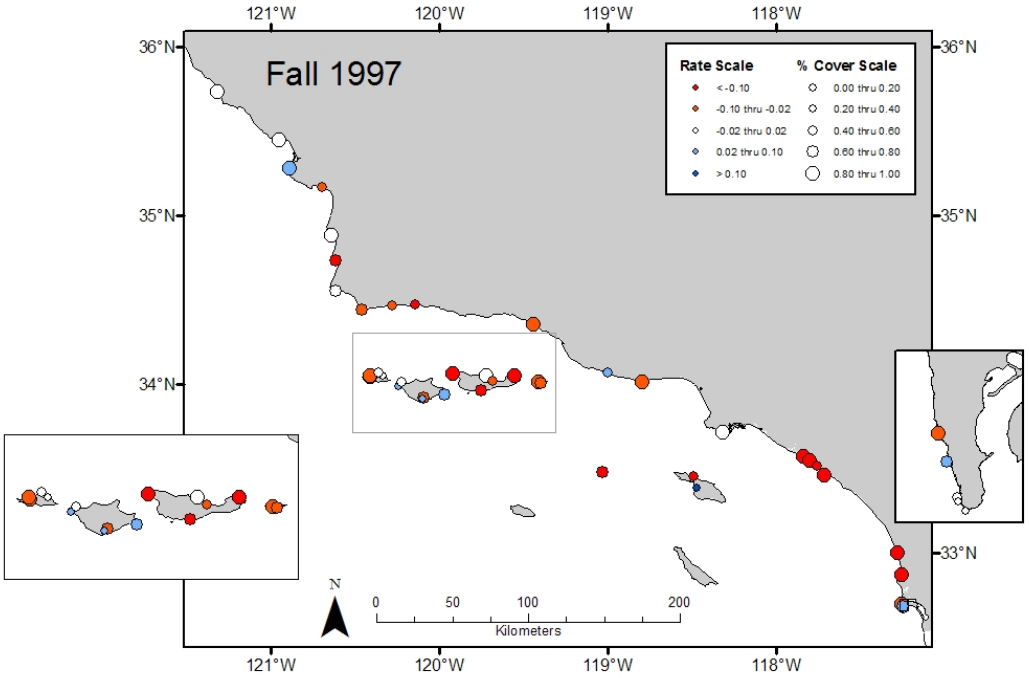

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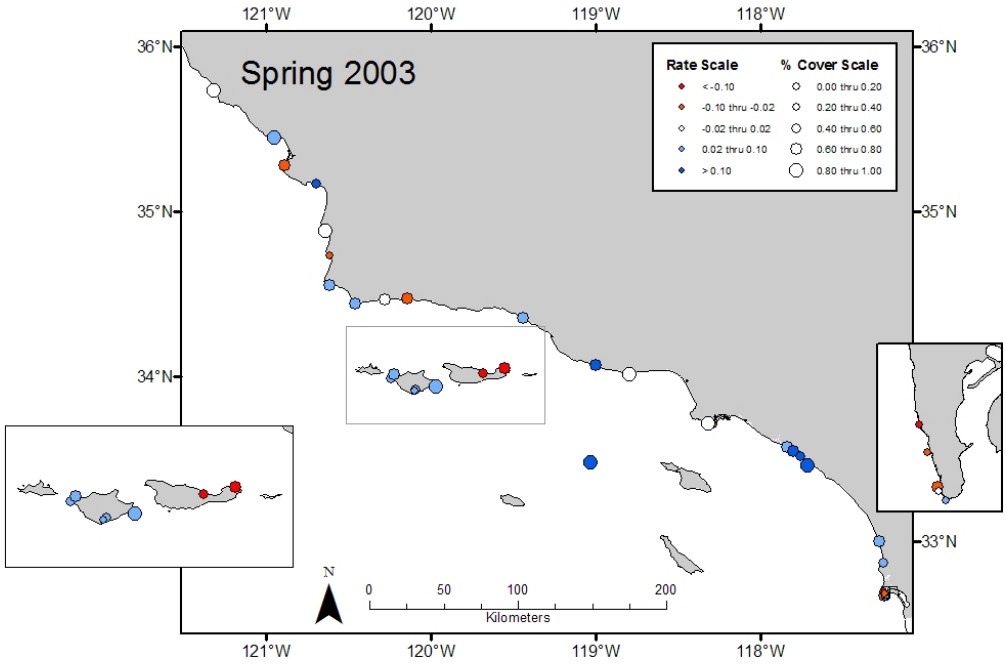

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CHAPTER IV

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Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: A precursor to invertebrate larval tracking

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Abstract

Elements incorporated into developing hard parts of planktonic larvae record the environmental conditions experienced during growth. These chemical signatures, termed elemental fingerprints, potentially allow for reconstruction of locations of larvae. Here, we have demonstrated for the first time the feasibility of this approach for bivalve shells. We have determined the spatial scale over which we are able to discriminate chemical signatures in mussels in southern California and characterized the temporal stability of these signals. Early settlers of *Mytilus californianus* and *Mytilus galloprovincialis* were collected from eight sites in southern California. Shells were analyzed for nine isotopes using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). We discriminated among mussels collected in two bays and the open coast using Mn, Pb, and Ba shell concentrations. 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. These signals were relatively stable on monthly and weekly time scales. These results indicate that trace elemental fingerprinting of shell material is a promising technique to track bivalve larvae moving between bays and the open coast or over along-shore scales on the order of 20 km. Identification of spatial variation in elemental fingerprints that is stable over time represents a crucial step in enhancing our ability to understand larval transport and population connectivity in invertebrates.

As marine biologists began to recognize the existence of planktonic larval stages of benthic adults during the first half of the 19th century, they began to evaluate the role of early life history in determining the abundance and distribution of benthic populations (e.g., Young 1990). Over time, marine ecologists have become increasingly concerned with the role of prerecruitment processes in structuring populations (e.g., Prytherch 1929; Roughgarden et al. 1988; Caley et al. 1996). 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. Direct tracking of all but a few invertebrate taxa using visual observation or artificial tagging has been challenging (reviewed by Levin 1990; Thorrold et al. 2002) due to the small size, low concentration, and relatively long planktonic durations of most larvae.

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 noncalcium elements into the carbonate matrix of their newly forming hard parts (shells, otoliths, statoliths). These elements are likely to be incorporated 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. If chemical signatures could be determined for individuals of known origin, the signals of larvae of unknown origin could be compared and their location of development determined. This type of tag is potentially found in all animals with structures capable of recording conditions in a given environment, and therefore overcomes many of the difficulties experienced with artificial tags (Levin 1990; Levin et al. 1993; Thorrold et al. 2002).

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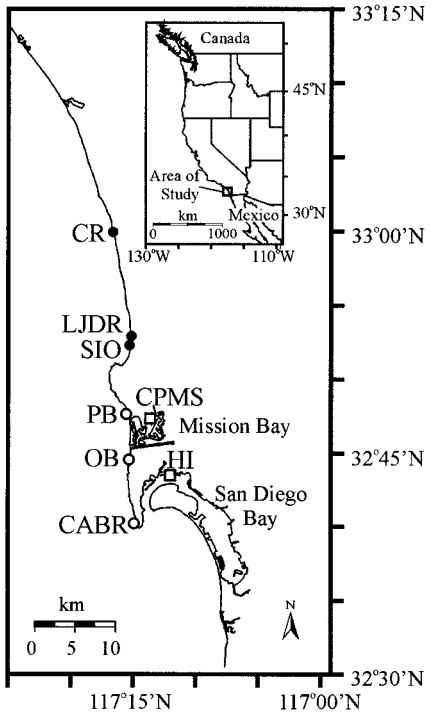

Fig. 1. Map of *Mytilus* mussel collection sites in San Diego County, California USA. Northern region (filled circles): CR = Cardiff Reef; LJDR = La Jolla Dike Rock; SIO = Scripps Institution of Oceanography Pier. Southern region (open circles): PB = Pacific Beach (Crystal) Pier; OB = Ocean Beach; CABR = 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.

Beginning in the 1980s, and increasingly since the 1990s, 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. DiBacco and Levin (2000) and DiBacco and Chadwick (2001) used chemistry of developing crab zoeae, dissolved whole, to discriminate between larvae spawned inside and outside of San Diego Bay. Zacherl et al. (2003*a*) analyzed the statoliths of larval gastropods in three sites in Chile and found sufficient spatial variability to discriminate among sites.

There is a long history of using molluskan microchemistry, especially of mytilid mussels, to monitor ocean environments, past and present (reviewed in Richardson 2001). The soft parts and byssal threads (Goldberg et al. 1978; Cossa 1989; Szefer et al. 2002), as well as the shells of mussels have been studied for use as marine pollution indicators (Koide et al. 1982; Puente et al. 1996; Richardson et al. 2001). Interest in mussel shell chemistry has focused mainly on its use as an environmental recorder, not as a tool for the study of mussel ecology. Intertidal mussels have also played a key role in our understanding of the ecology of rocky shore communities (e.g., Dayton 1971; Paine 1974). Here we explore the use of molluskan shell microchemistry as a tool for tracking larvae. Understanding of population connectivity in mussels may further expand the utility of these species in theoretical ecology studies.

Elemental fingerprinting is most powerful when it can be generalized to answer broad ecological questions on appropriate scales. This study tests the use of this method under realistic conditions with space and time scales that are applicable and important for many future ecological and applied studies. The focus of this study is the use of shell elemental fingerprinting to track larvae of mytilid mussels in San Diego County, California (Fig. 1). Our long-term goals are to use shell microchemistry to determine natal origins, larval trajectories, and population connectivity of mytilid mussels. This requires the development of a reference chemical signature for various locations or regions where larvae could potentially develop and a comparison of this signature to the larval shells of mussels that have settled in known locations. The research presented here seeks to validate the utility of this method using mollusk shells from sites near each other (within 50 km). We aim to determine the appropriate spatial scale at which there are differences in mussel shell chemistry that can be attributed to location. Documenting these differences is necessary for the method to be useful in tracking larvae.

An important secondary need is to determine how stable these signals are over time. If the signals are relatively stable, we will be able to determine a reference signal for each site for comparisons with unknown samples at a later date. If the signals are changing rapidly, it will be crucial to collect reference shells and unknown shells during similar time periods.

In this study, we collected recently settled mussels at eight sites in San Diego County (Fig. 1) and analyzed their shell microchemistries for multiple elements. Multivariate discriminant approaches were employed to ask whether combinations of elemental concentrations could be used to distinguish shells from the different locations and collection periods at various space and time scales. Specifically, we addressed two questions: (1) Can we use the microchemistry of *Mytilus* mussel settler shells to predict site of collection, and if so, at what scale? We asked whether it was possible to distinguish shells from two bays and the open coast and along the open coast. We addressed two spatial scales: regional (northern vs. southern open coast sites, ~20 km areas) and individual sites, and (2) How stable are elemental fingerprints over time, considered on (a) monthly and (b) weekly time scales? Supporting environmental data (water chemistry and temperature) were collected as possible agents creating the observed trends, although the mechanisms of elemental enrichment or depletion were not determined.

Methods

Mytilid mussels as model species—Mytilus californianus and *Mytilus galloprovincialis* were chosen as test species for this study because they are important structural components of rocky intertidal ecosystems (Suchanek 1979, 1992) and they have key roles as prey items (Paine 1974) and competitors for space (Dayton 1971). In the past decade, *M. californianus* has experienced an alarming decline in percentage cover at some sites in San Diego County (Engle and Davis 2000; Becker, B. J., unpubl.). These species have a larval shell that incorporates trace elements and is retained at least into the early plantigrade stage, thus leaving a potential record of where the shell developed. *Mytilus* settlers (defined as individuals smaller than 2.5 mm with a dissoconch that is discernible under a dissecting microscope) are fairly common and easy to collect year round. *M. californianus* larvae are present in the water column throughout the year, although the peak reproductive season is from October to March (Young 1942). Determining the spawning season of *M. galloprovincialis* using past literature is complicated by the fact that, until recently (McDonald and Koehn 1988), this species was misidentified as *M. edulis*. Fortunately, Coe (1946) noted a resurgent population of *M. edulis* on the Scripps Institution of Oceanography Pier, which he called *Mytilus edulis diegensis*; the species described by these observations is most likely to be the *M. galloprovincialis* studied here. Coe (1946) indicates that, although spawning occurred all year, it was concentrated in March through June and early winter, with the highest settlement in June and less settlement in winter.

Both *M. californianus* and *M. galloprovincialis* have larval durations of medium length—approximately 9–10 d for *M. californianus* (Strathmann 1987) and 16–24 d for *M. galloprovincialis* (Satuito et al. 1994). This intermediate larval duration makes them interesting model systems for comparative studies of population connectivity and larval retention.

Species identification—Mytilus settlers less than 2.5 mm could not be identified to species visually. Thus, mussel tissue samples were identified to species using a selective polymerase chain reaction (PCR) technique. DNA was extracted from the soft tissues of juvenile mussels using lysis buffer (65°C for 1 h, 95°C for 15 min). Primers targeting the 16S ribosomal RNA gene were developed using sequences from *M. californianus* and *M. galloprovincialis* listed in the National Center for Biotechnology Information web page (www.ncbi.nlm.nih.gov). The DNA was incubated in a forward primer unique to *M. californianus* (5′ GGTGAAGAGGCCTTTATGAAG 3′) and another unique to *M. galloprovincialis* (5′ GCTTTATCTTAATTGGAGCTT 3′), combined with a reverse primer common to both species (5′ CTAAAGCCAACATCGAGGTC 3′). The PCR reaction proceeded under the following conditions: 95°C for 120 s (denaturation), followed by 35 cycles of 95°C for 30 s, 50°C for 60 s, and 72°C for 90 s. One final elongation step was completed at 72°C for 5 min. This reaction was expected to yield a 223-base pair (bp) fragment for *M. californianus* and a 286-bp fragment for *M. galloprovincialis*. Primers were tested for accuracy on tissues of adults of known identity. The resulting products were run through a 2% agarose gel and stained with ethidium bromide. The species identification of mussels was determined from the presence and length of a PCR product.

Site selection—Eight sites were located in San Diego County within areas where *Mytilus* spawning stock was present and collection was feasible (Fig. 1). Sites are spread within a 45-km length of shoreline, the approximate distance a passive larva would travel in 28 d if average linear transport was 2 cm s^{-1}, a reasonable estimate of monthly averaged values in this area (Winant, C., pers. comm.). Two bay sites were selected, Harbor Island (HI) in San Diego Bay and Crown Point Mitigation Site (CPMS) in Mission Bay. Both bays receive little freshwater flow and have long water residence times in their inner basins, which tend to become hypersaline during the dry summer months (Largier et al. 1997). Exchange between these bays and the open ocean is driven by tidal pumping (Esser and Volpe 2002) that varies over a tidal cycle (Chadwick and Largier 1999). San Diego Bay is a large, highly industrialized harbor that is 24 km long, 4–5.8 km wide, and averages 6.5 m depth. HI is a human-made island created from artificially placed rip-rap near the mouth of San Diego Bay, where the depth averages greater than 10.5 m. Mussels from HI were collected directly from the rip-rap on the bay-facing side. Mission Bay is a shallow estuary (3.5 m average depth) that is mostly used for recreational purposes. CPMS is a restored salt marsh located near the back of Mission Bay. Mussels were collected from scattered small boulders.

Six sites were located on the open coast (Fig. 1). Cardiff Reef (CR) and La Jolla Dike Rock (LJDR) are both natural intertidal areas located at the bottoms of sandstone cliffs. Mussels were collected from a sandstone platform at CR and from a basaltic andesite boulder at LJDR. Scripps Institution of Oceanography pier (SIO) is located in La Jolla, about 600 m south of the LJDR site. Crystal (Pacific Beach) Pier (PB) and Ocean Beach Pier (OB) are located on the north and south sides, respectively, of the mouth of Mission Bay. The San Diego River empties at the outlet of Mission Bay, close to OB. At SIO, PB, and OB, collections were made directly from the pier pilings, at intertidal heights. Cabrillo National Monument (CABR) is a natural intertidal area located at the tip of Point Loma, just north of the mouth of San Diego Bay. The land margin is also sandstone at this site, and the mussels were collected from three different metavolcanic boulders throughout the park.

Sample collection—Collections of mussel settlers were made at most sites on 26 and 27 December 2001 or 9 January 2002 (SIO only) to compare spatial differences in elemental signatures, while keeping temporal signals relatively constant. Additional samples were collected on 1 May 2001 and 8 September 2001 at SIO to compare seasonal variation at a single site. High-frequency variation in shell chemistry was examined with samples collected at SIO for 5 consecutive weeks between 26 January and 21 February 2002.

Early mussel settlers were obtained from either byssal threads of adult mussels (CPMS, HI, SIO, PB, OB) or red algal turf (CR, LJDR, CABR). Samples were immediately

frozen in local seawater and thawed at a later date. Early settlers measuring less than 2.5 mm (less than 2–3 weeks after settlement, as interpreted from Coe and Fox 1942; Coe 1946) were removed using porcelain-tipped forceps under a dissecting microscope. Sorting was done in acid-washed Petri dishes using Milli-Q water. The average size of mussels analyzed was 1.49 mm (0.56 mm standard deviation). A total of 111 mussels were analyzed in this study, including 4–11 recruits from each site for the spatial analyses and 3–15 mussels from each time period.

Sample preparation—Using acid-dipped, porcelain-tipped forceps and tungsten probes, samples were split open, and the flesh was manually removed and retained for species identification. The valves were separated and one valve was put aside. The remaining valve was manually scraped of debris and transferred to a clean plastic vial. Samples were then soaked in 15% H_2O_2 (Trace Select; Sigma-Aldrich) buffered with 0.05 mol L^{-1} NaOH (Suprapur; VWR Scientific Products) overnight (approximately 18–36 h) in order to remove organic matter, including the periostracum, from the shell. Valves were then washed in quartz-distilled (QD) Milli-Q water three times. A low concentration (1%) of HNO_3 (Optima grade; Fisherbrand) was then added to the vial for 10 s. After this acid wash, the shells were rinsed in QD water three additional times and then stored in clean QD water. Shells were then mounted for laser analysis on a petrographic slide using a wet paintbrush and double-stick tape.

Elemental analysis of mussel shells—The elemental composition of a shell can be determined in two general ways—by digesting the shell and analyzing constituents in the resulting liquid or by analyzing the hard parts directly (Campana 1999). Recent technological advances in laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) allow direct analysis of precise regions of a shell. A laser is used to ablate small amounts of shell and the resulting vaporized particles are sent to a high-resolution mass spectrometer for analysis. It is therefore possible to look at specific parts of the shell, which correspond to different periods of the individual's development, without averaging the signal by digesting the whole shell.

Shells were analyzed using a New Wave UP 213-nm laser ablation unit attached to a Thermoquest Finnigan Element 2 double focusing, single collector, magnetic sector ICP-MS (inductively coupled plasma-mass spectrometer). We conducted some preliminary studies using over 15 different isotopes on glass and rock standards and eliminated those that did not yield repeatable values. Of the remaining isotopes, we focused on those that enabled us to distinguish among mussel samples from our sites during preliminary trials. In this study, nine isotopes were analyzed in every sample: ^{24}Mg, ^{48}Ca, ^{53}Cr, ^{55}Mn, ^{64}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U. Analyses of shell composition were performed on the outer margin of shells. A line was ablated beginning at the dorsal apex, and following growth lines as close to the margin as possible, toward the anterior part of the shell. This area represents the most recently formed shell material on the mussels and was chosen to minimize temporal differences caused by variation in mussel age. The ablated line measured approximately 350 μm, with a thickness (i.e., spot size) of 100 μm. The laser was set at 55% power, with a speed of 100 $\mu m\ s^{-1}$.

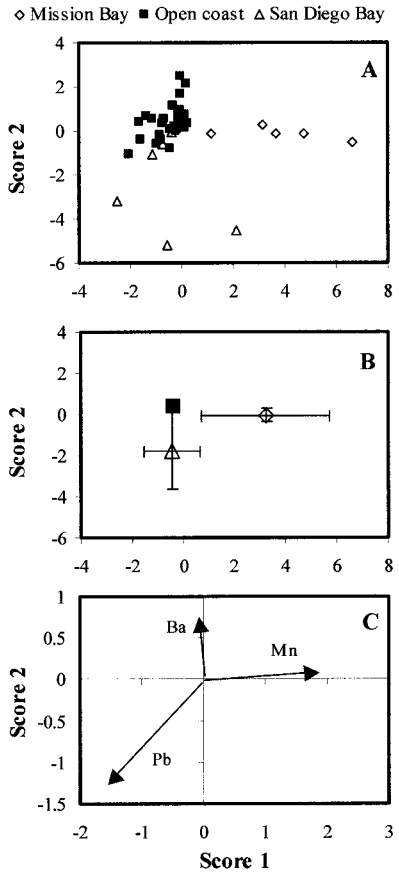

Fig. 2. Discriminant scores of element (Mn, Ba, Pb) to Ca ratios in shells of *Mytilus* mussel recruits collected between 26 December 2001 and 9 January 2002 at sites in San Diego County, grouped as Mission Bay, San Diego Bay, and Open Coast sites (Cardiff Reef, La Jolla Dike Rock, Scripps Institution of Oceanography Pier, Crystal (Pacific Beach) Pier, Ocean Beach Pier, and Cabrillo National Monument). (A) Scatterplot of discriminant function analysis (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.

Glass standards spiked with trace elements (National Institute of Standards and Technology Standard Reference Material 612, 614, and 616; NIST) were analyzed at the beginning and end of a run as well as once or twice in the middle of each run in order to account for machine drift. NIST standards were analyzed using a 600-μm line sampled at 55%

Table 1. Means (±1 standard error) of metal to calcium ratios in juvenile mussel shells collected in San Diego County, grouped by site and date. CPMS = Crown Point Mitigation Site (Mission Bay), HI = Harbor Island (San Diego Bay), CR = Cardiff Reef, LJDR = La Jolla Dike Rock, SIO = Scripps Pier, PB = Crystal (Pacific Beach) Pier, OB = Ocean Beach Pier, CABR = Cabrillo National Monument.

Collection site	Collection date	n	Mg:Ca (mmol mol^{-1})	Mn:Ca (mmol mol^{-1})	Sr:Ca (mmol mol^{-1})	Ba:Ca (μmol mol^{-1})	Pb:Ca (μmol mol^{-1})	U:Ca (μmol mol^{-1})
CPMS	27 Dec 2001	6	49.87±10.65	0.66±0.18	2.37±0.30	16.15±3.34	36.93±8.98	1.45±0.32
HI	27 Dec 2001	8	46.87±9.53	0.44±0.22	2.25±0.33	19.03±7.11	101.20±37.06	1.41±0.24
CR	27 Dec 2001	7	33.95±3.43	0.02±0.00	2.80±0.07	7.61±3.19	4.37±1.76	0.82±0.17
LJDR	27 Dec 2001	4	30.98±3.19	0.01±0.01	3.29±0.20	6.11±3.53	4.60±1.32	0.54±0.21
SIO	01 May 2001	7	23.46±4.21	0.01±0.01	3.38±0.28	7.11±2.36	5.65±2.24	1.64±0.44
	08 Sep 2001	15	30.78±3.47	0.02±0.01	2.89±0.18	3.72±1.07	6.45±1.61	1.44±0.48
	09 Jan 2002	5	15.64±4.36	0.00±0.00	2.86±0.29	2.31±1.06	6.55±1.72	1.96±0.46
	26 Jan 2002	8	12.40±1.30	0.01±0.01	1.55±0.32	1.69±1.20	24.21±12.57	0.90±0.24
	01 Feb 2002	6	12.84±1.63	0.03±0.01	1.82±0.32	4.48±1.64	18.95±5.36	1.78±0.59
	08 Feb 2002	8	11.32±1.00	0.03±0.01	1.44±0.20	4.16±1.27	7.78±2.90	2.58±0.85
	13 Feb 2002	9	10.65±0.48	0.08±0.03	2.85±0.32	5.16±1.90	31.22±7.86	5.85±0.70
	21 Mar 2002	3	15.72±1.38	0.10±0.06	2.06±0.49	0.00±0.00	16.39±8.19	4.21±1.63
PB	26 Dec 2001	11	30.68±6.27	0.06±0.01	2.26±0.19	22.77±5.79	22.26±5.08	1.31±0.27
OB	26 Dec 2001	7	38.47±5.88	0.20±0.05	1.41±0.16	41.35±8.62	67.95±8.57	2.34±1.53
CABR	27 Dec 2001	7	18.79±3.30	0.02±0.01	1.94±0.12	24.83±7.90	12.40±6.29	1.93±0.56

intensity, 25 μm s^{-1} line speed, and 100-μm spot size. In addition, U.S. Geological Survey (USGS) certified rock (quartz latite, USGS-QLO-1) that was melted and reformed for homogenization was run at the same time as the NIST standards. Because this reformed glass was relatively soft, a 300-μm line was run at 45% intensity, 50 μm s^{-1}, and 50-μm spot size.

To determine isotope intensities, a chromatogram was generated for each element in each sample using the Element Software, and resulting peaks were analyzed individually. A peak was defined as having a maximum value greater than three standard deviations above the mean of the background, and background levels were subtracted from peaks using linear regression of nonpeak values. We calculated the raw count per second (cps, area under the peak) for each isotope in each sample. The background-corrected cps values were then multiplied by a correction factor generated by the standard (NIST or QLO-1), using recorded run numbers and linear estimations of machine drift. The sample cps values were then divided by the counts of ^{48}Ca, a rare isotope of Ca, which was used as an internal standard in order to standardize for the amount of shell ablated. These ratios were used for all resulting analyses, except for determination of the tape and slide values. Resulting isotope ratios were converted to element ratios using relative abundances of naturally occurring isotopes. The elemental count ratios were converted to molar ratios using NIST glass results for SRM 612, 614, and 616 and available published concentrations (612: Pearce et al. 1997; 614 and 616: Horn et al. 1997; Ca values: NIST certified values).

It is important to note that these absolute values are dependent on the standard used for calibration. There are currently no matrix-matched and homogenous standards available for analyzing biogenic calcite (Campana 1999; Vander Putten et al. 2000); thus, NIST glass was used for this study. The relative ratios are consistent among samples used in these analyses (Campana 1999) and the results of the multivariate analyses are valid. However, these absolute values are not necessarily accurate and are difficult to compare across studies with different calibration standards. Vander Putten et al. (1999) discusses the drawbacks of using glass standards to analyze biogenic calcite but concludes that, until appropriate standards are developed, NIST provides good precision and allows for intrastudy consistency among samples. These authors later analyzed adult *Mytilus edulis* shells using NIST SRM 610 and 612 glass as standards and reported element ratios in the same order of magnitude as we found in this study, with the exception of Pb, which was lower than in our samples (Vander Putten et al. 2000). U was not analyzed in their study.

Table 2. Classification success (jackknifed) for using shell chemistry to determine where *Mytilus* mussel shells were formed, with sites grouped as San Diego Bay (SDB), Mission Bay (MB), and open coast (OC). Rows list the actual grouping, columns list the grouping predicted using the discriminant function analysis (DFA) model without replacement. Individual DFA scores are shown in Figure 2.

	Predicted grouping			Total per site	% correctly classified
	Open coast	Mission Bay	San Diego Bay		
Actual grouping					
OC	39		2	41	95
MB	2	4		6	67
SDB	4	1	3	8	38
Total	45	5	5	55	84

Table 3. Classification success (jackknifed) for using shell chemistry to determine where *Mytilus* mussel shells were formed, with individual open coast sites of San Diego County represented. Rows list the actual grouping, columns list the grouping predicted using the discriminant function analysis (DFA) model without replacement. The numbers of correct classifications are presented as individual sites (% correct sites) or with the sites grouped into northern and southern regions (% correct regions). Northern region sites are CR = Cardiff Reef, LJDR = La Jolla Dike Rock, and SIO = Scripps Institution of Oceanography Pier. Southern region sites are PB = Crystal (Pacific Beach) Pier, OB = Ocean Beach Pier, and CABR = Cabrillo National Monument. Individual DFA scores are shown in Figure 3.

	Predicted site								
	Northern region			Southern region			Total per site	% correct (sites)	% correct (regions)
	CR	LJDR	SIO	PB	OB	CABR			
Actual site									
CR	4	1	2				7	57	100
LJDR	1	3					4	75	100
SIO	1	1	1	1		1	5	20	60
PB		3	1	3	2	2	11	27	82
OB					6	1	7	86	100
CABR					1	6	7	86	100
Total	6	8	4	4	9	10	41	56	90

Contaminant avoidance—To evaluate the risk that adhesive and glass slide material could be ablated and included in the analysis, two to three lines were sampled on the tape and slide without a mussel sample, for each slide of mussels analyzed. For almost every isotope, the average of the tape value was less than 5% of the mussel value. Because there was an average of 162% more ^{53}Cr and 11% more ^{64}Zn in the slide than in the mussels, these isotopes were removed from further analyses.

Statistical tests—Resulting element ratios (X:^{48}Ca) were analyzed using a linear discriminant function analysis (DFA; Systat 9) to examine our hypotheses. First, in order to examine spatial variation, mussels collected at all eight sites between 26 December 2001 and 9 January 2002 were included in the analysis. All sites were initially grouped as San Diego Bay (HI), Mission Bay (CPMS), and open coast (all other sites). A second analysis was conducted at the site level using the open coast sites only. Seasonal variation was then examined by (a) considering shells collected at the various dates at SIO as unknowns, (b) determining their discriminant scores using the site DFA, and (c) evaluating how closely they matched the SIO site from the original analyses. If the signal is stable over time, these unknowns should be classified from the correct site (i.e., open coast and SIO). Weekly variation was examined by comparing 5 weeks of samples from SIO (26 January–21 February 2002) using a separate DFA. The weekly samples were pooled as a single February sample for the seasonal analysis and considered individually for the weekly analysis.

All DFAs were conducted in a stepwise manner, by running the analysis on all element ratios and dropping the least significant variable, as determined by the F to remove statistic. The DFA was then run again, and the next-least significant variable was removed. This was repeated until the F to remove statistic of all included element ratios was greater than 3.5.

Results are presented as (1) scores plotted as individual points representing single shells and (2) mean ± 95% confidence intervals. Cross-validation was achieved using a jackknifed classification matrix. Each sample was removed from the creation of the DFA model, and then the classification of the sample was determined using just its score. The data are presented as raw numbers classified in each group, as well as percentage correct values. The relative weighting of the elements in the DFA analysis is indicated

Table 4. Jackknifed classification success table for *Mytilus* mussels collected at Scripps Pier during different seasons, using a discriminant function analysis (DFA) model developed using shells collected in December 2001 and January 2002. Sites were grouped into San Diego Bay (SDB), Mission Bay (MB), and the open coast (OC) (Table 2 and Figure 2). SIO-Jan 2002 was the sample originally used to classify Scripps Pier in the DFA. SIO-Feb 2002 represents an average of 5 consecutive weeks from 26 January to 21 February 2002. Rows list the actual grouping, columns list the grouping predicted using the DFA model.

	Predicted grouping				
	Open coast	Mission Bay	San Diego Bay	Total per date	% correct
Actual grouping					
SIO-Jan 2002	5			5	100
SIO-May 2001	7			7	100
SIO-Sept 2001	15			15	100
SIO-Feb 2002	28		6	34	82
Seasonal, total	50		6	56	89

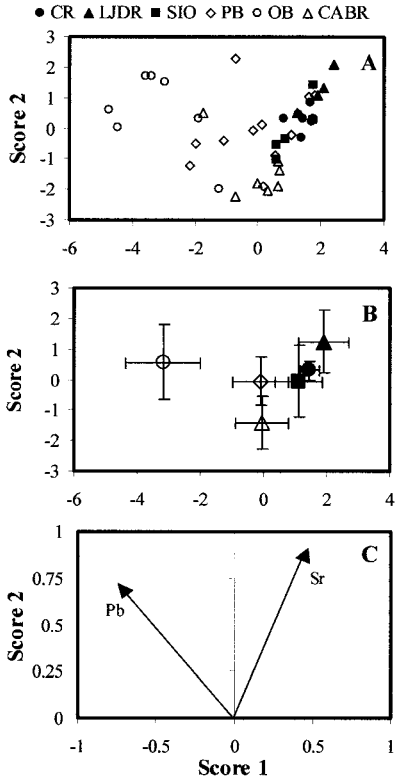

Fig. 3. Discriminant scores of element (Pb, Sr) to Ca ratios in shells of *Mytilus* mussel recruits collected between 26 December 2001 and 9 January 2002 at open coast sites in San Diego County. Sites are listed from north to south. The northern region is represented by filled shapes; the southern region 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 discriminant function analysis (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.

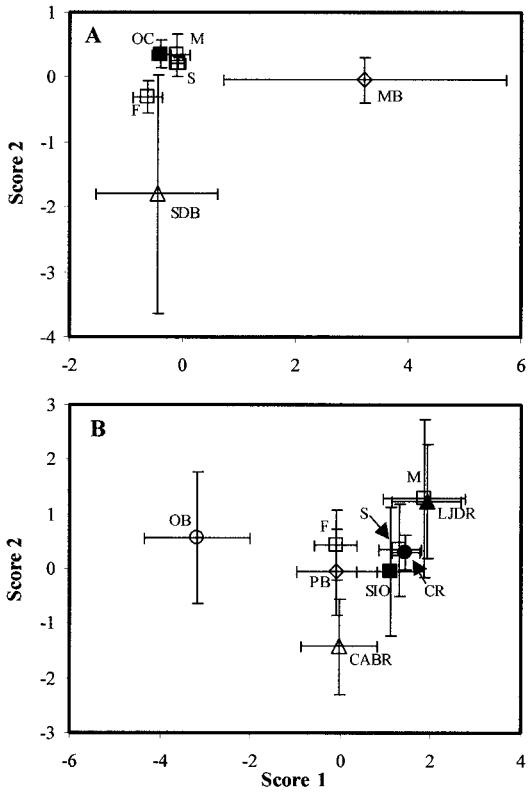

Fig. 4. Discriminant scores of element (Pb, Sr) to Ca ratios in shells of *Mytilus* mussel recruits collected from Scripps Institution of Oceanography Pier in May 2001, September 2001, and February 2002 compared with those collected at various sites between 26 December 2001 and 9 January 2002 in San Diego County. Scores were calculated for shells collected at SIO during various seasons using the same discriminant function analyses (DFAs) depicted in Figs. 2 and 3. All are plotted as averages with ± 95% confidence intervals. The standardized discriminant functions are given in Fig. 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 with Mission Bay, San Diego Bay, and open coast sites. The standardized discriminant functions are given in Fig. 3C; (B) averages of DFA scores of the various seasons compared with 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.

by the graphical representation of standardized discriminant functions.

Elemental analysis of water—Water samples were collected within 1 h of low tide from both bay sites (HI and CPMS) on 25 January 2001 and the six open coast sites on 26 January 2001 (Fig. 1). The samples were obtained from 15–30 cm below the sea surface off the bow of a small research vessel following the methods prescribed by Gasparon (1998) to avoid contamination. After collection, samples were stored in acid-washed high density polyethylene

Table 5. Jackknifed classification success table for *Mytilus* mussels collected at Scripps Pier during different seasons, using a discriminant function analysis (DFA) model developed using shells collected in December 2001 and January 2002 (Table 3 and Figure 3). Rows list the actual site, columns list the site predicted using the DFA model. The numbers of correct classifications are presented as individual sites or with the sites grouped into northern and southern regions. SIO-Jan 2002 was the sample originally used to classify SIO in the DFA. SIO-Feb 2002 represents an average of 5 consecutive weeks from 26 January to 21 February 2002. 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.

	Predicted grouping							
	Northern region			Southern region			Total per date	% correct (regions)
	CR	LJDR	SIO	PB	OB	CABR		
Actual grouping								
SIO-Jan 2002	1	1	1	1		1	5	60
SIO-May 2001		5	1			1	7	86
SIO-Sep 2001	1	6	4			4	15	73
SIO-Feb 2002	3	6	0	11	4	10	34	26
Months, total	4	17	5	11	4	15	56	46

(HDPE) bottles, placed on ice, and transported to the laboratory for immediate processing. All glassware, pipette tips, and sample containers were washed in 10% HNO_3 and rinsed three times in Milli-Q water prior to coming in contact with the samples.

Samples were filtered, acidified, and diluted following the general methods of Field et al. (1999), except where deviations had to be made to accommodate our particular analysis. Samples were first passed through a 0.4-μm ceramic filter. Between samples, the ceramic filter was acid washed, Milli-Q rinsed, and rinsed again using 100 ml of excess sample. Filtered samples were then spiked with Optima-grade nitric acid in a 9:1 ratio and stored in acid-washed, 15-ml polystyrene centrifuge tubes. Acidified samples were diluted 20-fold with 3% Optima nitric acid in QD water and spiked with a 1-ppb In internal standard (Spex Certiprep) before introduction to the ICP-MS. We analyzed samples via solution-based ICP-MS following the guidelines of Field et al. (1999) for instrument and induction parameters. The Element 2 software provided elemental concentration data that were later corrected for dilution in Microsoft Excel.

We incorporated matrix-matched external standards to produce calibration curves for Mn, Pb, and Sr (low resolution), and Ba (medium resolution) (Rodushkin and Ruth 1997). These curves were then used to determine the trace element makeup of coastal seawater samples. Standards were created by various dilutions of Multi-Element Standard 2A (Spex Certiprep), Ba and Sr (FisherChemical) stock standards, each spiked with 1 ppb In. In order to match the sample matrix, standards were diluted using 3% seawater in QD water that had been stripped of trace metals using Optima ammonium hydroxide (Fluka Chemika). To ensure the reliability of our results, reference waters CASS-4, NASS-5, PPREE1, and SCREE1 (Verplank et al. 2001) were included in the analysis using the protocols described above. Additionally, several test blanks were analyzed to account for any possible contamination that occurred as a result of our methodology.

Temperature data—Water temperature data were obtained immediately offshore of sites at SIO, PB, CABR, CPMS, and HI using Onset Stowaway TidbiT thermistors. Temperature was recorded every 1–4 min during our sampling periods. Two months of data (December 2001 and January 2002) are presented here to illustrate the spatial variation in temperature among sites.

Results

Species effect in elemental fingerprints—We expected to encounter many more *M. californianus* settlers than *M. galloprovincialis* settlers because sampling was done during winter for most sites and only at an exposed coastal site during other seasons. However, genetic analysis of mussel settlers indicated that, of the 111 mussels analyzed, 38 (34%) were *M. californianus* and 23 (21%) were *M. galloprovincialis*; 50 (45%) were not identifiable due to lack of soft tissue or lack of an unambiguous signal. All identified mussels collected in Bay sites were *M. galloprovincialis*. Of identified mussels from the open coast sites, 67% were *M. californianus* and 33% were *M. galloprovincialis*.

Dodd (1964, 1965) reported that adult *M. californianus* shells are composed of two calcitic layers with an aragonitic layer in between, while *M. "edulis"* (=*M. galloprovincialis* or *M. trossulus*) does not have an inner layer of calcite. Calcite is generally lower in Sr and higher in Mg relative to aragonite (Dodd 1967). Thus, we predicted that *M. californianus* shells should be enriched in Mg and depleted in Sr compared with *M. galloprovincialis* shells. We found no significant difference in Mg or Sr composition between the juveniles of the two species when considering all samples (ANOVA Mg: $F_{1,59} = 0.03$, $p = 0.86$; Sr: $F_{1,59} = 0.05$, $p = 0.82$) or when considering samples from SIO only to standardize for a site effect (ANOVA Mg: $F_{1,38} = 1.32$, $p = 0.26$; Sr: $F_{1,38} = 0.13$, $p = 0.72$).

The amount of Mn, Ba, or U between shells of the two species did not differ when considering all samples or samples from SIO only. Pb ratios in the shells of the two species,

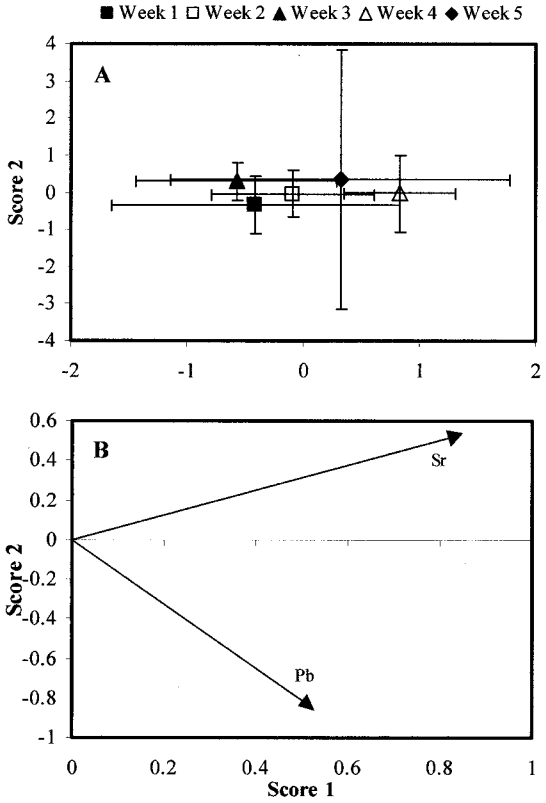

Fig. 5. Discriminant scores of element (Pb, Sr) ratios to Ca in shells of mussel recruits collected once per week between 26 January and 21 February 2002 at Scripps Pier, grouped as weeks. (A) Discriminant function analysis (DFA) scores, plotted as averages with ± 95% confidence intervals; (B) discriminant 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.

however, were significantly different when considering all samples (ANOVA, $F_{1,59} = 4.40$, $p = 0.04$), probably due to the disproportionately high proportion of *M. galloprovincialis* from the bay sites. When just SIO mussels were considered to minimize the site bias, the Pb ratios were not different between the species (ANOVA $F_{1,38} = 0.30$, $p = 0.59$). Because the chemistry of the mussel settler shells did not exhibit a species effect, the remaining analyses were conducted on both species without discriminating between them in order to improve statistical and interpretive power.

Spatial variation in elemental fingerprints—For each element, the molar ratio to calcium was determined (Table 1). A multivariate, multistep process was used to determine whether sufficient spatial variation in trace element composition exists for use as a tracer of larval trajectories and at what spatial scales. Mussel shells collected from San Diego Bay, Mission Bay, and the open coast sites were successfully discriminated 84% of the time (Table 2; Fig. 2) using the Mn : Ca, Pb : Ca, and Ba : Ca ratios in their shells. A 95% success rate was achieved in classifying mussels from the open coast, and only 2 out of 41 of the open coast mussels were misclassified. Our ability to classify Mission Bay was relatively good (10% by random chance, 67% in our model), but our success with San Diego Bay was lower (15% by random chance, 38% in our model). Resulting scores were graphed as a scatterplot (Fig. 2A) and as averages ± 95% confidence intervals (Fig. 2B). Mission Bay shells were distinct from those of San Diego Bay and the open coast mostly due to higher Mn and lower Pb values at that site; San Diego Bay separated from the open coast due to lower Ba and higher Pb in the former (see discriminant functions, Fig. 2C). Because there were three site groups, all of the dispersion in the data is explained by two DFA scores.

Shells from the six open coast sites were successfully distinguished 56% of the time using Pb : Ca and Sr : Ca when considered individually (Table 3; Fig. 3), although both OB and CABR were distinguished 86% of the time. When grouped at a larger spatial scale, however, mussel shells from northern and southern regions were successfully distinguished 90% of the time (Table 3). Shells from CR, LJDR, OB, and CABR were successfully assigned to the appropriate region with 100% accuracy (Table 3). Shells from the northern and southern regions are distinct (Fig. 3A,B), with PB and SIO having intermediate composition. The northern regions were characterized by higher Sr and lower Pb than the southern regions (see discriminant functions, Fig. 3C). Again, with two variables, all of the dispersion in the data is explained by two DFA scores.

Seasonal stability of elemental fingerprints—SIO shell samples from different months were correctly classified as open coast mussels 89% of the time (Table 4; Fig. 4A). May and September 2001 were correctly classified 100% of the time (Table 4; Fig. 4A) using the existing DFA based on Mn : Ca, Pb : Ca, and Ba : Ca ratios in mussel shells collected in December 2001/January 2002 (Fig. 2). The February shells, however, were misclassified as coming from San Diego Bay 18% of the time (Table 4).

More variation was found when evaluating classification success of open coast regions. SIO samples from May and September were correctly classified as coming from the northern region 86% and 73% of the time, respectively; shells from February were correctly classified as northern only 26% of the time (Table 5). The existing DFA based on Pb : Ca and Sr : Ca ratios for categorizing open coast sites (Fig. 3) was the source of the comparison. Samples collected at SIO pier in May and September 2001 clustered most closely to LJDR and CR, respectively, while samples collected in February clustered more closely with samples from PB (Fig. 4B), with 32% of the samples being classified as coming from PB (Table 5).

Weekly stability of elemental fingerprints—The week-to-week stability of the signal was found to be quite high. None of the measured elements generated distinctions among

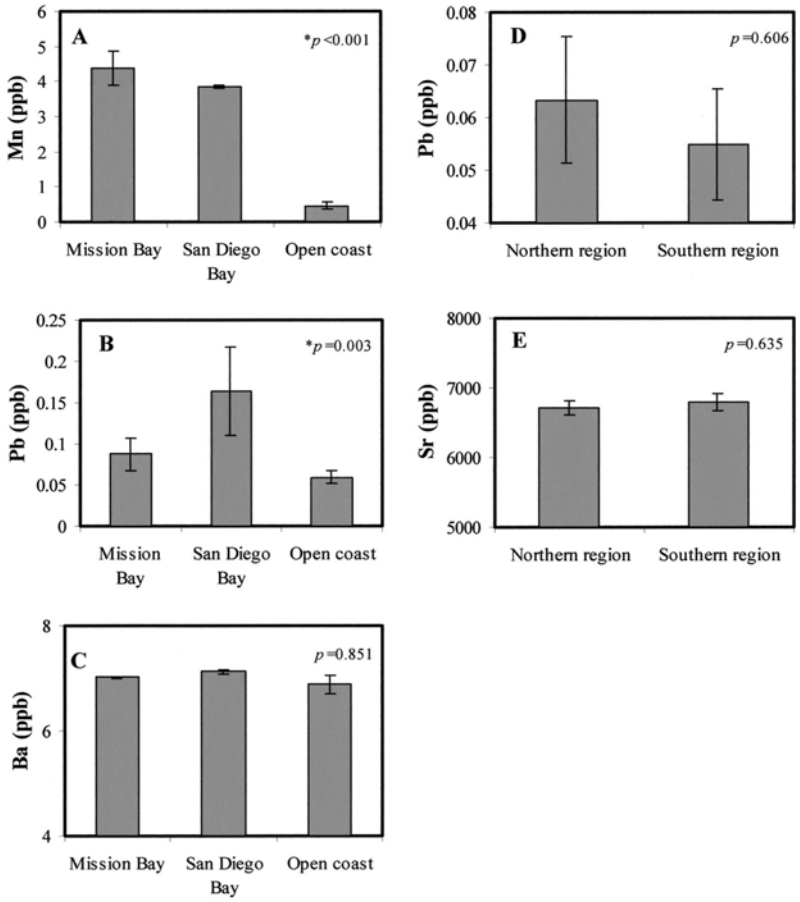

Fig. 6. Concentrations of elements (parts per billion) in seawater collected 25–26 January 2002 from the mussel collection sites in this study. Error bars represent ±1 standard error, p values are from ANOVA analysis, values with an asterisk are significant at $p < 0.05$. Panels (A), Mn, (B), Pb, and (C), Ba compare water composition between major bays and open coast sites. Panels (D), Pb, and (E), Sr, compare water composition between regions of the open coast.

weeks (i.e., all F to remove ratios were less than 3.5, the criterion used in the rest of the analyses in this paper); Sr : Ca and Pb : Ca were used in order to compare the weekly data with the sites-scale data (Figs. 3 and 4B). Weekly samples were distinguishable 38% of the time, and no notable pattern in time was noted (Fig. 5).

Elemental composition of local seawater—Seawater data from the eight mussel collection sites (Fig. 1) were examined in the context of the elemental fingerprinting results presented above, focusing on those elements used in the DFA. Water collected from both major bays contained eight to nine times more Mn than from open coast sites, and Mission Bay contained higher levels of Mn than San Diego Bay (ANOVA $F_{2,14} = 142.12$, $p < 0.001$; Fig. 6A). San Diego Bay water contained almost two times the amount of Pb as water from Mission Bay and open coast sites (ANOVA $F_{2,14} = 8.75$, $p = 0.003$; Fig. 6B). Ba concentrations were not significantly different among the bays and the open coast sites (Fig. 6C). Concentrations of Pb and Sr were not significantly different in seawater collected from the northern and southern open coast regions (Fig. 6D,E).

Temperature data—There were notable differences in water temperature at the five sites monitored (Fig. 7) over the weeks prior to the sampling period, while the mussels were forming shell material. Two weeks before sampling, HI was a degree warmer than the other sites but was comparable

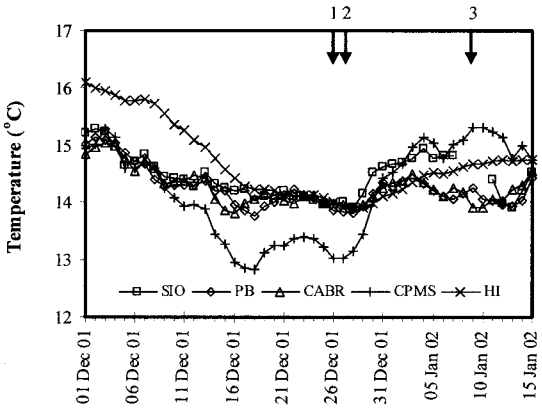

Fig. 7. Temperature data for San Diego County. Sampling times for this study are noted with numbered arrows. Daily averages of sea surface temperature immediately offshore of five sites, collected using a temperature logger located on a surface float; 1 = 26 December 2001; 2 = 27 December 2001; 3 = 9 January 2002. Thermistor temperature data were provided by John Largier, SIO.

with open coast sites during the week before sampling. CPMS was similar to open coast sites 2 weeks prior to sampling but was over a degree cooler during the week before sampling. The open coast sites remained between 14°C and 15°C, although CABR and PB (southern region) were as much as 0.4°C cooler than SIO (northern region) from 14–18 December 2001.

Discussion

Sources of variation in shell signatures—In this study, we used element ratios in mussel shells to classify individuals by location at various spatial scales. While it is not necessary to determine the factors responsible for a given signal in order to use the method for tracking larvae (Zacherl et al. 2003*a*), water samples and temperature data taken at the time of the study provide some insight into possible correlations and inconsistencies between environmental parameters and shell chemistry. Future studies should address these mechanisms in order to better understand the relationships between the environment and elemental fingerprints.

Three elements, Mn, Pb, and Ba, were used to distinguish among mussels collected from two major bays and open coast sites. Mission Bay mussel shells had relatively high, but variable, levels of Mn compared with the rest of the samples (Table 1). San Diego Bay mussels were also somewhat elevated in Mn, although less than those from Mission Bay (Table 1). Water taken from the bay sites in late January was similarly found to be elevated in Mn when compared with the open coast samples (Fig. 6A). Similar results were reported for San Diego Bay seawater by Esser and Volpe (2002), who document increasing levels of Mn with distance into the bay from the ocean in September 1999. They attributed the origin of elevated Mn in seawater to sediment–water interactions rather than local anthropogenic sources. DiBacco and Levin (2000) found that crab zoea from San Diego Bay had higher Mn concentrations than those from coastal sites or neighboring bays. Because elevated Mn in water seems to be related to higher Mn : Ca ratios in the shell and Mn appears to be consistently higher in the bays, this element signal might serve as a valuable marker of shell deposited and larvae developing in these bays.

San Diego Bay (HI) mussels were also characterized by high levels of Pb (Table 1). Water samples from late January reflected a similar pattern; San Diego Bay water had much higher Pb levels than water from all other sites (Fig. 6B). Flegal and Sañudo-Wilhelmy (1993) also found high levels of Pb in San Diego Bay water compared with coastal waters during June of 1989, with especially high levels off of Shelter Island, approximately 3 km west of HI. They attribute higher Pb in San Diego Bay to contaminants residing in the sediments of the area or surface runoff. Similarly to Mn, Pb : Ca in mussel shells could be a useful marker to discriminate shell material formed in San Diego Bay.

For other elements, there was no correlation between water and shell concentrations. No significant difference in Ba concentration was found between San Diego Bay mussels and other sites (Fig. 6C), although slightly lower Ba levels in San Diego Bay mussels played a minor role in discriminating these individuals from Mission Bay and open coast mussels (Fig. 2). Likewise, shells from the southern region of the open coast contained more Pb and less Sr than those from the northern region (Fig. 3), although water samples taken from the same areas did not show this pattern (Fig. 6D,E). There are at least three possible reasons for this apparent inconsistency. First, we examined a section of the shell (100 μm along the growth axis) that corresponds to a few days of mussel growth. It is therefore possible that the mussel shells are recording average water conditions that the individual water samples, taken at one moment in time, are not capturing. Second, mussels have the ability to bioaccumulate some metals in their shells, although bioaccumulation will depend on the element and species considered (e.g., Carriker et al. 1996). For example, Sturesson (1976) found that the carbonate matrix in *M. edulis* shells can contain over three orders of magnitude more Pb than ambient seawater. Perhaps small differences in lead are more pronounced in mussel shells but not detectable in water samples. Third, additional environmental factors, such as temperature and physiological processes, have been shown to influence the elemental concentrations, especially of Sr and Ba, in mollusk shells independent of seawater concentrations (e.g., Cardellicchio et al. 1998; Vander Putten et al. 2000; Zacherl et al. 2003*b*). There were notable differences in temperature among the study sites during the weeks before sample collection (Fig. 7).

Potential for application of elemental fingerprinting of bivalves: spatial differences—The most important prerequisite to using trace element signatures as a larval tracking tool is to determine the spatial scales at which the chemical signals are unique. Using the settled juveniles of mytilid mussels, we have determined that shells formed at different sites have distinctive chemistry, although not at all spatial scales. On the largest scale, considering whether a shell was deposited

on the open coast or in a bay, our accuracy was quite high (Table 2). The ability to classify Mission Bay was relatively good, especially given the small sample size from this site. Success with San Diego Bay was poor, possibly because HI is not far from the outlet of the bay, and coastal waters often bathed mussels during flooding tides. On the open coast, our results indicate that we are able to discern two major regions, northern (CR, LJDR, and SIO) and southern (PB, OB, and CABR), rather than individual sites. Each region spans approximately 20 km of shoreline. Two sites, SIO and PB, seem to act as transition sites. The relatively small sample size from individual open coast sites and HI could have led to poor discrimination, and additional studies should include a higher number of samples. Analyses could be run serially on mussel shells to determine first if they were from the open coast, and if so, what region they came from.

Potential for application of elemental fingerprinting of bivalves: signal stability over time—In order to use trace element signals collected at one time as predictors of unknown samples collected at another, it will be crucial to understand if and how the signals are changing with time. Our results indicate that, on the appropriate spatial scales (open coast vs. major bays and northern vs. southern regions), samples collected from SIO during months before and after our spatial sampling were correctly classified as coming from the open coast almost all of the time. An exception occurred for mussels collected in February.

Previous studies have examined the temporal variability of mussel shell and otolith chemistry on a seasonal or longer time scale. Seasonality was observed in the signals of Mg, Sr, Pb, Ba, and Mn in *M. edulis* from The Netherlands (Vander Putten et al. 2000). Gillanders (2002) reviewed several papers that found significant differences in fish otolith microchemistry between samples collected at monthly and yearly time scales for a number of elements, including Mn, Sr, and Ba. Similarly, spatial patterns in otolith microchemistry (Mn, Sr, Ba, and Pb) of three estuarine fish in southern California were confounded by seasonal variation (Swearer et al. 2003).

Based on analyses of shells of new mussel recruits in southern California, we infer that it will 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. An alternative approach would be to collect reference signatures over multiple seasons and years to create a time-integrated signal, as suggested by Gillanders (2002). This approach will probably not be applicable in southern California, which is characterized by long dry periods punctuated with large rainstorms, which lead to high temporal variability in surface runoff and other environmental conditions. The finding that shells collected weekly from a single site in a single month could not be differentiated based on their elemental fingerprints (Fig. 5) 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.

Future directions—We were able to characterize fingerprints of known origin by using new recruit shells (less than 2.5 mm). In order to use the technique to determine larval origins, the next step will be to determine the ability to distinguish trace element signals of larval shells from known waters. Mytilid mussel fertilization occurs externally in the water column (i.e., via free spawning). In contrast with species with benthic egg capsules, shell formation begins at some time after the individual embryo is transported from its parents. In laboratory cultures of these species, shelled, D-shaped veligers 100 μm in length are formed in less than 40 h (Becker, B. J., unpubl.). Veligers would travel less than 3 km in 40 h at an average transport velocity of 2 cm s^{-1}, a reasonable estimate for monthly averaged values in coastal southern California (Winant, C., pers. comm.). This distance is much smaller than the 20-km natal regions we are able to discriminate using shell chemistry.

Because naturally occurring, free-spawned planktonic larvae may have arrived from unknown locations, it will be necessary to generate reference larval elemental signals through in situ larval culturing. Outplanted larvae can serve as references of known origin, which can be compared with the larval shells of unknown origin from recent settlers. This will allow characterization of likely natal regions of juveniles. While the present feasibility study combined two species, it will be essential to work with only a single species to apply this method to ecological questions, such as degree of connectivity and self-recruitment, in order not to confound the patterns of organisms with different life histories.

The inability to determine larval origins has challenged marine ecologists for over a century. In the past two decades, interest in tracking larvae among adjacent populations has grown exponentially due to increasing realization of the importance of prerecruitment processes in structuring adult populations and, more recently, due to interest in spatially based management tools such as marine protected areas. 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 2003*a*), and shells (this study, Zacherl 2003*b*) 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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CHAPTER V

Determining natal origins and population connectivity of newly-settled mytilid mussels using trace elemental fingerprinting

ABSTRACT

Based upon the observation that passive particles in the ocean can be transported over considerable distances in a short period of time, it has long been believed that coastal benthic marine species with a planktonic larval stage are dispersed great distances and that their populations are demographically "open" or highly connected. There is a growing recognition of the importance of small-scale larval dispersal, and a general interest in defining the connectivity between marine populations, although direct evidence of "self-recruitment" has been difficult to collect. In this study elemental fingerprinting was used to determine the patterns of connectivity in mussel populations (*Mytilus californianus* and *M. galloprovincialis*) in San Diego County, California. In situ larval culturing was conducted at thirteen sites, spanning 75 km of shoreline and three embayments, to create reference signals of trace element chemistry (a combination of seven element ratios) in larval shells formed at known locations. This method expanded elemental fingerprinting methods to species with wholly planktonic larval development for the first time. These experimentally-generated elemental fingerprints were compared to the chemistry of retained larval shells of recently-settled juveniles in order to determine the natal origins of 232 juveniles of

both species collected at thirteen coastal sites. From these results, it appears that mussel larval retention occurs over small scales (10 to 30 km) and on conservation-relevant time scales (less than one generation). However, these populations could not be considered demographically "closed". The two mussel species exhibit different connectivity patterns. Most of the *M. californianus* originated from the northern part of the study area, suggesting that these populations follow a "single source" model of larval replenishment. *M. galloprovincialis* populations in San Diego County appear to originate from a larger number of sources including bays and southern sites, although a smaller sample size led to more equivocal results. The details of life-history differences and distribution between the two species provide possible mechanisms leading to different connectivity patterns. The promise of elemental fingerprinting as a larval tracking tool is beginning to be realized, greatly improving our understanding of the connectivity between geographically separated populations. Ultimately, this information will be crucial for scientifically-based management of marine resources.

INTRODUCTION

It is a reasonable assumption that benthic marine species with planktonic larvae, which can spend days to months at the whim of currents, would be transported great distances and would disperse quite widely. This logical concept led marine ecologists for much of the 20th century to presume that most coastal benthic populations were "open" (reviewed in Caley et al. 1996, see Table 5.1 for definition of terms), that most of the new individuals settling into a given location originated elsewhere, and that

localized adult production and recruitment were decoupled (Swearer et al. 2002, although, see Crisp 1958, Iles and Sinclair 1982, Knowlton and Keller 1986, Scheltema 1986, and Sammarco and Andrews 1988 for earlier exceptions in open-coast organisms). In other words, marine populations were considered to be highly "connected" through larval transport. Recent technological advances coupled with recognition of the importance of larval behavior and mortality, physical variability, and oceanographic retention features have led to the beginning of a paradigm shift in recent years (reviewed by Swearer et al. 2002, Levin submitted), focusing on evidence of and mechanisms leading to "closed" populations, where "self-seeding" or "self-recruitment" occurs at smaller spatial scales. It is not expected that all benthic populations are dichotomously either open or closed. Populations likely represent a continuum of connectivity and are "ajar" to varying degrees and at different scales. In this study, a relatively new method, elemental fingerprinting, was used to explore the population connectivity of mussel populations (*Mytilus californianus* and *M. galloprovincialis*) in San Diego County, California.

IMPLICATIONS, EVIDENCE AND DESCRIPTION OF MARINE POPULATION CONNECTIVITY

Understanding the degree of larval connectivity and retention within marine populations has crucial implications for the study of evolution and ecology (Warner 1997, Strathmann et al. 2002, Levin submitted). For example, if connectivity between many benthic populations is considerably lower than previously thought, then it is

likely that the scale at which gene flow and adaptation occurs is also smaller. On ecological time scales, general population dynamics (reviewed in Caley et al. 1996), including specific parameters such as local density dependence (Strathmann et al. 2002) and predator-mediated coexistence of species (Caswell 1978), will be greatly affected by the amount of recruitment from outside of a given population. Although occasional long-distance dispersal has important consequences (Levin submitted) in terms of genetic heterogeneity, biogeography and colonization events, sporadic exchange among widely distributed populations will have less influence on population demography than a large proportion of self-recruitment (Strathmann et al. 2002).

It is crucial that marine resource and fisheries managers determine the appropriate scale of larval dispersal and connectivity among populations and stocks (Fairweather 1991, Warner and Cowen 2002). For example, managing fisheries as larger, open populations when there is a high degree of self-recruitment can lead to recruitment overfishing (Strathmann et al. 2002). Our ability to control and predict the effects of direct human disturbance (Strathmann et al. 2002), climate change, emerging diseases, and the spread of invasive species (Levin submitted) will be greatly improved with knowledge of the scale of population connectivity.

Over the past two decades, there has been increasing interest in the use of spatially-based management tools, such as marine protected areas (MPAs), to conserve marine natural resources. Although MPAs are designed with a number of different goals in mind, many are expected to serve as a source of larval production to enhance populations outside of the MPA (Planes et al. 2000). As populations outside

of reserves are increasingly exploited, the degree of self-recruitment within a given MPA as well as the connectivity between protected populations and among protected and exploited populations, will determine the sustainability and utility of specific MPAs. Therefore, larval connectivity is a crucial parameter to include when designing MPAs or reserve networks (Planes et al. 2000, Warner et al. 2000, Shanks et al. 2003).

While discussing conceptual issues related to designing marine reserves, Carr and Reed (1993) define four general models (Figure 5.1) of "larval replenishment" or larval connectivity among local marine populations, that represent a continuum between "open" and "closed". In the "multiple source" model, local populations contribute larvae to a common larval pool, mix, and eventually return back to settle in the various populations in similar proportions. This can be modified to the more general case of a high level of exchange between populations, regardless of whether the larvae spend the planktonic period in a single common pool. A "single source" model occurs if one population contributes most of the new recruits to the other populations, which do not contribute much to their own replenishment. "Limited distance" populations contribute to their own replenishment and nearby populations, but have little connectivity with more distant populations. This would include exchange between continuous populations and "stepping stone" relationships between more disjointed populations. If populations only replenish themselves and contribute little to other populations, the system would be considered "closed". These generalized models could be expanded to a number of other more-complicated

situations, such as two essentially open populations separated by a barrier (Hellberg et al. 2002). In addition, these patterns might change over time. For example, "Hedgecock's Sweepstakes Hypothesis" (Hedgecock 1994) describes a case where a small number of individuals, whose reproduction is advantageously timed, contribute the bulk of new settlers to a given population, but due to random chance the lucky parents vary between cohorts. This could be considered a single source population model where the source changes through time. The number and configuration of appropriate MPAs would vary depending on which of these model types dominates in a specific set of populations.

Although determining which of these simple models of population connectivity occurs is important, it is quite difficult. Commonly, mathematical models that consider larval connectivity use a scale of larval dispersal estimated from laboratory-determined pelagic larval duration (PLD) and mean advective current flow (Sponaugle et al. 2002, see Scheltema 1986, Widdows 1991, Carr and Reed 1993, for examples). These estimates are likely to be highly inaccurate for predicting actual dispersal distance for a variety of reasons. PLDs determined in laboratory cultures do not necessarily reflect realistic conditions, can vary greatly with local environmental conditions, and often do not account for the high level of plasticity in most aspects of larval life history (Scheltema 1986, Hadfield and Strathmann 1996). The use of mean advective flow to describe actual transport oversimplifies much of the complexity found in existing oceanographic data (Sponaugle et al. 2002, Largier 2003). Although the importance of larval behavior in affecting transport into and out of estuaries has

long been recognized (e.g., Prytherch 1929, Bousfield 1955, review in Scheltema 1986), the complexity of including behavior in coastal estimates of transport distances is often ignored. Accounting for additional parameters, such as larval mortality, habitat availability, and diffusion within models of transport can lead to much lower larval transport rates (Scheltema 1986, Cowen et al. 2000, Largier 2003, Shanks et al. 2003, Levin submitted). Shanks et al. (2003) compared reported PLDs and empirically determined mean transport distances and found that in most cases, larvae did not travel as far as would be expected by simple advection.

However, the logic and simplicity of using just PLD and average currents has led ecologists to consider self-recruitment of open-coast populations of species with longer PLDs (weeks) on smaller (10s of km) scales highly unlikely (e.g., Widdows 1991, Caley 1996). A number of recent studies using "indirect" methods (sensu Warner and Cowen 2002) have contradicted this notion that self-recruitment is a rare event (reviewed in Swearer et al. 2002, Levin submitted). For example, the persistence of isolated, endemic, and upstream species (Scheltema 1986, Mullineaux and Mills 1997, Swearer et al. 2002), as well as a lack of relationship between geographic isolation of species and their PLD (as reviewed in Sponaugle et al. 2002), challenge the notion that larvae that are planktonic for a long period of time are necessarily transported away from their natal origins. Tracking the spread of introduced species can help define the scales of successful larval transport and often the spread of these species is slower than would be predicted (e.g., Crisp 1958, Geller 1994, McQuaid and Phillips 2000). A number of modeling studies focusing on coral

reef fish species have also concluded that self-recruitment is possible and often necessary to sustain populations (e.g., Schultz and Cowen 1994, Cowen et al. 2000).

On longer time scales, speciation of populations within potentially overlapping dispersal ranges (reviewed by Hellberg et al. 2002) also implies that limited dispersal of planktonic larvae commonly occurs. Since genetic heterogeneity can be greatly reduced by as few as one migrant per generation, molecular evidence of lack of gene flow between adjacent populations implies a lack of connectivity over evolutionary time scales (Slatkin 1987). In other words, a lack of genetic differentiation does not imply that populations are completely "open", but evidence of differentiation indicates a strong lack of connectivity between populations over many generations. A small number of molecular genetic studies have indeed demonstrated some level of differentiation between populations that superficially appear to have the capability to exchange larvae (reviewed in Hellberg et al. 2002, also Taylor and Hellberg 2003).

Direct evidence of self-recruitment has been more challenging to collect, mostly due to the difficulty of tracking all but the largest and shortest-lived marine larvae for their whole PLD (e.g., Olson 1985, reviewed in Levin 1990, Gaines and Bertness 1993, Thorrold et al. 2002, although see Paris and Cowen 2004 for a recent example using reef fish). Artificial mark-recapture studies, in theory, should provide invaluable and unequivocal information on larval population connectivity, but have had limited success due mostly to the difficulty of recapturing marked larvae (Levin 1990, Thorrold et al. 2002). To date, the most successful example of artificial tagging of larvae is Jones et al. (1999). Of the 10 million damselfish embryos they stained on

Lizard Island on the Great Barrier Reef, they recovered 15 marked recruits, which corresponded to 15-60% self-recruitment. This study is widely credited as one of the first two to provide direct evidence that coral reef juvenile fish with a relatively long PLD (18-21 days) can return to their natal site.

One way to improve the recapture rates of larvae is to use a "natural" tag, something unique that is related to the environment (such as trace elements, isotopic ratios, growth patterns, or genetic markers) that essentially marks all of the larvae in a given area. For example, "elemental fingerprinting" takes advantage in differences in the chemistry of growing hard parts, such as otoliths, scales, shells, and statoliths imparted by waters in different locations (reviewed by Campana 1999, Campana and Thorrold 2001, Thorrold et al. 2002, Levin submitted). The clear advantage of using this approach is that all organisms receive the tag, and it also limits the amount of handling and potential artificial behavior or mortality due to the tag itself (Thorrold et al. 2002). This sort of approach has been increasingly used to study the movements, stock structures, and behaviors of juvenile and adult fish using the trace elemental chemistry of their otoliths (e.g., Campana et al. 2000, Thorrold et al. 2001, Forrester and Swearer 2002). A smaller number of studies have used this approach as a larval tracking tool for fish (e.g., Radtke et al. 1990, Milton et al. 1997). Swearer et al. (1999), analyzed the chemistry and growth of the otoliths of recently-settled bluehead wrasse on the island of St. Croix to discriminate between larvae developing in coastal and open ocean waters. They assumed that larvae in coastal waters would grow quicker and retain more trace elements as they grow, and that they could be considered

as self-recruits if they remained in coastal water during their development. Their results indicated that populations on the leeward side of the island received much of their recruitment from local sources, despite the long PLD of this species (approximately 45 days). This study, which was published in the same journal issue as Jones et al. (1999), is also one of the first to provide direct evidence of self-recruitment in coral reef fish.

Although most elemental fingerprinting studies have focused on fish, there are many invertebrates that form larval hard parts that can potentially contain natural tags for larval tracking. DiBacco and Levin (2000) and DiBacco and Chadwick (2001) used the elemental fingerprints of whole crab zoeae collected at the mouth and inside San Diego Bay to determine whether they originated in the embayment or along the open coast. They successfully applied the method to determine the effect of vertical position and tidal cycle on transport, and to explore the consequences for bay-ocean larval exchange. In order to determine the natal origin of a settled individual, a larval hard part needs to be retained after settlement, such as a molluskan statolith or larval shell; unfortunately, decapod larvae molt their carapaces a number of times during the planktonic stage. Zacherl et al. (2003) has successfully demonstrated that *Concholepas concholepas* "near-hatching" larvae collected from egg capsules at three sites in Chile contained spatially-distinct chemical signatures in their statoliths, indicating that these structures, which are retained after settlement, could contain useful natural markers for determining origins of gastropod settlers. Zacherl (in press), verified that *Kelletia kelletii* protoconchs, collected from benthic egg capsules

at various sites, contained unique chemical signatures. To date, these are the only studies to employ trace elemental fingerprinting in invertebrate larvae (although see Killingly and Rex 1985 for a study using stable isotopes to asses development zone in deep-sea gastropods). None have applied the method to determine a natal origin of settled juveniles. In this study, the retained larval shell chemistry of settlers, combined with the elemental fingerprints of larval shells raised in situ, were used to determine the natal origins of mussel settlers collected in San Diego County.

It is important to note that much indirect and direct evidence makes a compelling case that self-recruitment often occurs in specific situations, although generalizing to broader contexts will take considerably more study (Mora and Sale 2002). There is also a strong need not to ignore past evidence of long-distance population connectivity (e.g., Scheltema 1968, Shanks et al. 2003), especially as a mechanism for colonization (e.g., Scheltema 1986) and maintaining biogeographic patterns (e.g., Scheltema 1971, Scheltema 1995). There are likely to be systems where either long-distance transport or self recruitment dominates, as well as those where a combination occurs (Scheltema et al. 1996, Largier 2003).

DEVELOPMENT OF LARVAL TRACKING TECHNIQUE

The conceptual approach of most elemental fingerprinting studies is to empirically determine reference signals from the possible sites of origin, and then compare these to elemental signatures of a structure that is retained in later life stages, when the individual is collected at a known site. A significant challenge to the

realization of this method becomes the determination of appropriate reference signals from known locations.

There are a number of ways to accomplish this task. If the organism of interest does not migrate great distances as an adult, the hard parts formed by post-settlement individuals at a known collection site can be compared to those formed during a larval dispersal phase (e.g., comparing bivalve dissoconch and prodissoconch chemistry). In past studies (Becker et al. 2005), it has been demonstrated that mussel dissoconch (or post-settlement) shell chemistry from eight sites in San Diego County can serve as a marker for four 20-km "natal regions" of collection using elemental fingerprinting. Unfortunately, mytilid dissoconch and prodissoconch (or larval shell) chemical signatures are chemically distinct (Figure 5.2), probably due to the mineralogical differences between larval (aragonite, Bayne 1976, Fuller and Lutz 1988) and adult shell (aragonite and calcite, Dodd 1964). In order to use dissoconch shell as a reference signal for mussels, studies must be done on the differing chemical responses of these shell parts to identical environments in order to model a predicted prodissonch elemental fingerprint.

Some marine organisms, including many gastropods, have a mixed life history that includes an encapsulated benthic stage in eggs or egg capsules that remain at the natal origin for some time before they hatch to become dispersing larvae (Pechenik 1979, Levin and Bridges 1995). Assuming that larvae developing in these capsules can incorporate signals from their environment that are retained after settlement, the collection of larvae of known origin can be achieved by collecting these egg capsules

before hatching. The work of Zacherl et al. (2003) focuses on gastropod species, such as *Kelletia kelletia*, with a benthic portion of their larval stage. Although this approach shows great promise for species with this type of life history, it will not be applicable to the large number of species with entirely planktonic larval phases, including mytilid mussels.

A similar concept to collecting benthic egg capsules would be to collect planktonic larvae at known locations to determine their chemical signature. This approach would be difficult, since plankton samples would be time consuming to sort, and identifying a large number larvae to species is difficult to do without destroying them or exposing them to a number of chemicals (e.g., Coffroth and Mulawka 1995, Paugam et al. 2000) that might lead to contamination for trace metal analysis. More importantly, this method would be flawed since larvae could have been transported great distances before capture, so the known location of the signal would be dubious.

Larvae can be cultured in controlled conditions in order to generate a specific signal. For example, cultures could be created using seawater from various locations (e.g., DiBacco 2000). This approach would be challenging, since it would be complicated to control for real-time temperature conditions and to mimic specific food conditions with sufficient nutrition for the growing larvae.

Alternatively, larvae can be outplanted and grown in the field, which would control for these environmental factors and create a more accurate simulation of actual conditions. In this study larvae of mytilid mussels were raised in situ to somewhat realistically mimic the conditions that wild larvae experience, and to determine the

trace element signatures imparted to larval shell laid down at different locations. A number of researchers have successfully raised larvae in the field for other purposes (reviewed in Davis et al. 1996), such as determining near-natural mortality (Planes 2001), starvation (Olson 1985, Olson 1987), predation (Johnson and Shanks 2003), and growth rates (Stoecker et al. 1983)).

In this study, the natal origin of mytilid mussel juveniles were determined by comparing the chemistry of their prodissoconchs with that of larvae cultured in situ at 13 known sites throughout San Diego County. These natal origins were used to explore the connectivity between local populations. Specifically, the model or models of larval connectivity that best describe the *M. californianus* and *M. galloprovincialis* populations in San Diego were determined: single source, multiple source, self-seeding, or nearest neighbor.

In order to address this main goal, a number of smaller questions were addressed. Can larvae cultured at various sites (or in various "natal regions") be discriminated using shell chemistry? Most importantly, is there evidence that self-seeding can occur on small (~20 km) spatial scales? This is the scale at which the chemistry of juvenile dissoconch was successfully used to discriminate between sites in the past (Becker et al. 2005), and represents the appropriate minimum scale at which this method can be used. Clearly every population will be completely open when examined at very small spatial scales and completely closed when considered across its range. Warner and Cowen (2002), while describing an NCEAS working group evaluating the evidence of larval retention in marine populations, decided to

focus their efforts on a spatial scale of kilometers to tens of kilometers and less than a generation time; similar scales are considered in this paper.

The use of in situ larval culturing can expand the application of trace elemental fingerprinting for tracking larvae to species with a wholly planktonic larval phase. This study is unique in a number of additional ways. This is the first application of the method to determine the natal origin of a settled invertebrate. Many sites (13) within a relatively small length of coastline (approximately 75 km) were used, providing for greater resolution of chemical signals than has often been used as a reference. Many studies using larval tracking focus on areas with large chemical differences expected a priori, such as estuaries. I focus on some embayments that fit this description, but also discriminate between two natal regions along a mostly linear exposed coastline. Many of the recently identified systems with larval retention features are located on isolated islands or reefs with some known physical retention mechanism and an expectation of the need for self-recruitment for population persistence (e.g., Sammarco and Andrews 1988, Schultz and Cowen 1994, Scheltema et al. 1996, Jones et al. 1999, Swearer 1999, Cowen et al. 2000). Sponaugle (2002) stated that "where sites are largely contiguous, the degree to which self-recruitment is important is largely unknown". This study greatly expands our understanding of mussel population connectivity in San Diego County, but also more generally provides one of the first explorations of invertebrate population connectivity in the coastal zone.

METHODS

DESCRIPTION OF SPECIES

In this study, I determine the natal origins of early settlers of two species of mussel, *Mytilus californianus* Conrad (commonly called the California or sea mussel) and *M. galloprovincialis* Lamarck (the bay, blue, or Mediterranean) mussel. These species were chosen as a model system for a number of reasons. There is a conservation need to understand population connectivity in mytilid mussels in San Diego County. In the past decade, *M. californianus* has been experiencing an alarming decline in cover within plots in Cabrillo National Monument, a National Park within the study area (Engle and Davis 2000, Chapter 2 of this dissertation). Due to their formation of large beds that house over 300 taxa of animals and plants (Suchanek 1979, Suchanek 1992), and their role as prey (Paine 1974) and competitors for space (Dayton 1971), mussels are important components of rocky intertidal ecosystems. In addition, mytilid mussels settlers are easy to collect and spawn, making them good species for this study.

The distribution of *M. californianus* is limited to the northeastern Pacific, from the Aleutian Islands to northern Mexico (Seed 1992). *M. californianus* can live subtidally but is commonly found on exposed rocky shores in the middle-intertidal (Coan et al. 2000).

M. galloprovincialis is part of the *M. edulis* complex, a group of three closely related species of bay mussels: *M. edulis*, *M. trossulus*, and M. *galloprovincialis*. Until recently, it was believed that all bay mussels on the west coast of North America

were *M. edulis*. It was discovered in 1988 that on this coast there were actually two species of bay mussel, *M. trossulus* and *M. galloprovincialis*, and *M. edulis* was not present in wild populations (Mcdonald and Koehn 1988). Generally, *M. trossulus* is found in northern California and further north, *M. galloprovincialis* is found in southern California and further south, and an area of hybridization is found between them in central California (Mcdonald and Koehn 1988, Geller 1994, Suchanek et al. 1997). However, more fine-scale studies have found many exceptions to that general pattern. For example, Suchanek et al. (1997) determined that although Scripps Pier contained only *M. galloprovincialis*, San Diego Bay contained a combination of species. It is believed that *M. galloprovincialis* is native to the Mediterranean and northern Europe (Geller 1999) and that it was introduced to southern California in the 20th century (e.g., Coe 1946).

Although members of the *M. edulis* complex have been the subject of many ecological and physiological studies, the confused taxonomy of these species means that in articles prior to 1988, *M. edulis* is used to describe the other two species. Studies describing *M. edulis* and *M. trossulus* are relevant to this study of *M. galloprovincialis* for two reasons. Firstly, much of what is known about the ecology and life history of *M. galloprovincialis* in southern California is reported in past literature that described this species as *M. edulis*. Secondly, the sibling species in the *M. edulis* complex are closely related, especially *M. edulis* and *M. galloprovincialis* (Hilbish 2000, Martínez-Lage et al. 2002) and are capable of interbreeding (Seed 1992). They are therefore likely to share many ecological and developmental

characteristics (although see Seed 1992 for exceptions). Many more studies have been conducted on the life history and development of *M. edulis* than other mytilids, and it is assumed that these studies can be applied to *M. galloprovincialis* as well. When earlier work on *M.* "*edulis*" is described, a likely actual species identity will be provided, assuming that bay mussels studied on the west coast of North America were either *M. galloprovincialis* (south of San Francsico, CA) or *M. trossulus* (north of San Francisco, CA) and those studied in northern Europe were *M. edulis*. In this study all local bay mussels will be referred to as *M. galloprovincialis*, although the possibility exists of an occasional *M. trossulus* or hybrid juvenile being included in the San Diego Bay sites; all larvae were spawned from individuals collected from SIO pier where only *M. galloprovincialis* has been reported (Suchanek et al. 1997).

M. californianus is mostly found in wave-exposed coastal areas, and are generally not tolerant of conditions in bays and harbors (Harger 1968). *M. galloprovincialis* is found in calm water as well as on the open coast, where it competes with the larger congener. When in wave-swept environments, *M. californianus* is generally the better competitor, as it is bigger, more robust, and more tolerant of high wave action (Suchanek 1981), and is usually the dominant mussel in local open coast populations. Members of the *M. edulis* complex act as "fugitive" species compared to *M. californianus*; they are smaller and more mobile, grow faster, mature earlier, and are quite tolerant of various environmental conditions (likely *M. galloprovincialis*, Harger 1968; likely *M. trossulus,* Suchanek 1981). Other aspects of the life histories of these species will be considered throughout this paper.

IN SITU LARVAL CULTURING

Larvae of *M. californianus* and *M. galloprovincialis* that were spawned in the laboratory were cultured for one week at thirteen sites throughout San Diego County. Individuals were outplanted as embryos without visible shells, so the resulting larval shells were formed entirely in the field. These shells were analyzed for a suite of trace and minor elements in order to characterize spatially-distinct elemental fingerprints.

Spawning

M. californianus and *M. galloprovincialis* adults were collected from the Scripps Institution of Oceanography (SIO) pier on May 11, 2003 for spawning that evening. All tools and containers used for spawning and culturing mussel larvae were made with non-metal materials that had been previously acid washed in nitric acid; no glue or adhesives were used to construct the various sieves and larval traps. Throughout the process, mussels were only exposed to seawater that had passed through the general SIO sand filters, additional 20-µm and 1-µm filters and then an aquarium UV sterilizer (hereafter called "sterilized seawater") Care was taken not to mix gametes from the two species.

Individuals were "roughed up" (including knocking off fouling barnacles and pulling on byssus) and then plunged into sterilized seawater that was heated in a water bath (at 20-22^{o}C, ambient water was approximately 17^{o}C). Multiple mussels of the same species were heat shocked in the same acid-washed container. The first

individuals initiated spawning within 2 hours. All spawning individuals were isolated into separate containers of fresh sterilized seawater, with up to two individuals of the same sex in a single container. A trace amount of sperm from the appropriate species and some *Isochrysis* sp. (Instant Algae Premium 1800, Aquatic Ecosystems) were added to the buckets of individuals that had not spawned yet to encourage spawning. Over a period of 5 hours, a total of one male and one female *M. galloprovincialis* spawned and four male and one female *M. californianus* spawned.

Eggs were fertilized in batches in order to minimize the aging of gametes. Larger pieces of debris (e.g., pseudofeces) were removed by filtering the eggs through a 125-µm sieve. Sperm was added to the filtered eggs until a faint discoloration was noted in the water. The sperm from all four *M. californianus* males were used in each fertilization batch of that species. Fertilization was only allowed to occur for 10-25 minutes in order to minimize polyspermy, which mytilid mussels are susceptible to. At that point, the egg-sperm mixture was filtered through a 35-µm sieve so that the eggs were retained on the mesh and the unused sperm passed through; the eggs were then rinsed into a clean container and aerated.

Each batch of eggs was added to a single large plastic container which represented a mixture of all of the batches. The embryos were later split into multiple clear polycarbonate buckets, diluted, and aerated, but not fed. All buckets were put in a water bath of flowing seawater with a temperature logger (Onset StowAway TidbiT), collecting data every 10 minutes. The total time from the beginning of the first to the end of the last fertilization was approximately two hours. Four hours later,

multi-celled embryos appeared healthy and were swimming in spirals. The concentrations ranged from 200 to 800 individuals per milliliter.

Larval home design

Larvae were outplanted in "homes" which consisted of a 1.5 inch PVC pipe coupler, a 14 cm-long piece of pipe with an open cap on either end. The caps were removed, a small square of 35 μm nitex mesh was placed over the end, and the caps were then screwed back on. The inner volume of this trap was 215 ml. Prior to this experiment, the homes were leached of contaminants in flowing seawater for approximately three months, and soaked in 10% nitric acid for 10-14 days. The mesh was rinsed well with Milli-Q water, soaked in 1% nitric acid for 24 hours, and then soaked in Milli-Q water for approximately one week. In order to make the home negatively buoyant, a small (2 ounce) metal fishing net weight that had been dipped in "Plastidip" (Performix) and soaked in seawater was attached. Two homes of *M. californianus* embryos and one home of *M. galloprovincialis* embryos were outplanted at each site.

Two types of laboratory controls were established for each species. Larvae were raised in the laboratory in larval homes, set up with all of the same connectors and weights as the field homes, in buckets of sterilized seawater. In addition, larvae were raised in buckets without larval homes to test for home effects. All laboratory samples were aerated and placed in a water bath of flowing seawater. The temperature logger placed in this bath earlier continued to collect temperature data in the laboratory

throughout the experiment. Once per day the water was changed and larvae were fed *Isochrysis* sp. A sample was also taken from all laboratory cultures and fixed in a small amount of formalin at that time to determine shell growth rates.

In order to reduce bias between larval homes and sites, homes were filled in a random order and care was taken to fill the homes with a mixture from all buckets of larvae of a species. Approximately 100,000 larvae were put in each home. This large number of larvae was used to compensate for the high expected mortality rate in the field. Homes were transported onto boats in large painter's buckets that were double lined and filled with sterilized seawater. The complete home-filling process was begun seven hours after the first batch of eggs were fertilized and took 2.5 hours to complete.

Outplanting

Thirteen sites were selected in San Diego County, California; most were offshore of a known source of mytilid mussels. Eight of the sites were on the exposed coast (Agua Hedionda, AH; Cardiff Reef, CR; La Jolla Dike Rock, LJDR; Scripps Pier, SIO; Pacific Beach/Crystal Pier, PB; Ocean Beach Pier, OB; Cabrillo National Monument, CABR; and Imperial Beach Pier, IB; see Tables 5.2 and 5.3, Figure 5.3). Buoys were placed at 10 m depth (from mean lower low water, MLLW) just offshore of a known adult population of mytilid mussels. Three sites were located at five to six meters water depth in San Diego Bay (SDB), a large embayment (4500 hectares at high tide, maximum depth 18 m, Fodrie submitted) that serves as a highly active

industrial, commercial and military port. Chula Vista (CV) is a site near the back of SDB with no known source of adult mussels. The other two sites, Harbor Island (HI) and Shelter Island (SHI) are located off of artificial islands near the mouth of the bay. One site was located in Mission Bay (MB), a medium-sized, shallow bay (900 hectares at high tide, 4.0-5.5 m average depth, Fodrie submitted) that is mostly used for recreational purposes. Crown Point Mitigation Site (CPMS) was located towards the back of MB in 3.5 m of water. Agua Hedionda Lagoon (AL), was located in 5.5 m of water in the outer section of a small (83 hectares at high tide, 3-4 m average depth, Fodrie submitted) embayment in Carlsbad, California; the site is in close proximity to a mussel aquaculture facility and a large power plant. Eight of these sites (CR, LJDR, SIO, PB, OB, CABR, CPMS, HI) were just offshore of coastal sites studied for trace elemental fingerprinting signals in juvenile mussel shells (Becker et al. 2005).

In order to minimize travel time from the laboratory to the sites, four boat crews were used to transport the larval homes. The AL larval homes were transported by car to Carlsbad and then deployed by boat in the same manner as the other sites. The larval homes were deployed on to the buoys in a manner that minimized their exposure to air. The total length of time from fertilization to deployment ranged from 8.5 to 15 hours.

Larval homes were outplanted on one buoy per site (Figure 5.4). Buoys were designed to standardize depth and minimize shaking due to swell. The buoys were constructed of nylon braided line with a surface and subsurface float, below which the larval homes and a temperature logger were attached. The buoy was designed so that

the larval homes would be attached 2 m below MLLW. Using predicted tides during this period, it was estimated that the larvae remained between 1.5 and 4 m below the surface, which is the approximate depth at which mytilid larvae have been found in nature (*M. edulis* in the White Sea, Dobretsov and Miron 2001).

After seven days in situ exposure to seawater at each site, the homes were retrieved, although the buoys with the temperature loggers were left in place for an additional week. The buoy with temperature logger at CPMS was lost between retrieval of the larvae and final pickup. Once back at the laboratory, the contents of the homes were filtered using the existing mesh and water collected from that site and examined for mussel larval survivorship and later for shell growth (see Appendix A). The control cultures were processed in a similar manner. Since the larvae were added as shell-less embryos, it was assumed that all larval shells found at the end of the deployment were formed in situ at the deployment site. The contents of the larval homes were stored in an acid-washed, 50 ml centrifuge tube at -20^{o}C.

Sample preparation for elemental analysis

All sample preparation of larval shells was done in a clean room, using acid-washed, non-metal supplies. Homes were processed in random order, as predetermined by a random-number generator. The larvae were separated from the rest of the organic and inorganic debris remaining in the tube through filtration (i.e., larvae were retained on acid-washed, 35-µm nitex mesh, rinsing away smaller

particles with Milli-Q water) and visually sorted with a pipette under a dissecting microscope.

The sorted larvae were put in a small Petri dish (one per site) and the remaining Milli-Q water was removed using a 10 µl pipette under the dissecting microscope, while being careful not to remove any individuals. From this point on, only quartz distilled Milli-Q (QD) or trace-metal free reagents diluted in QD water were applied to the larvae. Larvae were treated with hydrogen peroxide (15%, Suprapure, EM Chemicals through VWR) buffered in NaOH (0.05 N, Suprapure, EM Chemicals through VWR) for 10-11 hours in order to remove all organic material from the shells. Using a pipette under a dissecting microscope, the larvae were then rinsed in QD water three times. The larvae were then transferred in a minimal amount of QD water to a petrographic slide covered in double-sided tape (Scotch Brand), and allowed to dry under a Class 100 laminar flow hood (AirClean Systems). Slides were separated into six sections, and the larvae from a single home were mounted in each section. After the liquid evaporated, the location of the larvae was marked on the underside of the slide using a permanent marker, and the samples were stored in closed Petri dishes inside zip lock bags under a Class 100 laminar flow hood until analysis.

It should be noted that since these shells are very small and fragile (110 µm $\pm$ 12 µm length, 80 µm $\pm$ 10 µm width, mean $\pm$ 1 SD), a large number of shells were lost in this process. However, it was experimentally determined that this peroxide cleaning was necessary to provide for material that was comparable to juvenile prodissoconch.

COLLECTION AND IDENTIFICATION OF EARLY SETTLERS

Early settlers of mytilid mussels were collected at the same thirteen sites around San Diego County and their prodissoconchs were analyzed for minor and trace elements. The larval shell chemistry of these wild-caught juveniles was compared to the elemental fingerprints characterized using the larval outplanting to assess larval origins.

Collection

Juveniles were obtained from the intertidal zone by collecting substrate that mussels are known to settle into (adult mussels and turf-forming algae) from 13 sites in San Diego County, California during the period of June 3-6, 2003 (Table 5.2). In most cases (AL, AH, CR, LJDR, SIO, PB, OB, CABR, IB, SHI, HI), the collection sites were located directly inshore of the larval home buoy site. At CV, no suitable material could be found in the intertidal zone. Across the bay from this site on Coronado Island (2.2 km west of CV), some scattered boulders were found to contain a small number of adult *M. galloprovincialis*, which were collected to serve as a paired site for CV larval homes. At CABR, samples were collected separately from three management zones (CABR I, II, and III) of the National Park that administers this rocky bench. At AL the mussel sample was a clump of live but dislodged *M. californianus* found on the muddy shore of the lagoon; this sample was likely recently washed in from the large population of *M. californianus* at the mouth of the lagoon that was used as the paired site for AH.

Samples were frozen (-20^{o}C) in zip lock bags within two hours of collection and were sorted two to three months later. The material was examined under a dissecting microscope in Milli Q water in an acid-washed plastic dish and mussel juveniles (less than 3 mm maximum length) were removed using acid-dipped ceramic-tipped forceps (Fine Science Tools). Approximate settlement levels (defined as number of mussels found at time of collection less than 3 mm maximum length) were determined in both settling substrates by standardizing for search effort (Appendix B). Individuals were sorted into approximate size classes, and stored in acid-washed vials at -20^{o}C.

Since these juveniles were collected 14-17 days after the end of the outplanting period, it will be useful to know how old these individuals were at the time of collection. *M. edulis*, a species closely related to *M. galloprovincialis* (Hilbish 2000, Martínez-Lage et al. 2002), are competent to settle at approximately 260 µm regardless of temperature, although they can grow bigger if metamorphosis is delayed (in Denmark, Bayne 1965). Field-determined growth rates of young *M.* "*edulis*" (likely *M. trossulus*) range from about 8-12 mm/month (in Washington, Suchanek 1981), so 3 mm *M. galloprovincialis* juveniles are assumed to be approximately one to two weeks old. Coe and Fox (1942) estimate that *M. californianus* on SIO pier can grow 4-5 mm in the first month after settlement, so 3 mm *M. californianus* juveniles are approximately 18 days old. Therefore, juveniles 3 mm and smaller should have been developing in the plankton during the same approximate time frame as the larvae cultured in situ.

Sample preparation

All further processing of settler shells was done in a clean room using acid-washed, non-metal materials. Individuals from various sites were processed, mounted, and analyzed in random order, as determined by a random number generator. Mussels were kept on ice when not in the freezer to minimize repeated freezing and thawing of soft parts. The juvenile shells were split, and the soft parts removed and frozen for molecular genetic identification. The left valve was retained in a clean vial for future analyses and the right valve was further processed for chemical analyses for this study.

Beginning at this step of processing, only QD water and trace-metal free reagents diluted in QD water were applied to the juvenile mussels. Shells were placed in individual vials, to which 15% H_2O_2 (Suprapure, EM Chemicals through VWR), buffered in 0.05 N NaOH (Suprapure, EM Chemicals through VWR) was added for approximately 18 hours. The remaining liquid was removed with a 100 µl pipette (under a dissecting microscope to avoid accidental removal of the sample). Shells were rinsed in QD water, and 1% HNO_3 (OPTIMA grade, Fisher Scientific) was added for less than 10 seconds. The acid was removed and the samples were rinsed three times in QD water. They were stored in the vials containing a small amount of QD water under a class 100 laminar flow hood until they were mounted. Shells were mounted on to a petrographic slide covered in double-sided tape using a wet paint brush. Twenty to thirty individuals, in random order, were mounted on a single slide. The small amount of QD water used to transfer the shells was allowed to evaporate in

the clean room, and the slides were placed in closed Petri dishes inside of zip lock bags, stored in a laminar flow hood until analysis.

A total of 230 juvenile mussels were used in this analysis, with an average of 18 juveniles per site. More than 10 juveniles were analyzed for all sites except three (CV, CPMS, and SHI), where too few settlers were found. Mussels ranged in length from 663 to 3009 μm (1595 ± 544, mean ± 1 SD). In addition, one very large individual (6 mm) from CV was used since it was the only individual found at that site.

Identification

Visually, small individuals of *M. californianus* and *M. galloprovincialis* are very difficult to identify to species. A PCR-based assay, as described in Becker et al. (2005), was utilized to determine the species of mussel in 99 (43%) of individuals. It should be noted that this assay does not discriminate between *M. galloprovincialis* and the similar *M. trossulus*; it is possible that some of the San Diego Bay mussels were *M. trossulus* or hybrids between the two species (Suchanek et al. 1997).

The remaining 131 individuals did not amplify using the molecular genetic assay, probably due to the freezing and thawing associated with sorting and splitting. In order to identify these unknown mussels, a Discriminant Function Analysis (DFA, Systat 9) was conducted using the PCR-identified mussels as known grouping variables. The predicting variables used included site (quantified as percentage of identified *M. californianus* from the site), dissoconch shell chemistry (as described later in the methods), shell length/width ratio, and the angle of the hinge relative to the

ventral margin (as described in Martel et al. 1999, Figure 5.5). The accuracy of this combination of variables, as determined by using identified mussels as unknowns and predicting species identities, was quite high (54 of 58 *M. californianus* and 28 of 33 *M. galloprovincialis* identified correctly, 90% average classification success, Figure 5.6). The resulting discriminant functions were applied to the remaining 122 mussels intact enough to measure all of the variables in order to predict species identification.

Nine of the mussels were broken during processing, and although parts of the shell could be used for chemical analyses, accurate measurements of morphology were not possible. In these cases, a separate DFA using location, settlement substrate, and shell chemistry was used to identify the individuals. This method was less successful (57 of 63 *M. californianus* and 31 of 40 *M. galloprovincialis* identified correctly) than the complete DFA, but still provided for good predictive ability (86% average classification success).

CHEMICAL ANALYSIS

Larval and juvenile shells were sampled using a New Wave UP 213 nm laser ablation unit and analyzed using a Thermoquest Finnigan Element 2 double focusing, single collector, magnetic sector ICP-MS (Inductively Coupled Plasma-Mass Spectrometer). Methods were similar to those reported in Becker et al. (2005). The LA-ICPMS system was modified so that 2% HNO_3 (OPTIMA) was aspirated through the nebulizer, and this aerosol was mixed was the sample gas (He) in the spray

chamber. These conditions greatly improved signal strength and stability over sending the "dry" sample directly into the ICPMS with Ar sample gas.

Juveniles were analyzed using two laser lines (Figure 5.5), both with the same laser settings (30% power, 10 Hz, 20-μm spot size, 15 μm/s). The first line was on the dissoconch starting at the dorsal apex and ending 150 μm towards the posterior along a growth line. The second line was on the early prodissoconch (larval shell), and measured 75 μm perpendicular to the axis of growth. Larvae were analyzed individually with a single 75 μm line (25% power, 10 Hz, 40 μm spot size, 15 μm/s). This analysis completely destroyed the shells. Since samples from a single home were mounted together and six homes were mounted on a single slide, an individual from each home was sampled in rotating order in an effort to reduce bias.

The isotope menu consisted of ^{26}Mg, ^{48}Ca, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{88}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U. In order to correct for interferences in ^{138}Ba (and as a marker for the slide material), ^{118}Sn was also included. The slide and tape were sampled to determine if this set of isotopes would include contaminants from the slide. These results were examined in terms of raw counts, since Ca could not be used as a proxy for the amount of glass material being ablated. Because of this the same laser settings used for the various shell types were repeated on the slide. For most laser settings, the average amount of most isotopes in the slide was less than 1% of the average amount in the shells, and for almost all it was less than 6%. The exception was the amount of ^{63}Cu in the slides and tape, which averaged 12% of the juvenile prodissoconch average. As

in past studies (Becker et al. 2005), Sn was found to be a major component of the slides, and it was therefore not used in these analysis.

Glass standards spiked with trace elements (National Institute of Standards and Technology Standard Reference Material 612, 614, and 616; NIST) were analyzed at the beginning, middle, and end of each run day to account for machine drift and convert isotope intensities to "absolute" ratios. The laser settings for standards were 50% power, 20 Hz, 50 μm spot size, 10 μm/s, and 300 μm line length.

To determine isotope intensities, a chromatogram was generated for each element in each sample using Element Software, and resulting peaks were analyzed individually. A "peak" was defined as having a maximum value greater than three standard deviations above the mean of the background, and background levels were subtracted from peaks using linear regression of non-peak values. I calculated the raw count per second (cps, area under the peak) for each isotope in each sample. The background-corrected cps values were then multiplied by a correction factor generated by the standard (NIST), using recorded run times and linear estimations of machine drift. The sample cps values were then divided by the counts of ^{48}Ca, a rare isotope of Ca, which was used as an internal standard in order to standardize for the amount of shell ablated. These ratios were used for all resulting analyses, except for determination of the tape and slide values. It is important to note that due to a lack of matrix matched standards, the "absolute" ratios determined by this method, although consistent within studies using these standards, might not be directly comparable with studies using different standards (Becker et al. 2005).

STATISTICAL ANALYSIS

Resulting element ratios (X:^{48}Ca) were statistically analyzed with linear DFA to examine differences between the chemistry of larval shells raised at the various sites and to determine the effects of species and control treatment on the elemental signatures. A step-wise procedure was utilized to choose the element ratios to include in the analyses of larval shells by site (Becker et al. 2005). A DFA was done with all ratios (except Mg:Ca, which was not used at all), and repeated dropping one ratio at a time until all were above an *F to Remove* of 3.5. In order to maximize relevance, DFAs to examine species and controls were done with the same elements as used for the sites, regardless of the *F to Remove* value.

WATER COLLECTION AND ANALYSIS

During both deployment and retrieval of larval homes, two samples of local seawater were collected near every larval home buoy. In order to avoid contamination from the boat, water was collected at the bow while slowly moving against the prevailing current (Gasparon 1998). Bottles were rinsed three times with ambient water, filled and capped, and then placed on ice during transport. Water was filtered and acidified (Becker et al. 2005), and stored for later analysis.

Samples were filtered, acidified and diluted following the general methods of Field et al. (1999), except where deviations had to be made to accommodate this particular analysis. Samples were first passed through a 0.4-µm ceramic filter.

Between samples the ceramic filter was rinsed in acid and Milli-Q, and rinsed again using 100 ml of excess sample. Filtered samples were then spiked with Optima grade nitric acid in a 9 to 1 ratio and stored in acid-washed, 15 ml polystyrene centrifuge tubes. Acidified samples were diluted 40 fold with 3% Optima nitric acid in QD water and spiked with a 1 ppb In internal standard (Spex Certiprep Inc.). Samples were analyzed via solution-based ICP-MS following the guidelines of Field et al. (1999) for instrument and induction parameters. The element menu was Mn, Co, and U. The Element 2 software provided elemental concentration data that were later corrected for dilution in Microsoft Excel.

RESULTS

EVALUATION OF OUTPLANTING SUCCESS

Larval survival and growth rates

The larval survival rates (as determined by formation of larval shell) in the field, relative to laboratory samples, were quite high (1.84% survival of field larvae, 8.05% of larvae raised in homes in the lab), given the large number of potential sources of mortality in situ, such as predators, shaking, infection, and lack of food (see Appendix A). Natural mortality rates are reported to be around 10-20%/day (20-50% survival after 7 days, Widdows 1991). The outplanting experiment yielded larval shells that were mostly greater than 100 μm long that were formed entirely in the field at known locations. The size of the larval shells recovered from the field (average of 108.5 ±

12.7 μm long x 77.6 ± 10.1 μm wide, mean ± 1 SD) were comparable in size to those raised in homes in the laboratory (Appendix A).

While studying the artifacts of in situ larval culturing of the crown of thorns seastar, *Acanthaster planci*, Olson et al. (1988) suggested that it was important to limit the density of larvae inside in situ culturing chambers. In this study, I used extremely high concentrations of larvae (reported maximum natural concentrations of *Mytilus* larvae range from 1500-40,000 larvae/m^3, Bayne 1976), and mortality or growth rates within the homes cannot be used to infer actual mortality rates in nature. The goal of the outplanting was to culture as many individuals as possible to maximize the statistical power of the reference signal.

Shell chemistry of controls: Home and species effects

The effects of the larval homes (as determined by comparing larvae raised in the laboratory in larval homes or loose in buckets), although detectable, were minimal when compared to the differences between sites (see Appendix A). The differences between the species were somewhat more problematic, and could lead to site misclassifications. Since sufficient *M. californianus* larvae from diverse sites were successfully analyzed, elemental fingerprints developed using just this species were used to evaluate natal origins of *M. californianus* juveniles. In order to improve sample size and site representation for *M. galloprovincialis* (which were reared successfully at fewer sites), elemental fingerprints were developed using larvae of

both species but without using Co/Ca (which led to more differences between species) in the evaluation (see Appendix A).

CREATION OF LARVAL REFERENCE MODEL

M. californianus

The distinctness of the shell chemistry of *M. californianus* larvae raised in situ at sites in San Diego County was quite variable depending on the site based on DFA site classification success which averaged 49% and ranged from 0% classification success at PB to 100% at CPMS (Table 5.4).

Becker et al. (2005) found that for the study area, fingerprinting success is likely to be greatest if defined on a regional scale. This approach improves the accuracy of the signal greatly, and is more biologically appropriate due to the delay in shell formation of early larvae. A number of studies have shown that *Mytilus* larvae experience maximum shell growth during the early part of their development (Bayne 1965, Chícharo and Chicharo 2000), so once shell formation is initiated, the larvae are expected to grow a lot of shell relatively quickly. In laboratory cultures raised during the outplanting, D-shaped veligers grew to 100 μm in less than 40 hours after fertilization. This short delay in shell formation also implies that the first day of larval transport will not be "recorded" by shell chemistry. Therefore, larvae could be transported away from their natal site, but it is unlikely that a larva would be transported out of the approximately 20 km natal region in this period of time.

For this study, the regional assignments that led to the most predictive success (Table 5.4, Figure 5.7) were northern coastal (AL, AH, CR, LJDR), southern coastal (SIO, PB, OB, SHI, IB), outer bay (HI) and inner bays (CV, CPMS). When grouped in this manner, the predictive success of the elemental fingerprints was quite high (average 80%, 9 out of 12 sites higher than 75%).

In order to determine the robustness of these classifications, relative to random chance, the data were randomized (i.e., the element ratios for a single mussel were kept together but assigned a different site using a random number generator) ten times and the same DFA was run. The classification successes were then averaged and the variability determined using 95% confidence intervals (Figure 5.8). Using random data, average classification successes were much lower than the actual data described above (average 6% $\pm$ 0.57 by site, 31% $\pm$ 1.71 by region). At almost every site, the actual classification success was considerably higher than the randomly generated value, whether considered by site or region. The exception was PB, where actual site classification success was lower than the randomly generated value; the regional classification success for PB (100%) was considerably higher than that of the random data set (47% $\pm$ 5.44).

The element ratios most important in discriminating between *M. californianus* larval shells varied among regions (Figure 5.7). Due to the large number of sites considered, the resulting DFA model was complex, with the first four scores accounting for 96% of the variability. The inner bays were most distinct from the others in score 1, mostly due to high Co/Ca. The southern region and the outer bay

were distinguished by score 2 (mostly due to high Pb/Ca in the southern coastal region and high Cu/Ca in the outer bay) and score 3 (mostly due to high Mn/Ca in the outer bay). The northern region was best distinguished from the southern and outer bay regions by score 4, due to higher U/Ca and lower Ba/Ca and Sr/Ca in larval shells from the north.

M. californianus and *M. galloprovincialis* combined

Due to low sample size, elemental fingerprints were created using larval shell chemistry of *M. californianus* and *M. galloprovincialis* combined in order to compare with *M. galloprovincialis* juveniles. Similar to the *M. californianus* larvae, the distinctness of the elemental fingerprints of *M. galloprovincialis* and *M. californianus* shells combined (Table 5.5, Figure 5.9) varied with site (ranging from 0% to 73% classification success for PB and CABR, respectively), although the general site distinctness was lower for this analysis (42% average classification success). Assignment of cohesive regions for the combined species was not as clear as it was for the single species analysis, but the same scheme used for the single species analysis was a reasonable approach that provided for maximum comparability. When sites were grouped into these regions, the average classification success was considerably higher than for sites alone (67%), and varied between sites (ranging from 43% at PB to 90% at OB). It is interesting to note that the larval shell elemental fingerprints of larvae raised at CABR are somewhat distinct from the other sites, even within the southern coastal region. In almost every case, the classification successes were higher

than those generated using ten random data sets (site average 7% ± 0.58, region average 32% ± 1.79, mean ± 95% CIs, Figure 5.10). When considering classification site, PB was the only site where the actual classification success was similar or lower than the randomly generated one, whether considered by site or region.

The most important elemental ratios contributing to regional discrimination in the two-species analysis were somewhat similar to those in the one-species case (Figure 5.9). Higher Mn/Ca and Cu/Ca in the bay sites and higher Pb/Ca and Ba/Ca in the southern coastal sites accounted for most, but not all, of the variation (74% of dispersion attributed to DFA scores 1 and 2) between the regions. The higher U/Ca and Mn/Ca in larval shells raised at CABR led to the distinctness of the elemental fingerprint from this site. As in the single species analysis, the DFA was quite complex, with the first four scores accounting for 93% of the variation.

Environmental Data

The average temperature of all sites for the outplanting period was 16.1 ± 2.1°C (mean ± 1 SD, Figure 5.11). There were very large average temperature differences among the sites, with over a six degree temperature range between the warmest (CV, 20.8 ± 0.4°C) and coolest (PB, 14.0 ± 1.2°C) site. During the outplanting period, sites in the northern coastal region were considerably warmer (16.9 ± 0.9°C average) than the southern coastal region (14.4 ± 1.3°C average without SIO). The SIO site, which was placed in the southern coastal region using shell chemistry, was quite similar to the northern coastal region in terms of temperature (17.1 ± 1.2°C average). The

temperature at SHI, a site within SDB that was placed into the southern coastal region using shell chemistry, was also quite similar to that of the southern coastal sites. CV, a shallow site located near the back of SDB, was considerably warmer than all of the other sites. Unfortunately the temperature logger at CPMS, another shallow site located at the back of MB, was lost before it was retrieved. During the period from May 24 to 28, after the larval outplanting was over, all of the sites (except CV) were quite similar in terms of temperature.

There were very large temperature fluctuations within each site that correlated with tidal cycles. The difference between the minimum and maximum temperature during one day at a given site averaged 2.3^{o}C ($\pm$ 1.4^{o}C) and could be as high as 6.4^{o}C. Throughout the outplanting period, the warmest data point was 21.8^{o}C and the coolest was 10.7^{o}C.

The concentrations of Co, Mn, and U from seawater collected on the first and last day of larval outplanting are shown in Figure 5.12. Although there were significant differences between sampling times for one of the elements (Co, p<0.01, ANOVA, SYSTAT 9), the only element that differed significantly between regions was Mn (p<0.01, ANOVA, SYSTAT 9). Seawater Mn concentrations were generally highest at the inner bay sites (CV and CPMS) during both sampling days, and in the outer bay site (HI) on the last day.

SETTLEMENT AND IDENTIFICATION OF JUVENILES

Settlement by site and species

There were clear geographic patterns in the number of identified *M. californianus* and *M. galloprovincialis* settlers (Figure 5.13, see Appendix B). Of the mussels identified with PCR, no *M. californianus* were found in Mission or San Diego Bays. In Agua Hedionda Lagoon, all of the mussels that settled in attached algae were *M. galloprovincialis*, while all of the mussels found in a clump of *M. californianus*, likely dislodged from the outer mouth of the lagoon, were *M. californianus*. Samples from all open coast sites contained a mixture of both species. At most sites, more *M. californianus* were identified than *M. galloprovincialis* (Figure 5.13). However, at two southern coastal sites, CABR and OB, there were mainly *M. galloprovincialis* (93% and 75%, respectively). The most southerly site (IB), unlike its nearest neighbors, was dominated by *M. californianus* (88%). The amount of material sorted differed between sites; see Appendix B for a discussion of the relative settlement rates at each site.

DETERMINING NATAL ORIGINS OF JUVENILES

Mytilus californianus

The majority (88%) of *M. californianus* juveniles collected at sites in San Diego County originated from the northern coastal region, as determined by comparing the prodissoconch chemistry of these individuals to larval shell elemental fingerprints of individuals raised in situ (Table 5.6, Figure 5.14). More specifically, most of the

juveniles (81%) appeared to have originated from the Agua Hedionda area. Only 10% of the juveniles were predicted to have originated from the southern coastal region, mostly from the OB site. Two out of the three (67%) *M. californianus* settlers found at CABR and OB were identified as having a southern origin, but this sample size was too small to generalize about these sites. At IB, the most southerly site and one that was dominated by *M. californianus* settlement, 95% of the juveniles were predicted to have originated in the north. Surprisingly, three of the 125 *M. californianus* juveniles were predicted to have originated from the bays.

From this analysis, it appears as if self-recruitment of *M. californianus* has occurred within San Diego County, possibly at the site level (13 out of 125 juveniles or 10.4% originated at the site of collection), and probably at the regional level (64 of 125 juveniles or 51.2% originated at the region of collection). There is no evidence, however, of mostly closed mussel populations at the site level in most locations. The exceptions are at the two Agua Hedionda Sites, AH and AL (70% and 88% originate at AH and AL, respectively) and OB, where the single *M. californianus* juvenile was predicted to have originated at OB. On a regional level, the northern sites do appear to be mostly "closed" (87% of northern juveniles originated in the northern region), whereas the southern region has more varied sources that differ by site (SIO, PB, and IB have mostly northern origins, OB and CABR might have a more southern influence, although sample sizes are low).

Mytilus galloprovincialis

Compared to *M. californianus*, there appears to be a greater diversity of origins of *M. galloprovincialis* juveniles, as determined by comparison of prodissoconch chemistry to elemental fingerprints of outplanted larvae (Table 5.7, Figure 5.15). Of the 108 individual *M. galloprovincialis* juveniles analyzed, 45% had a northern coastal origin (mostly AH), 47% had a southern coastal origin (mostly OB), and 7% had a bay origin (mostly CPMS). There did not appear to be a clear pattern in region of origin as a function of collection site. Of the 28 *M. galloprovincialis* juveniles collected at CABR, about half originated from the northern coastal region (46%), half originated from the southern coastal region (54%), and none originated from the bays. There was no apparent pattern in origin region within the three management zones of the park.

As with *M. californianus* juveniles, there is some evidence that self-recruitment of *M. galloprovincialis* occurred during this study, with 10 individuals (9%) originating from the site of collection and 48 (44%) from the region of collection (Table 5.7, Figure 5.15). There was little evidence that *M. galloprovincialis* populations are mostly closed, when considered by site or region; in most cases, the number of individuals at a given site with the local or outside origins were fairly even. Harbor Island, the one bay site with a large sample size of *M. galloprovincialis* (21 individuals), was the only site with more than one individual predicted to originate in a bay (5 individuals or 25%), indicating that there is some degree of self-recruitment within the bay sites, although it is difficult to determine whether these individuals originated in San Diego or Mission Bay (Table 5.5).

DISCUSSION

EVALUATION OF OUTPLANTING EXPERIMENT

Determination of prodissoconch reference signals

I successfully cultured mussel larvae in situ and therefore acquired larval shell material formed at thirteen known geographic locations. The chemistry of these larval shells was distinct when considered by individual site and especially so when considered by region, differing notably from a random site assignment (Figures 5.8 and 5.10). The precision of the determined elemental fingerprints was greater when considering *M. californianus* alone compared to both species. This additional error in the determination of the background elemental fingerprints should be noted when interpreting the assignment of natal origins to *M. galloprovincialis* settlers.

The assigned transition sites between specific regions were slightly different from those applied to mussel juvenile dissoconchs collected in 2001 (Becker et al. 2005). For example, SIO and PB, which in the previous study were assigned to the northern and southern coastal regions, respectively, were both assigned to the southern coastal region in this study. In the previous study, these two sites were considered "transition" areas, and the classification success for both was quite low. It is, therefore, not surprising that their regional classification would vary, especially given the very low sample sizes at these two sites. The temperature at SIO was more similar to sites further north throughout the outplanting period, so it is possible that this site could be considered part of the northern region; there were very few juveniles

predicted to come from this site (1 *M. californianus* and 9 *M. galloprovincialis*), so this ambiguity did not affect the results greatly. The site at AL, although in an embayment, lumped with northern coastal sites rather than the bay sites. This is likely related to the small size and short residence time of water in the Agua Hedionda Lagoon. The residence time is likely artificially shortened since within AL there is a large power plant that draws an average of approximately 150 million liters per day (400 MGD, S. LePage, pers. comm.) of seawater from the lagoon for cooling purposes, effectively leading to an inequality between the import and export of water to and from the lagoon. This site had generally lower classification success by region, perhaps because the area represents a combination of the conditions experienced elsewhere. Similarly, the SHI site, although in San Diego Bay, was more closely related to southern coastal sites. The differences in chemistry of larval shells from sites varying distances into San Diego Bay is not surprising given the oceanography of this area. Although the inner bay is characterized by long seawater residence times (Largier et al. 1997), the outer part of the bay is highly influenced by tidal flows and with considerably more exchange with the open coast (Chadwick and Largier 1999).

The specific element ratios driving these patterns are quite complex. Some of the chemical signals are similar to those found in mussel dissoconch in this area in 2001 (Becker et al. 2005), such as generally higher Mn/Ca in mussels from the bays and higher Pb/Ca in mussels from the southern coastal region. The Sr/Ca ratios in larval shells were lower in the northern region during this study and higher in the previous study. This could be because the relationship between Sr incorporation and

temperature is opposite in aragonite and calcite (with higher temperatures, more Sr in calcite and less Sr in aragonite, Dodd 1965). The northern sites were warmer than the southern sites in both time periods, but the earlier study utilized the chemistry of juvenile shell (a combination of aragonite and calcite) while this study only included the aragonitic larval shell. Two of the element ratios, Co/Ca and Cu/Ca were not analyzed in the earlier study so cannot be compared. Of the four elements analyzed in seawater (Mn, Cu, Co, and U), only Mn differences between regions were significant, with higher levels in the bays. Shells of outplanted larvae in the bays generally contained more Mn than those from other regions. There are a number of reasons why there could be differences in shell chemistry from locations that do not have discernable differences in seawater chemistry, such as temporal integration, bioaccumulation, and additional environmental factors (discussed in Becker et al. 2005).

Determination of Natal Origins

The majority of *M. californianus* settlers in this study, regardless of collection site, are predicted to have originated in the northern coastal region; in fact most were assigned to the two furthest north sites in the study area, AL and AH. It is therefore possible that the actual natal source for most mussels comes from locations further north than the border of this study. It should be noted, however, that with the exception of Oceanside (Pier and Harbor, seven to ten kilometers north of AH), much

of the coastline near to and north of AH is sandy, and is not suitable adult mussel habitat.

Three *M. californianus* settlers were identified as originating in San Diego Bay, where there is no known source of this species. These results could have a number of explanations. Young larvae could have been transported into San Diego Bay (for example, from a population of *M. californianus* on the Zuniga Jetty, bordering the southern side of the mouth of SDB), where they were retained for much of their development. Johnson (2003) found *M. californianus* larvae within a harbor environment despite the lack of settlers found in this area. These individuals with a bay signal could also reflect inaccuracy in the original elemental fingerprints; six larvae were originally misclassified as originating at HI or CV when they had been cultured in situ on the open coast.

The *M. galloprovincialis* settlers appear to have a greater diversity of origins, with an approximately even number originating in the northern and southern coastal regions. Surprisingly, only 7% of the *M. galloprovincialis* appear to have originated in the bays and only six mussels were identified as coming from AL, given that most of the adult *M. galloprovincialis* live in these embayments. It is possible that mussels spawned in the bays or the lagoon were transported out of the embayments before shell development began (low residence times in outer Mission and San Diego Bays are documented by Largier et al. 1997 and Chadwick and Largier 1999), and continued their development offshore, where they picked up their chemical signature. Indeed, the two sites where the greatest number of settlers were predicted to originate

(AH and OB) are just offshore of embayments. DiBacco (2000) found that crab larvae (*Pachygrapsus crassipes*) raised in the lab in seawater from San Diego and Mission Bays experienced higher mortality than those in water from the open coast. It is possible that larvae that spend time in bays experience additional mortality or sub-lethal developmental effects, that lead to a selective advantage for larvae from the open coast. Another possibility for why so few *M. galloprovincialis* were found to originate in embayments is error in the original elemental fingerprints. Of the 51 analyzed larvae cultured in the bay sites, 16 were misclassified as coming from the open coast. Origins attributed to AL and AH, in particular, should be considered as part of the same region rather than by site, since the resolution of the elemental fingerprints is much higher by region than by site.

SELF-RECRUITMENT IN MUSSEL POPULATIONS

Mussel larvae have long been considered to be highly dispersed (Bayne 1976) based on a simple calculation of PLD and average currents, with dispersal distances on the order of 150 to 500 km (Widdows 1991). A number of studies have indirectly explored the actual dispersal ranges of various mussel species in various systems using different methodological approaches.

One striking and particularly relevant example is the spread of a single invading population of the non-native mussel *M. galloprovincialis* in South Africa over a period of four years; the spread was less than 100 km per year, and after four years, 90% of individuals were found within 5 km of the original population (McQuaid and Phillips

2000). The dispersal was highly directional and correlated to wind data, suggesting that *M. galloprovincialis* were transported like passive particles. Their limited transport was attributed to the variability of wind-driven currents.

Gilg and Hilbish (2003) used a combination of population genetics and oceanographic modeling to estimate the approximate scale of larval duration of *M. edulis* and *M. galloprovincialis* hybrids in southwest England. They found that dispersal typically occurs over distance of about 30 km and is usually less than 60 km.

By tracking the variability in mussel recruitment of four species of intertidal mussels on a variety of spatial scales on both coasts of South Africa, Harris et al. (1998) concluded that prerecruitment processes act at a relatively small scale (1-25 km). They also found a relationship between higher adult biomass and higher recruitment rates. However, since they defined their recruits as individuals from 1-10 mm in length, their approach did not allow them to discriminate among the effects of larval transport, differential settlement patterns, and post-recruitment processes. These results indicating self-recruitment is occurring in mussel populations on scales of 10s of km are consistent with these studies from other systems.

In addition, there are two studies that use drift tube tracking to approximate larval transport rates in San Diego County; these indicate that physical retention of passive particles on a time scale of weeks is possible and likely in this area. Levin (1983) released plastic drift tubes in Mission Bay once per season during spring tides for a year in order to simulate passive dispersal of polychaetes. Each tube contained a note informing the person who finds it to contact the researchers. Of the tubes

recovered outside of the bay, the vast majority of them traveled south, except during winter storms when they were transported very far north. During May 1980, the same month as this experiment, the tubes were recovered close to the mouth of the bay (between La Jolla and the Mexican border) and moved an average of 2.3 $\pm$ 4.3 km within two weeks. These results are consistent with the observation of mostly southerly transport of mussel larvae during May 2003. They also illustrate that during different oceanographic conditions, very different transport trajectories can exist.

Tegner and Butler (1985) used identical drift tubes to study the dispersal of abalone in southern California. They released 1200 test tubes throughout the Southern California Bight. Of the 175 tubes released in San Diego county (La Jolla), 64% (spring) and 70% (fall) were found within one kilometer of the local release area, with an average time of recovery of 3.9 to 7.6 days respectively. Additionally, of the 83 tubes recovered in the area, 90% of them originated locally. They concluded that although some transport was possible over long distances, the majority of drift tubes floated only a few kilometers and landed in habitat that was suitable for green abalone. This study shows in a general way that physical mechanisms leading to retention of passive particles exist in this area.

There are a number of known physical mechanisms that could lead to self-recruitment despite the high likelihood of advection of larvae away from their natal origin (reviewed by Sponaugle et al. 2002), some of which are likely to occur in San Diego County. Physical retention mechanisms include the complexities of flow in coastal environments, such as tidally-oscillating currents, variability with depth or

time, and eddies in the lee of headlands (Winant and Bratkovich 1981, Chadwick and Largier 1999, Dibacco and Chadwick 2001).

CONNECTIVITY MODELS FOR *M. CALIFORNIANUS* AND *M. GALLOPROVINCIALIS*

Since most of the *M. californianus* juveniles in the study region appear to have originated from a single region, these populations are best described by the "single source" model (Figure 5.16), with the northern coastal region as the main source. Determining the appropriate connectivity model for *M. galloprovincialis* is more difficult than in *M. californianus*. However, it appears as if the "multiple source" model is the most parsimonious given the greater diversity of origins of *M. galloprovincialis* juveniles (Figure 5.16).

DIFFERENCES BETWEEN SPECIES: ALTERNATE HYPOTHESES

In order to generate hypotheses addressing reasons for the differences between the connectivity patterns of these two species, it is necessary to contrast what is known about their life histories. Since I studied these two species in the same location and at the same time, physical mechanisms alone without some biological difference in how the larvae of both species interact with physics can not explain the dissimilarities found between their connectivities. For example, a number of studies have shown that the prevailing average surface current is usually southward just offshore of San Diego County (Winant and Bratkovich 1981, Roughan et al. in press); this observation is

consistent with the connectivity pattern determined in *M. californianus* (most settlers originated upstream) if simple average surface currents explain the trajectory of these larvae. *M. galloprovincialis* settlers had a range of sources, and therefore a more complicated explanation must be invoked. Biological mechanisms influencing the larval trajectories include timing of spawning, differences in PLD, larval behavior, and distribution of spawning stock (reviewed by Sponaugle et al. 2002, Kingsford et al. 2002).

Timing of spawning

Differences in the timing of spawning (on seasonal or tidal scales) between the two species could lead to large differences in initial transport trajectories (Sponaugle et al. 2002, Kingsford et al. 2002). On longer time scales, differences in spawning season could expose larvae to very different physical transport conditions. Curiel-Ramírez and Cáceres-Martínez (2004) report that both *M. californianus* and *M. galloprovincialis* from northern Baja California are reproductive at some level all year, with *M. galloprovincialis* fertility peaking from October to March and *M. californianus* peaking from December to August with a maximum in February through June. This and a number of additional studies of *M. trossolus* (Suchanek 1981, Petersen 1984), which is closely related to *M. galloprovincialis* (Hilbish 2000, Martínez-Lage et al. 2002), have noted that they tend to completely spawn in seasonal pulses, while *M. californianus* will partially spawn throughout the year. It should be noted that in their literature review of these species on the west coast of North

America, Curiel-Ramírez and Cáceres-Martínez (2004) document a surprisingly diverse list of spawning seasons in the region determined with a number of different methods. Environmental influences can undoubtedly lead to interannual and geographic variability in spawning season. Since both species were collected during the same time period (June), these seasonal differences in timing of spawning could not have led to differences in connectivity patterns. However, this study was not conducted during the peak reproductive season for *M. galloprovincialis* (and indeed I had more trouble inducing spawning in this species) and the patterns found might not be representative of typical or demographically relevant larval connectivity.

In order for timing of spawning to have affected the larval transport of the two species during this study, there must have been a difference over small time scales that exposed the larvae to different transport conditions (e.g., tidal phase, varying currents). Very little is known about the natural spawning timing of either species, probably because it apparently occurs during high tide in often treacherous conditions for swimmers. Interestingly, Gosselin (2004) observed two mass spawning events of *M. californianus* at low tide. Although it is highly unlikely that spawning at low tide is a common occurrence (consider the number of hours that researchers have observed intertidal mussel populations at low tides and the lack of reports of this behavior), this report has some interesting implications. At least 35% of the mussels within a small area (10s of meters) spawned synchronously, but individuals just outside of these areas could not be induced to spawn. Therefore, the widely-reported observation that *M. californianus* is a "dribble-spawner" might be a mischaracterization, and perhaps

individual populations of this species spawn synchronously at different times throughout the year (Gosselin 2004). Synchronous spawning in *M. californianus* populations in the northern coastal region could have led to the observed pattern of most of the juveniles originating in that area, and perhaps the major source of mussels in this region will vary depending on which populations are spawning at that time (i.e., a "Sweepstakes" model of larval connectivity). In order to test this hypothesis, elemental fingerprinting experiments, such as in the present study, would need to be repeated. This aspect of mussel life history is virtually unstudied in nature and will be an important factor when attempting to identify life history differences influencing population connectivity.

Planktonic Larval Duration (PLD)

The length of time that larvae are in the plankton and their flexibility in settling time after competence is a major determinant of transport distances and therefore connectivity (Sponaugle et al. 2002). Although members of the *M. edulis* complex are some of the best-studied bivalves in terms of larval development (Lutz and Kennish 1992), the variable life history in these species complicates prediction of its actual PLD (Bayne 1976). Bayne (1965) gives a detailed description of larval development of *M. edulis* (from Denmark) fertilized and cultured in a laboratory. He found that the time to competence varied greatly, ranging from 16 to 70 days from fertilization, depending on temperature and food supply. At 16^{o}C (the average temperature during this outplanting was 16.1, but varied greatly, Figure 5.11), with optimal food

conditions, mussels reached the pediveliger stage (and were competent to settle) after 16-20 days. A similar PLD estimate was made for *M. galloprovincialis* raised in the laboratory (14-21 days at 18-20^{o}C, Satuito et al. 1994). Bayne (1965) also noted that *M. edulis* pediveligers could delay metamorphosis after reaching competence for quite a long time (an additional 2-40 days, depending on temperature) if settlement substrates were not available and still settle successfully. Later studies documented that this delay could be as long as 45 days at 16^{o}C (Pechenik 1990). By tracking cohorts in the plankton in southern Portugal, Chícharo and Chicharo (2000) estimated that *M. galloprovincialis* larvae were in the plankton for six weeks (19-27.5^{o}C), but they felt that the lack of settlement sites in the area had led to a significant delay of metamorphosis. From these reports, it appears as if the practical PLD for *M. galloprovincialis* could be as short as two weeks and as long as two months, depending on whether suitable settlement substrate is encountered. Further complicating this estimate is the documented secondary settlement in *M. edulis*; pediveligers settle when less than 0.5 mm , and can use long byssal threads to control resuspension into the plankton until they get bigger, approximately 1-2 mm, before they settle permanently (Bayne 1964a, Verwey 1966). Closely related to *M. edulis*, *M. galloprovincialis* has also been demonstrated to experience secondary settlement (Cáceres-Martínez and Figueras 1998). However, there is some evidence that secondary settlement might be the exception rather than the rule for *M. edulis* complex species in various locations (Bayne 1976), that it might be a completely passive

process (Cáceres-Martínez et al. 1994), or that it mostly occurs on local scales (Cáceres-Martínez and Figueras 1998).

M. californianus larval development is not as well documented as it is for *M. galloprovincialis*. Skidmore and Chew (1985, citing the Master's Thesis of Falmagne 1984) report that *M. californianus* are competent to settle after 17-24 days, with no mention of a specific temperature. There is one estimate of PLD as low as 9 days (Strathmann 1987), although she refers to "pers. comm." and no peer reviewed documentation of this number could be found. Suchanek (1981) used an estimate of 2-4 weeks for *M. californianus* in Washington, but this was not specifically verified.

The size of the prodissoconch of juveniles might reflect the PLD of the individual. Martel et al. (2000) documented that the prodissoconch I is bigger in *M. californianus* than *M. galloprovincialis*, although the prodissoconch II was about the same size in *M. galloprovincialis* and *M. californianus* collected in southern California. This implies that the larval duration is similar in the two local species. There is less information about the delay of metamorphosis of *M. californianus* larvae, although Trevelyan and Chang (1983) found successful settlement in the laboratory after 35-45 days at 17 and 20^{o}C, possibly indicating that this species can extend its PLD after competence. Bartlett (1972) reported a delay in metamorphosis of *M. californianus* larvae of less than approximately 5 weeks at 15^{o}C. It is reported that no secondary settlement occurs in this species (Petersen 1984). More clarification will be needed to confidently define a PLD range for *M. californianus* in this area, but from

these studies the best estimate is approximately 2 to 4 weeks from fertilization to competence, possibly with a delay between competence and settlement without a secondary settlement period.

If the time for both species to develop to competence is similar, but *M. galloprovincialis* has more flexibility in delaying permanent settlement, this difference could lead to quite different larval trajectories for the two species (e.g., Sponaugle et al. 2002). *M. galloprovincialis* from all regions settled more widely throughout the county, perhaps reflecting a longer time in the plankton, which could have led to increased mixing in a general larval "pool" before returning to the coast. Alternatively, *M. galloprovincialis* being transported past potential sites were more able to take advantage of chance proximity to suitable settlement habitat. This could be an advantageous strategy for *M. galloprovincialis*, the more fugitive species (*M. trossulus*, Suchanek 1981 and *M. galloprovincialis* Johnson 2003) to maximize colonization success. Another explanation is that although juveniles of both species were collected simultaneously, the *M. galloprovincialis* could have been in the plankton for longer and experienced different physical conditions or more mixing during the earlier time period. Although more study will be needed to define the delay of metamorphosis of *Mytilus* pediveligers, potential differences between the species could help explain the differences between the connectivity patterns.

Larval Behavior

The behavior of larvae during coastal transport has been shown to greatly alter their resulting trajectories (Sponaugle et al. 2002, Paris and Cowen 2004). It is more likely that larval behavior will be an important factor determining transport trajectories if larvae have swimming or sensing abilities (Kingsford et al. 2002). Bivalve larvae, as compared to more highly developed fish and decapod larvae, have relatively little mobility or sensory abilities (swimming velocities on the order of 0.1 cm/sec, Chia et al. 1984), and it is unlikely that the veligers can control their transport in most horizontal flows. However, there is evidence that *Mytilus* larvae can swim strongly enough to affect vertical position (Bayne 1976). In the coastal waters off of San Diego, where depth-stratified flows in different directions are known to occur (Winant and Bratkovich 1981, Pringle and Riser 2003), larvae could use vertical migration to alter their horizontal trajectories. If there are differences in the vertical positioning of the two species (with ontogeny, tidal cycles, diel cycles, etc.), this could lead to observed differences in their connectivities.

Unfortunately, differences in larval behavior between the two species are not known. Bayne documented the responses of *M. edulis* (which is closely related to *M. galloprovincialis*) larvae cultured in the laboratory to light, gravity (Bayne 1964b) and pressure (Bayne 1963) at the various stages of its life history. Although larvae generally sink if they do not swim to the surface, during most of their larval duration they respond to these three cues by moving towards the surface. There are some exceptions to this tendency to swim up during the ontogeny of *M. edulis* larvae.

Before the shell is formed (estimated to be 20-24 hours), the trochophores did not respond to light or gravity; Bayne attributed this to a need to stay away from visual predators at the surface before forming a protective larval shell. When individuals reached the pediveliger stage, they sank, probably in order to find suitable settlement habitat. At higher temperatures (above 20^{o}C), none of the stages responded to light and some even stayed below the surface; this was probably a strategy to avoid overheating.

When tracking *M. edulis* in the White Sea, Dobretsov and Miron (2001) found the majority of larvae between 1.5 to 3 m depth, which correlated well with a peak in food sources, and most of them were above the thermocline at 4.5 m. When they were ready to settle, pediveligers swam up rather than down; Dobretsov and Miron (2001) felt that this was a location-specific adaptation to maximize exposure to appropriate settlement sites. Cáceras-Martínez and Figueras (1998) found large numbers of *M. galloprovincialis* larvae of various stages at 1 m from the surface, the only depth at which they looked. Verwey (1966) documented observations of *M. edulis* (in The Netherlands) that indicate that short-term vertical migrations, related to diel cycles and tidal currents (i.e., a similar response to maximum currents during ebb and flood), might occur during various stages of larval development.

I was not able to find any references regarding vertical swimming in *M. californianus* larvae, except a note that they tend to sink to the bottom when they were competent to settle (Skidmore and Chew 1985, citing the Master's Thesis of Falmagne 1984). Ajtai (pers. comm.) observed *M. californianus* and *M. galloprovincialis* larvae

cultured in the laboratory both swim to the surface. Since mussel larvae do have the ability to influence their transport trajectories with vertical migration, future comparative studies of mussel larval behavior could help explain the differences between the species.

Distribution of spawning stock

The origin, structure, connectivities, and amount of self-seeding can also be related to distribution and relative size of sources. For example, if two populations receive the same total number of self-seeding larvae, but one is closer to an additional source of recruits, the additional subsidy of larvae from other sources will reduce the percentage of self-seeding at the less-isolated site. An isolated population will therefore appear to have a higher degree of self-seeding (lower connectivity), even if the physical and biological conditions are identical. Likewise, measured population connectivity could be an artifact of the amount of spawning stock at nearby sites.

There are notable differences in spawning stock between the two study species and among the regions included in this study. *M. californianus* adults are found exclusively on the open coast, and tend to have larger populations to the north, especially at AH and CR. This difference in abundance likely translates to considerably higher production of larvae to the north. There could be a similar percentage of larvae (relative to number produced) being retained in the northern and southern coastal regions, but the net number of larvae from the north is much greater. *M. galloprovincialis* dominates mussel populations in the outer bays, which are

located in both the northern and southern parts of this study area. Therefore there is a greater distribution of spawning stock throughout southern coastal region. This pattern in adult distribution could explain the predicted pattern in natal origins in this study.

In addition, the distribution of the settlers of the two species might be related to their origins. The higher settlement of *M. californianus* in northern sites (Appendix B) might reflect the greater success in larval transport in self-seeding populations. In contrast, *M. galloprovincialis* settlers were found at all sites, and were also found to have a greater number of sources. Perhaps this reflects a more "shot-gun" approach for *M. galloprovincialis* dispersal and settlement, which could be related to its ecological role as more of a fugitive species (Suchanek 1981). Similarly, Johnson (2003) found that bay mussel settlers (*M. galloprovincialis* and *M. trossulus*) were broadly found in wave-swept and protected areas of the central California coast, while *M. californianus* settlers were only found on the open coast. The lack of mussels predicted to originate from the inner bays as well as the low number of settlers found in these sites, despite the highly retentive characteristic of the inner part of San Diego and Mission Bays (Levin 1983, Largier et al. 1997) is probably due to the low abundances of adult mussels in these areas.

Summary

This study demonstrates that there are differences between the population connectivities of these two congeners, and generates a number of interesting and

important questions about the details of how their larval life history interacts with circulation to drive metapopulation dynamics. Future comparative studies of the initiation of spawning, processes that delay metamorphosis, vertical migration, and adult distribution, will be important in identifying the biological mechanisms behind larval transport of southern California mussels and any other marine organism with a planktonic larval phase.

QUESTIONS, CHALLENGES AND LIMITATIONS

This work greatly expands our understanding of southern California mussel ecology in particular, and our ability to directly determine the population connectivity of mollusk populations in general. With this advancement, a number of general questions and challenges arise.

There are a few refinements that will improve the resolution and interpretability of elemental fingerprinting using outplanted larvae. The temporal stability, both seasonal and interannual, of the elemental fingerprints will need to be determined with repeated studies. Becker et al. (2005) found that the chemistry of mussel juvenile dissoconch remained stable over weekly and monthly periods, although individuals collected in one month (February) had a different signature than the others (May, September, and December). Since shell is formed throughout the PLD, while larvae are being transported, controlled studies of a) the timing of shell formation relative to transport distances and b) variations in shell chemistry during ontogeny, will improve the interpretability of this work. In addition, the role of maternal effects (e.g.,

chemical signatures imparted onto larvae from yolk material) in *Mytilus* larvae needs further exploration (e.g., DiBacco 2000). Larval outplanting was relatively resource-intensive but is likely to become more efficient with repeated trials. With improvement in efficiency, the geographic range, replication, and length of outplanting should be extended to improve the accuracy of results. In addition, the use of additional markers (e.g., additional trace elements and stable isotopes) is likely to improve successful classification of background elemental fingerprints.

The power of elemental fingerprinting as tool for studying population connectivity derives from the direct determination of natal origins in a discrete period of time. Unfortunately, this lack of temporal integration also precludes the ability to draw generalized conclusions about the contribution of different natal regions to the total population based on a single experiment. Settlement rates into populations with dispersing larvae are often highly variable, and it is possible that rare recruitment events (both in intensity and origins) could strongly influence the demographics of a given population (Dayton and Tegner 1984). In addition, this study focused on a specific size range of juveniles (600 to 3000 μm) with no consideration of selective mortality related to species, cohort, or natal origin either pre-settlement or post-settlement. For example, the large number of *M. galloprovincialis* that were found settling in open coast sites might not have survived to reproduce (Johnson 2003), and thus might not contribute to the dynamics of the population. Repeated fingerprinting activities, paired with simultaneous monitoring of settlement and adult demography

should help determine not only the origin of settlers but the relative importance of specific cohorts and their larval sources to the persistence of populations.

Genetic approaches to determining population connectivity tend to be more biased towards rare mixing events and only individuals that survive to reproduce contribute to the determined patterns. A recent large-scale examination of genetic population structure of *M. californianus* throughout its range on the west coast of North America (Engel 2004) found surprisingly little geographic structure and a large amount of chaotic small-scale variability. This result implies that on evolutionary time scales and throughout its biogeographic range, *M. californianus* populations are panmictic and completely "open", but that on smaller scales the connectivity between populations can vary. In addition, Engel (2004) compared the genetic variability between size classes at a single site in central California and found more heterogeneity within one population than between all of the populations she examined, implying that the sources of new recruits varied from cohort to cohort. She did not find that each cohort was particularly homogeneous, as would be expected if a single source replenishment model was the common pattern over many generations. The elemental fingerprinting approach, which is much more sensitive to self recruitment and short-term patterns, indicates that mussel larval retention does occur on small scales (10s of km) and on time scales relevant to conservation (less than one generation), and that a single source can contribute the majority of new offspring to a single cohort across a broader region. The results of Engel (2004) imply that this pattern is probably not stable over evolutionary time-scales. Contrasting fingerprinting with genetics results

is a clear demonstration of the value of examining a similar question on different scales and using a variety of approaches. Interdisciplinary studies that supplement elemental fingerprinting with genetic approaches and population and oceanographic modeling, will ultimately be the key to understanding larval connectivity and its consequences (see Levin submitted).

FUTURE DIRECTIONS AND APPLICATIONS

This study is already being expanded to include physical and metapopulation modeling (L. Levin, pers. comm.) in order to compare predicted connectivity based on ocean physics with realized connectivity determined using elemental fingerprinting. These investigations will allow for exploration of the influence of behavior and physics in larval transport and place these results in a larger context in terms of population dynamics. This study will be repeated a number of times to characterize the temporal changes in connectivity patterns in this region.

Determining degree of connectivity in marine populations is currently one of the "greatest challenges" in marine ecology (Swearer et al. 2002). The promise of elemental fingerprinting (with or without an in situ larval culturing component), as a larval tracking tool is beginning to be realized. As we are now able to define the actual connectivity between marine populations, our insight into the role of larval dispersal determining the abundance and distribution of species will become clearer. With greater understanding of how populations are connected, we will vastly improve our ability to manage and conserve them for future generations.

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Table 5.1: Definition of terms used in this paper. Throughout there is a general focus on coastal, benthic population with dispersing, planktonic larval stages, ignoring adult migration.

Population: A single species group that is spatially separated from other individuals of the same species

Natal region: A zone that imparts similar chemical signatures to larvae, and thus is considered as a single area of origin

Settlement: The process of successfully completing the planktonic larval stage, joining a benthic population, metamorphosis, and beginning dissoconch shell growth.

Early or Recent Settler: An individual that has achieved settlement, but still measures less than 3 mm maximum shell length.

Open: Degree to which new settlers entering a given population were spawned in a different population

Closed: Degree to which new settlers entering a given population were spawned in that population

Larval connectivity: The amount of exchange of successful larvae among populations or natal regions, quantified by the number of settlers in a given location that originate from outside of that area; higher connectivity implies more "open" populations and less self-seeding. Will be shortened to "connectivity" in this paper.

Self-recruitment or Self-seeding: The settlement of a larva into the same population where it was spawned. Will depend on a pre-defined scale (e.g., site, natal region).

Natal Origin: The location (either population or natal region) where a given settler was spawned and/or grew to a D-shaped veliger stage.

Planktonic Larval Duration (PLD): Length of time required by a planktonic larva in order to achieve competency and permanent settlement.

Elemental Fingerprinting: Use of a chemical signature, usually a combination of trace element ratios to calcium, in the hard part formed by an organism at a given time to predict the location of that organism at that time. Determined using Discriminant Function Analysis.

Table 5.2: Description of sites used for outplanting mussel larvae between May 12 and May 19, 2003 and for collecting juveniles between June 3 and 6, 2003 in San Diego County, California.

Site	Abbreviation	Bay vs. Open Coast	Outplanting Description	Depth water (m from MLLW)	Latitude (°N)	Longitude (°W)	Juvenile Collection Habitat	Settlement Substrate
Agua Hedionda Lagoon	AL	Agua Hedionda Lagoon	In lagoon near mussel farm	5.3	33.141	117.339	Muddy shore of lagoon	Algae and mussels
Agua Hedionda	AH	Open Coast	Offshore of lagoon	10.0	33.142	117.349	Rip rap at mouth of lagoon	Algae and mussels
Cardiff Reef	CR	Open Coast	Offshore of rocky reef	9.8	33.000	117.285	Rocky reef	Algae and mussels
La Jolla Dike Rock	LJDR	Open Coast	Offshore of rocky reef	9.5	32.873	117.256	Rocky reef	Algae
Scripps Pier	SIO	Open Coast	Offshore of pier	9.6	32.868	117.259	Pier	Algae and mussels
Pacific Beach (Crystal) Pier	PB	Open Coast	Offshore of pier	9.8	32.794	117.267	Pier	Mussels
Ocean Beach Pier	OB	Open Coast	Offshore of pier	10.7	32.749	117.264	Pier	Mussels
Cabrillo National Monument	CABR	Open Coast	Offshore of rocky reef	9.5	32.668	117.252	Rocky reef, 3 sites within 1 km of each other	Algae
Imperial Beach Pier	IB	Open Coast	Offshore of pier	9.7	32.579	117.142	Pier	Mussels
Shelter Island	SHI	San Diego Bay	Offshore of rip-rap island, near SIO ship facility	5.5	32.706	117.236	Rip rap	Algae and mussels
Harbor Island	HI	San Diego Bay	Offshore of rip-rap island	6.3	32.724	117.207	Rip rap	Algae and mussels
Chula Vista	CV	San Diego Bay	Offshore of marina	4.8	32.624	117.106	Small boulders in muddy substrate, across bay (Coronado Island)	Mussels
Crown Point Mitigation Site	CPMS	Mission Bay	Offshore of salt marsh	3.5	32.785	117.229	Small boulders in muddy substrate	Mussels

Table 5.3: Distances (km) among sites (Figure 5.3) used for outplanting mussel larvae between May 12 and May 19, 2003 and collecting juveniles from June 3-6, 2003 in San Diego County, California. AL=Agua Hedionda Lagoon, 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 Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island, HI=Harbor Island, CV=Chula Vista, CPMS=Crown Point Mitigation Site. Printed with permission from Linda Rasmussen.

	AH	**AL**	**CR**	**LJDR**	**SIO**	**PB**	**OB**	**CABR**	**IB**	**SHI**	**HI**	**CV**	**CPMS**
AH													
AL	0.72												
CR	15.92	16.64											
LJDR	30.07	30.78	14.15										
SIO	30.67	31.38	14.75	0.60									
PB	40.99	41.71	25.07	10.93	10.33								
OB	45.99	46.71	30.07	15.93	15.32	5.00							
CABR	55.79	56.51	39.87	25.73	25.12	14.80	9.80						
IB	71.56	72.28	55.64	41.50	40.90	30.57	25.57	15.77					
SHI	63.64	64.36	47.72	33.57	32.97	22.64	17.65	7.85	16.83				
HI	67.04	67.76	51.12	36.98	36.37	26.05	21.05	11.25	20.23	3.40			
CV	81.79	82.51	65.87	51.73	51.13	40.80	35.80	26.00	34.98	27.06	30.46		
CPMS	48.27	48.99	32.35	18.21	17.61	7.28	3.87	13.67	29.44	21.52	24.92	39.67	

Table 5.4: Jackknifed classification success of a DFA comparing shell chemistry of mussel larvae raised in situ at sites in San Diego County. DFA was conducted using only *M. californianus* larvae and only element ratios that met the F-to-Remove criterion (Mn/Ca, Co/Ca, Cu/Ca, Sr/Ca, Ba/Ca, Pb/Ca, and U/Ca). Classification success grouped by site (delimited by solid lines) and by region (northern coastal, southern coastal, outer bay and inner bays, as delimited by dotted lines). Northern coastal region: AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region: SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region: HI=Harbor Island (San Diego Bay). Inner bay region: CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). DFA scores and element ratios used are shown in Figure 5.7.

		Predicted Site													Classification Success (%)	
		AL	AH	CR	LJDR	SIO	PB	OB	IB	SHI	HI	CV	CPMS	Sum	Site	Region
Actual Site	AL	8	3			1	2	1	1		1			17	47	65
	AH	1	10		3				1		1			16	63	88
	CR		1	3										4	75	100
	LJDR		1		8			3		2	1			15	53	60
	SIO					1	2							3	33	100
	PB							2	1					3	0	100
	OB				4		4	19		2	1			30	63	83
	IB	1	1			2	6	5	6	8		1		30	20	90
	SHI	2	1			4	3	1	8	9	1			29	31	86
	HI		2			2	1		1		18			24	75	75
	CV			1	4		1	1				7	2	16	44	56
	CPMS												6	6	100	100
	Total													193	49	80

Table 5.5: Jackknifed classification success of a DFA comparing shell chemistry of mussel larvae raised in situ at sites in San Diego County. DFA was conducted using both *M. californianus* and *M. galloprovincialis* larvae and only element ratios that met the F-to-Remove criterion, excluding Co/Ca (Mn/Ca, Cu/Ca, Sr/Ca, Ba/Ca, Pb/Ca, and U/Ca). Classification success grouped by site (delimited by solid lines) and by Region (northern coastal, southern coastal, outer bay and inner bays, as delimited by dotted line). Northern coastal region: AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region: SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region: HI=Harbor Island (San Diego Bay). Inner bay region: CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). DFA scores and element ratios used are shown in Figure 5.9.

		Predicted Site														Classification Success (%)	
		AL	AH	CR	LJDR	SIO	PB	OB	CABR	IB	SHI	HI	CV	CPMS	Sum	Site	Region
Acutal Site	AL	5	3	2		3	6	1			1	1	1		23	22	43
	AH		7		2	1	2			1		2	1		16	44	56
	CR	1		2					1						4	50	75
	LJDR				10		2	1					2		15	67	67
	SIO				1	4	3	5				2	1	2	18	22	67
	PB	3			1			1		2					7	0	43
	OB				2	5		21			1			1	30	70	90
	CABR		2						11			2			15	73	73
	IB	3					1	12	1	7	3		2	1	30	23	80
	SHI		1		3	5	3	1		7	6	1	2		29	21	76
	HI		2				1					15	4	2	24	63	63
	CV	1	1		5			1			1		4	3	16	25	44
	CPMS					4								7	11	64	64
	Total														238	42	67

Table 5.6: Determination of natal origin of *M. californianus* juveniles collected at sites in San Diego County using shell chemistry of *M. californianus* larvae outplanted offshore for one week (Table 5.4, Figure 5.7). Northern coastal region: AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region: SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR II=Cabrillo National Monument (management Zone II), IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region: HI=Harbor Island (San Diego Bay). Inner bay region: CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). No CABR larvae were used in this analysis, so self-recruitment could not be determined for this site.

		Predicted Natal Origin																	
		AL	AH	CR	LJDR	SIO	PB	OB	IB	SHI	HI	CV	CPMS	Sum	% Northern	% Southern	% AL and AH	% Self-recruiting (site)	% Self-recruiting (region)
Juvenile Collection Site	AL	4	3		1									8	100	0	88	50	100
	AH	2	5					2			1			10	70	20	70	50	70
	CR	7	13		2			1						23	96	4	87	0	96
	LJDR	12	7		3			4				1		27	81	15	70	11	81
	SIO	3	8											11	100	0	100	0	0
	PB	2	16		1			2				1		22	86	9	82	0	9
	OB							1						1	0	100	0	100	100
	CABR II		1			1								2	50	50	50	ND	50
	IB	3	16		1			1						21	95	5	90	0	5
	Total													125	88	10	82	10	51

Table 5.7: Determination of natal origin of *M. galloprovincialis* juveniles collected at sites in San Diego County using shell chemistry of *M. californianus* and *M. galloprovincialis* larvae outplanted offshore for one week (Table 5.5, Figure 5.9). Northern coastal region: AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region: SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument (3 management zones), IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region: HI=Harbor Island (San Diego Bay). Inner bay region: CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay).

		Predicted Natal Origin																		
		AL	AH	CR	LJDR	SIO	PB	OB	CABR	IB	SHI	HI	CV	CPMS	Sum	% Northern	% Southern	% Bays	% Self-recruiting (site)	% Self-recruiting (region)
Juvenile Collection Site	AL		6					3	1				1		11	55	36	9	0	55
	AH		4			2		2	2						10	40	60	0	40	40
	CR		1			1		1	1						4	25	75	0	0	25
	LJDR		1		1										2	100	0	0	50	100
	SIO		4		1			2							7	71	29	0	0	29
	PB		2			1		1							4	50	50	0	0	50
	OB		4			1		4						1	10	40	50	10	40	50
	CABRI	3	7		1	1		10							22	50	50	0	0	50
	CABRII		1			1		1							3	33	67	0	0	67
	CABRIII		1					1	1						3	33	67	0	33	67
	IB		1		1			1							3	67	33	0	0	33
	SHI	1				1		3							5	20	80	0	0	80
	HI	2	6			1		4	3					5	21	38	38	24	0	0
	CV			1											1	100	0	0	0	0
	CPMS							1				1			2	0	50	50	0	0
	Total														108	45	47	7	9	39

Figure 5.1: General models of larval replenishment representing a gradient from open to closed populations. Each grey circle represents a spatially distinct species. Solid arrows represent a large amount of larval exchange; the dotted arrows represent a low level of exchange. After Carr and Reed (1993).

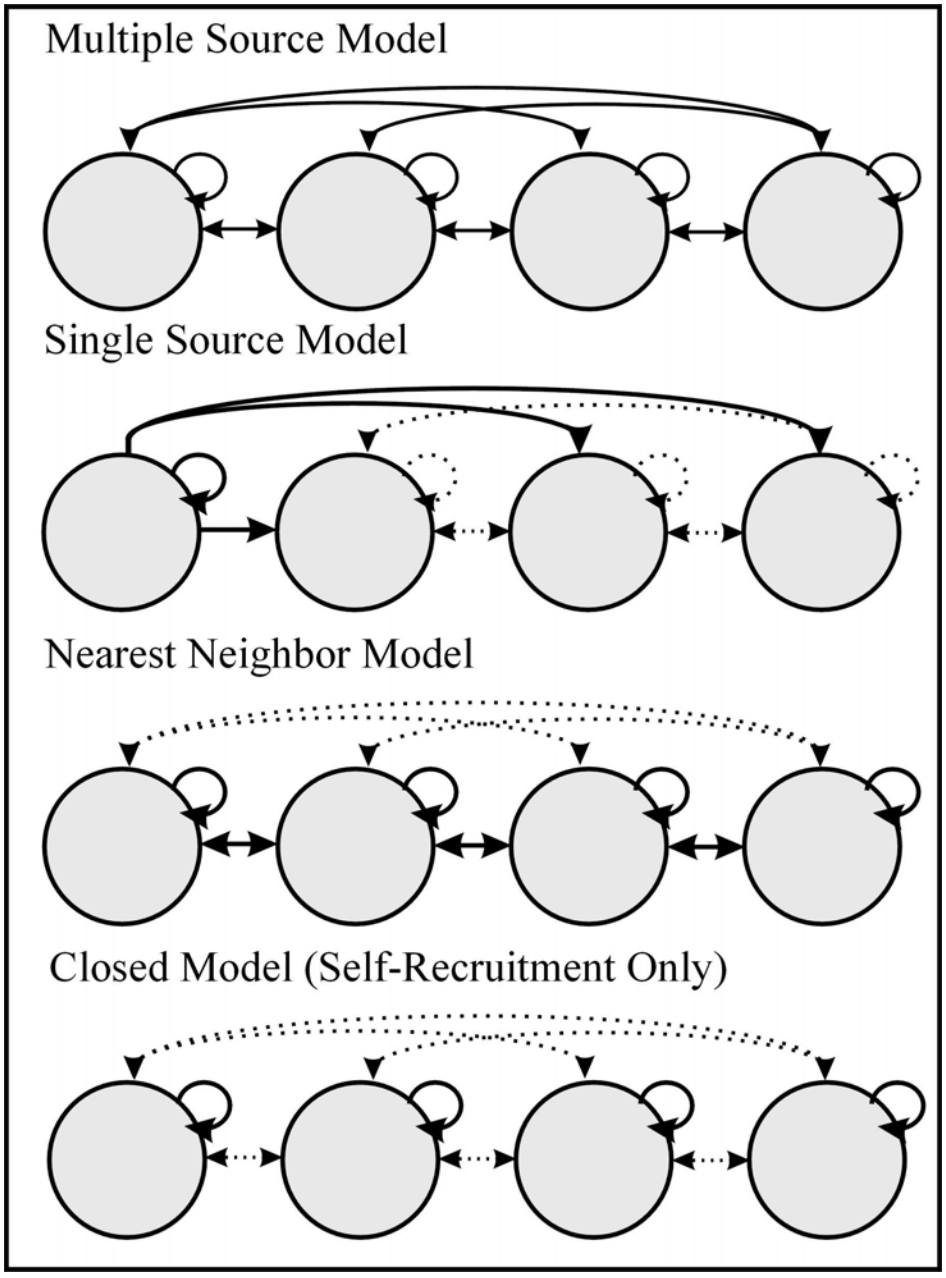


Figure 5.2: Discriminant function analysis (DFA) comparing shell chemistry of different parts of the shells of mussel juveniles collected from different sites in San Diego County, regardless of species. (A) Individual scores of mussel shell chemistry. (B) Canonical discriminant functions (standardized by within variances) of used element ratios. The first DFA score accounts for 99.6% of the total dispersion in the data. Corresponding DFA classification successes are 95% for juveniles, and 99% for early and late prodissoconch (combined).

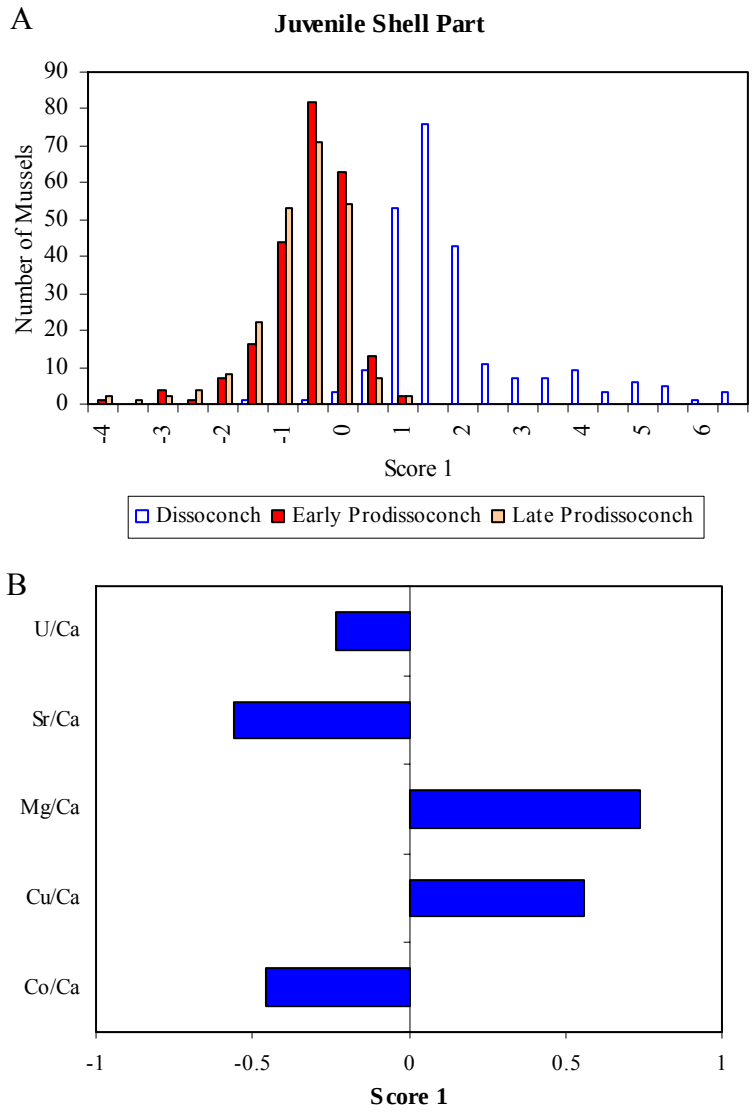


Figure 5.3: Map of sites. Northern coastal region (blue): AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region (red): SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region (green): HI=Harbor Island. Inner bay region (pink): CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). Labels point to offshore stations, Xs represent corresponding intertidal stations.

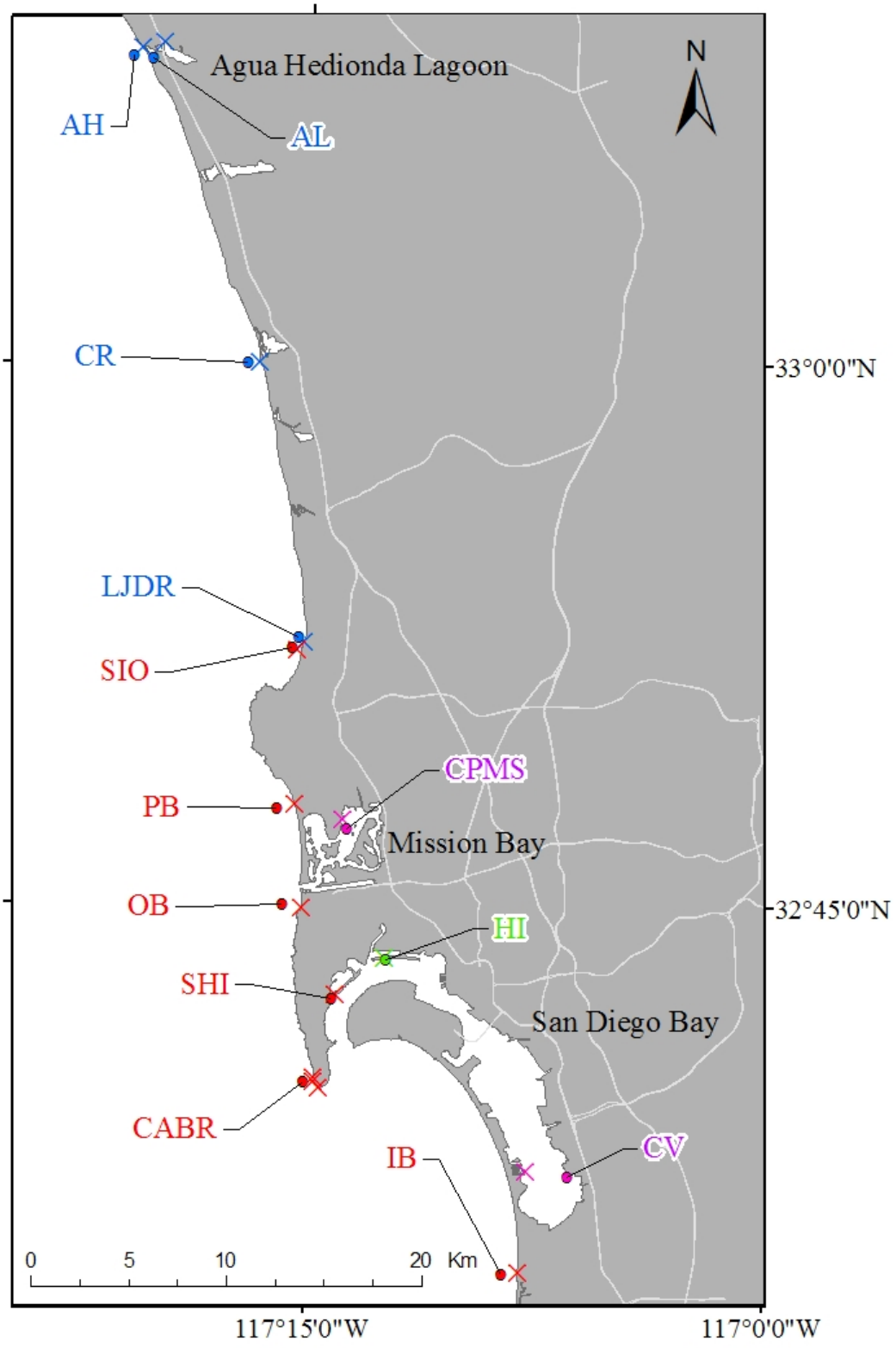
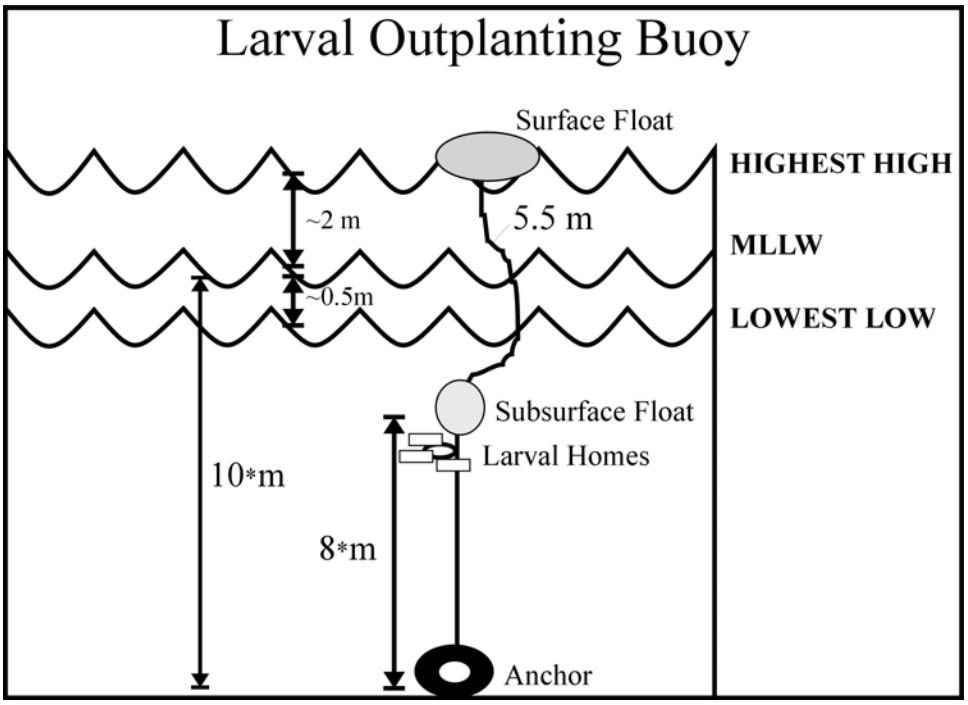

Agua Hedionda Lagoon
AH
AL
CR
LJDR
SIO
CPMS
PB
Mission Bay
OB
HI
SHI
San Diego Bay
CABR
IB
CV
N
33°0'0"N
32°45'0"N
0
5
10
20
Km
117°15'0"W
117°0'0"W

Figure 5.4: (A) Diagram of buoy setup for outplanting mussel larvae. (B) Photo of an example of larval homes that were deployed on the buoy so that they were located 2 m below mean lower low water (MLLW). Coastal sites were located in 10 m water depth. *For shallower bay sites, the distance between the bottom and the subsurface float was adjusted to maintain 2 m between the larval homes and the surface.

A


Larval Outplanting Buoy
Surface Float
HIGHEST HIGH
~2 m
5.5 m
MLLW
~0.5m
LOWEST LOW
Subsurface Float
Larval Homes
10*m
8*m
Anchor

B

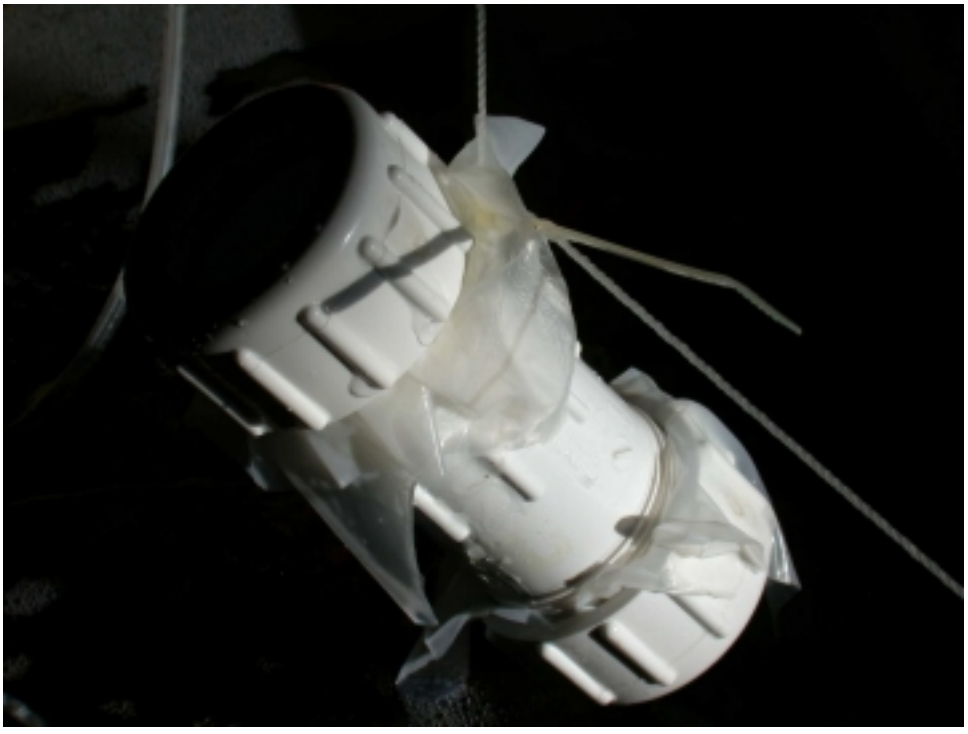


Figure 5.5: Diagram of typical mytilid mussel juvenile. Lines on shell represent laser sampling line positions (J=juvenile, EP=early prodissoconch). Dotted lines represent orientations used to determine the angle (A) of the hinge (line H) relative to the flat ventral margin (line VM), as described by Martel et al. (1999).

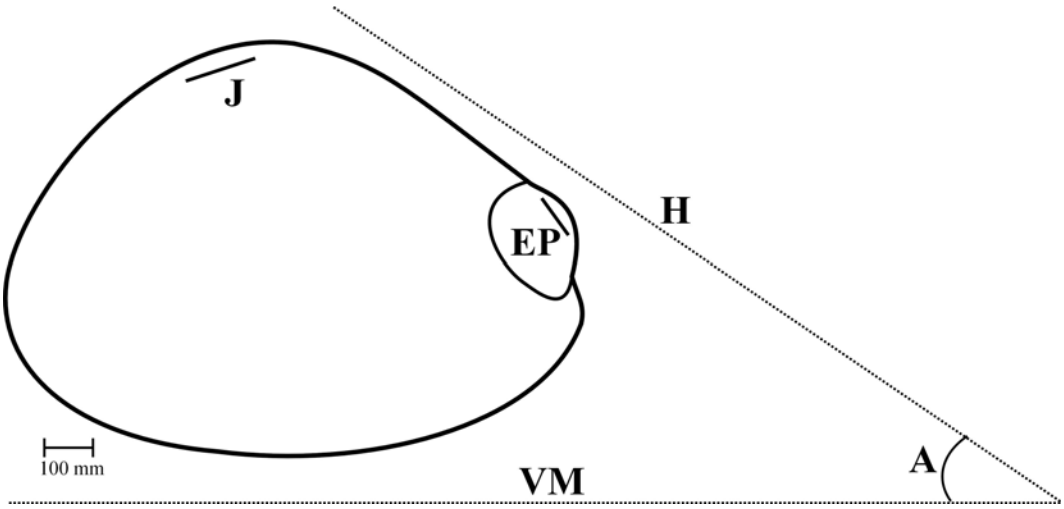


Figure 5.6: DFA of mussels of species identity (as determined by PCR). (A) Individual scores for each species: C= *M. californianus* and G= *M. galloprovincialis*. "Known" identities were determined using PCR. (B) Canonical discriminant functions (standardized by within variances) of characteristics used to make the predictions: Site (as quantified by percentage of *M. californianus* identified from the site), length/width ratio (L:W), hinge angle (as described by Martel et al. 1999, Hinge), and dissoconch chemistry (Co/Ca was only significant element ratio). The first DFA score accounts for 100% of the total dispersion in the data. Corresponding DFA classification successes for known species were 93% for *M. californianus* and 85% for *M. galloprovincialis*.

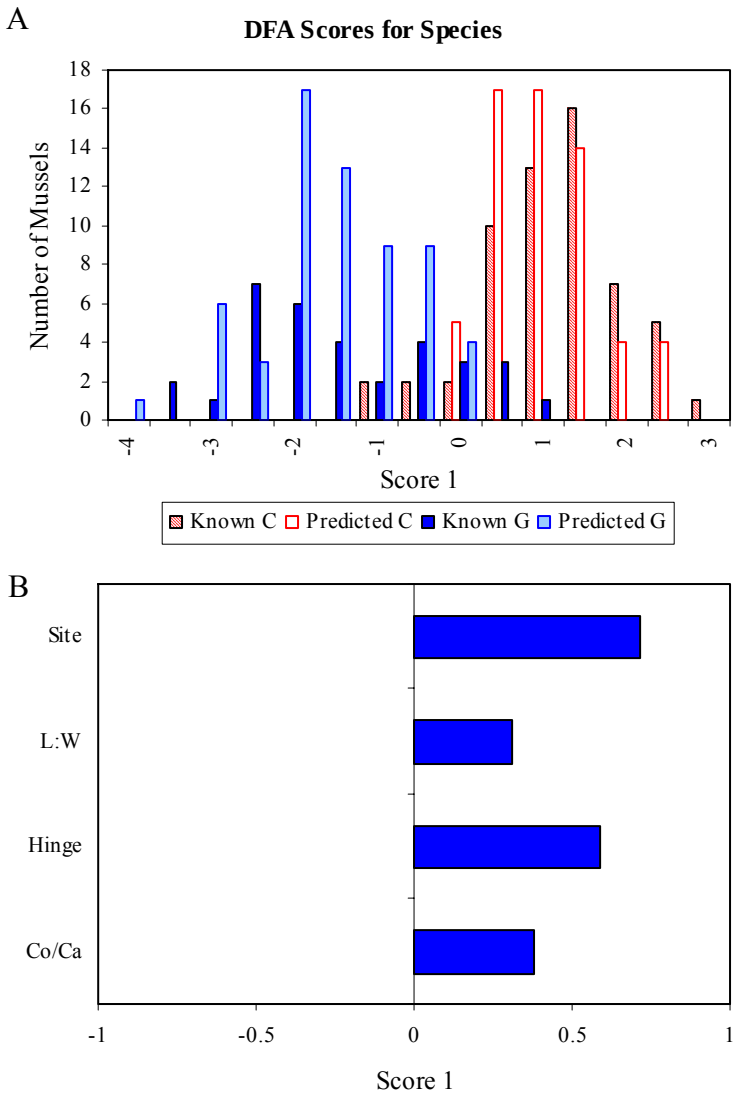
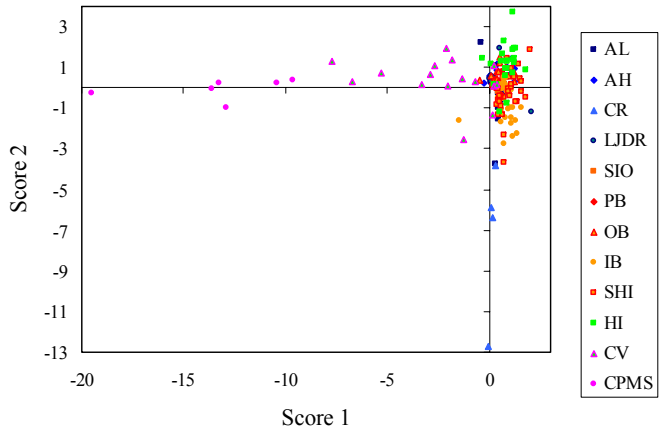


Figure 5.7: DFA comparing shell chemistry of mussel larvae raised in situ at sites in San Diego County. DFA was conducted using only *M. californianus* larvae and only element ratios that met the F-to-Remove criterion. Panels (A) and (B) show individual scores of mussel shell chemistry, sites are shown using the same icon, regions are shown using a color family. Northern coastal region (Blues): AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region (Reds and Oranges): SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region (Green): HI=Harbor Island (San Diego Bay). Inner bay region (Pinks): CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). Panels (C) and (D) show canonical discriminant functions (standardized by within variances) of used element ratios. The first two DFA scores account for 80.4% and the third and fourth scores account for 15.7% of the total dispersion in the data. Corresponding DFA classification successes are shown in Table 5.4.

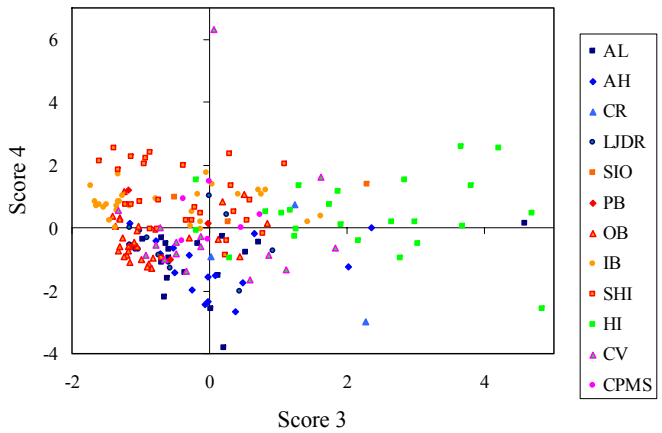
A

Scores 1 and 2: *Mytilus californianus*

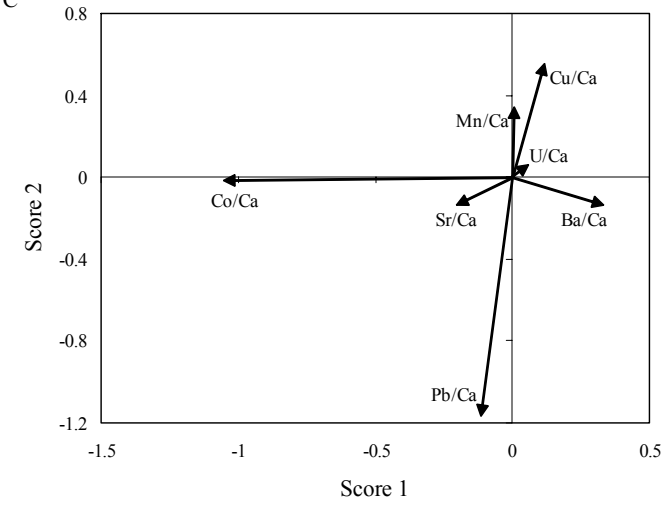


B

Scores 3 and 4: *Mytilus californianus*



C



D

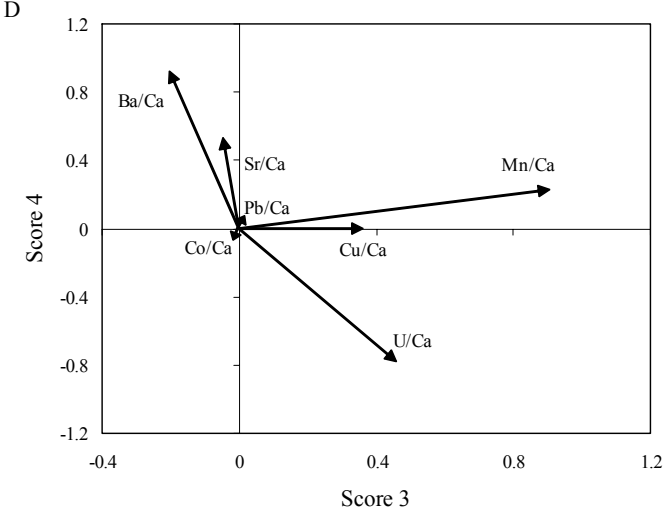


Figure 5.8: Average results of randomization procedure for DFA classifying *M. californianus* field-cultured larvae by site. Shell chemistry data for individual mussels were randomly assigned to sites ten times, and classification successes were averaged. Graphs show actual results presented in Table 5.4 compared to the average randomized result. Classification success grouped by site (A) or region (B). Northern coastal region: AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region: SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region: HI=Harbor Island (San Diego Bay). Inner bay region: CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). Bars represent ± 95% confidence intervals.

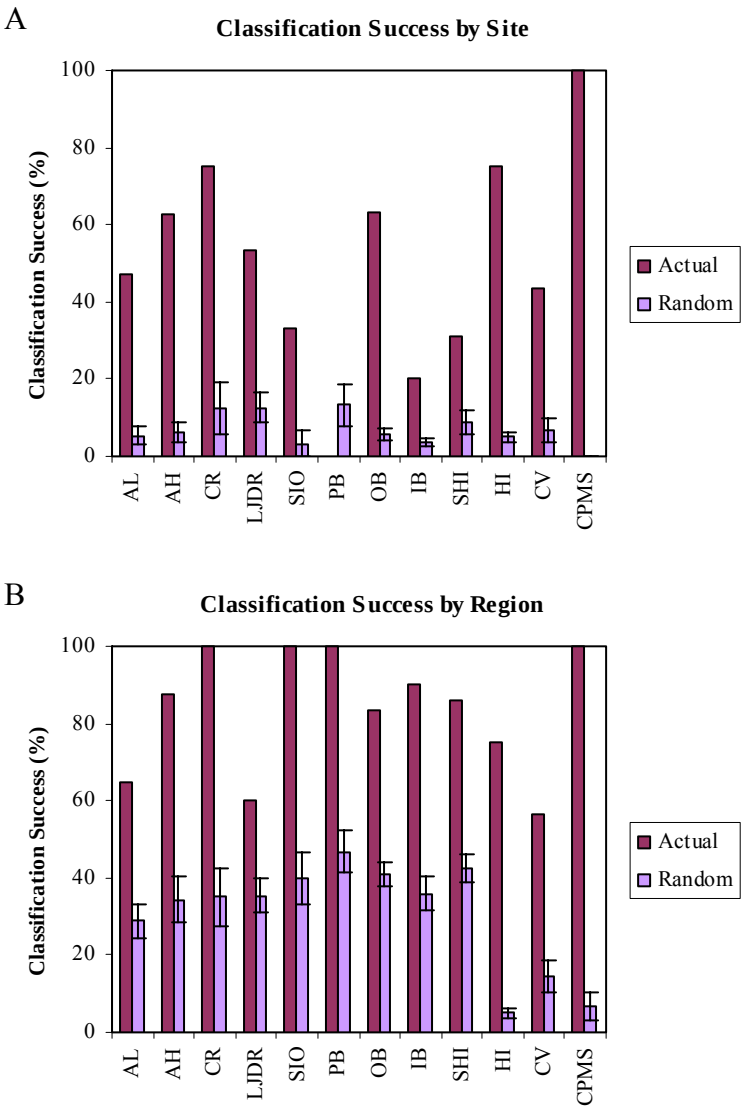
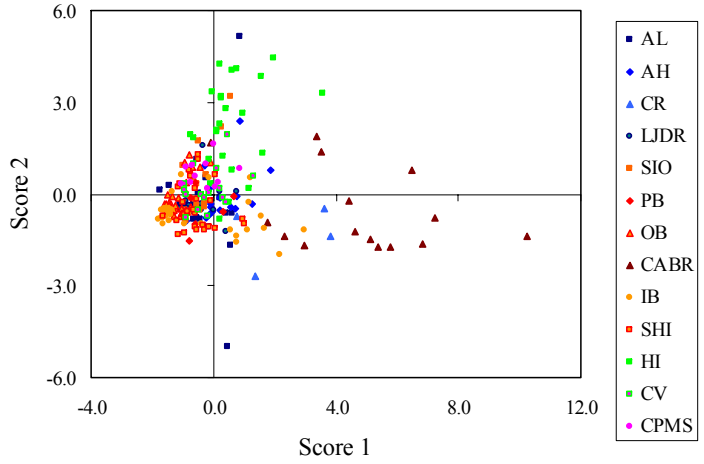
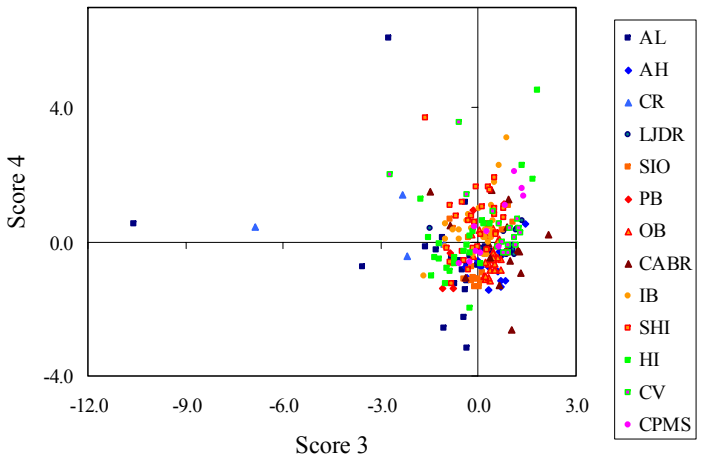


Figure 5.9: DFA comparing shell chemistry of mussel larvae raised in situ at sites in San Diego County. DFA was conducted using both *M. californianus* and *M. galloprovincialis* larvae and only element ratios that met the F-to-Remove criterion were used, excluding Co/Ca. Panels (A) and (B) show individual scores of mussel shell chemistry, sites are shown using the same icon, regions are shown using a color family. Northern coastal region (Blues): AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region (Reds and Oranges): SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region (Green): HI=Harbor Island (San Diego Bay). Inner bay region (Pinks): CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). Panels (C) and (D) show canonical discriminant functions (standardized by within variances) of used element ratios. The first two DFA scores account for 73.8% and the third and fourth scores account for 19.1% of the total dispersion in the data. Corresponding DFA classification successes are shown in Table 5.5.

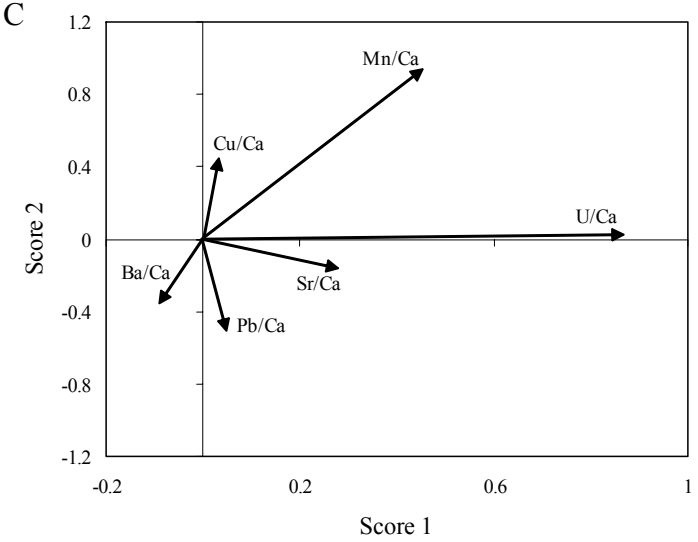
A


Scores 1 and 2: Both Species
Score 2
Score 1
6.0
3.0
0.0
-3.0
-6.0
-4.0
0.0
4.0
8.0
12.0
AL
AH
CR
LJDR
SIO
PB
OB
CABR
IB
SHI
HI
CV
CPMS

B


Scores 3 and 4: Both Species
Score 4
Score 3
4.0
0.0
-4.0
-12.0
-9.0
-6.0
-3.0
0.0
3.0
AL
AH
CR
LJDR
SIO
PB
OB
CABR
IB
SHI
HI
CV
CPMS

C


Score 2
Score 1
1.2
0.8
0.4
0
-0.4
-0.8
-1.2
-0.2
0.2
0.6
1
Mn/Ca
Cu/Ca
U/Ca
Ba/Ca
Sr/Ca
Pb/Ca

D

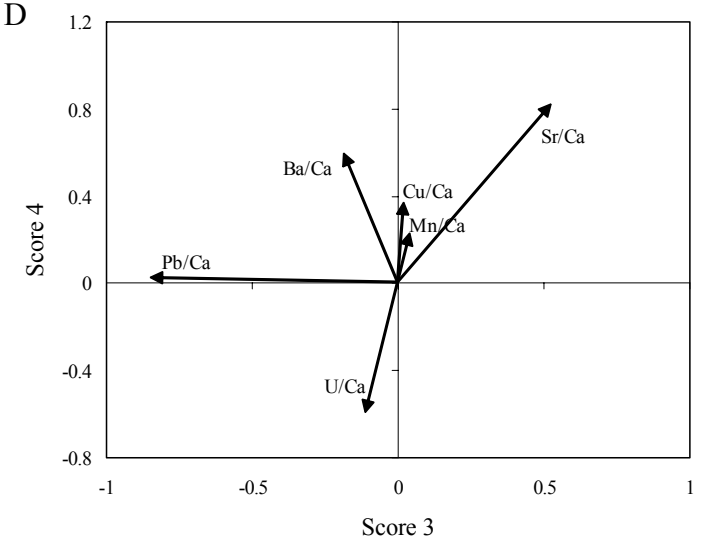

Score 4
Score 3
1.2
0.8
0.4
0
-0.4
-0.8
-1
-0.5
0
0.5
1
Ba/Ca
Cu/Ca
Sr/Ca
Mn/Ca
Pb/Ca
U/Ca

Figure 5.10: Average results of randomization procedure for DFA classifying *M. californianus* and *M. galloprovincialis* field-cultured larvae by site. Shell chemistry data for individual mussels were randomly assigned to sites ten times, and classification successes were averaged. Graphs show actual results presented in Table 5.5 compared to the average randomized result. Classification success when grouped by (A) site or (B) region. Northern coastal region: AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region: SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region: HI=Harbor Island (San Diego Bay). Inner bay region: CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). Bars represent ± 95% confidence intervals.

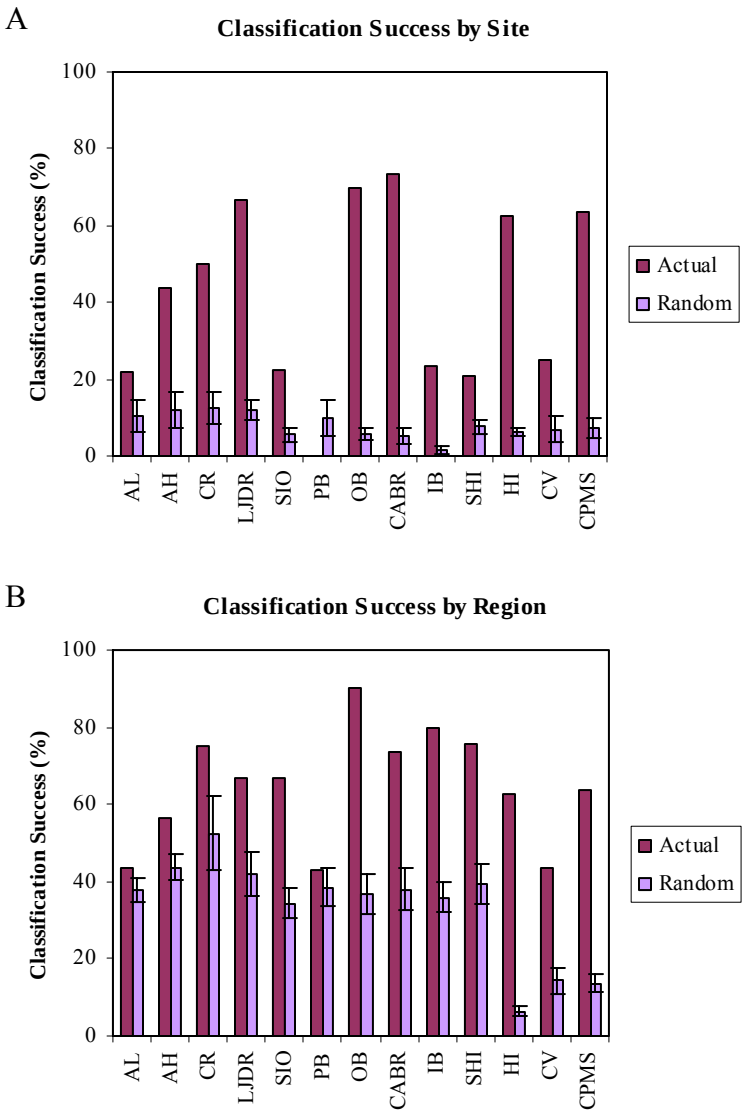


Figure 5.11. Temperature (24-hour moving average) at outplanting sites and in laboratory water bath during in situ larval culturing experiment (May 12-19, 2003, indicated by arrow) and the following week. AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay). Temperature logger at CPMS in Mission Bay was lost.

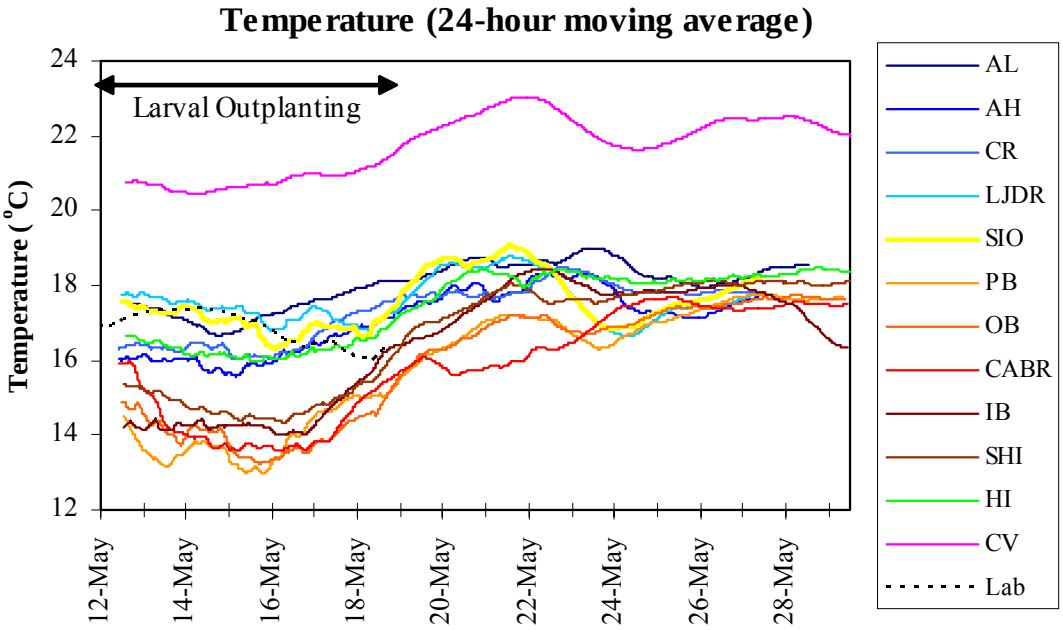
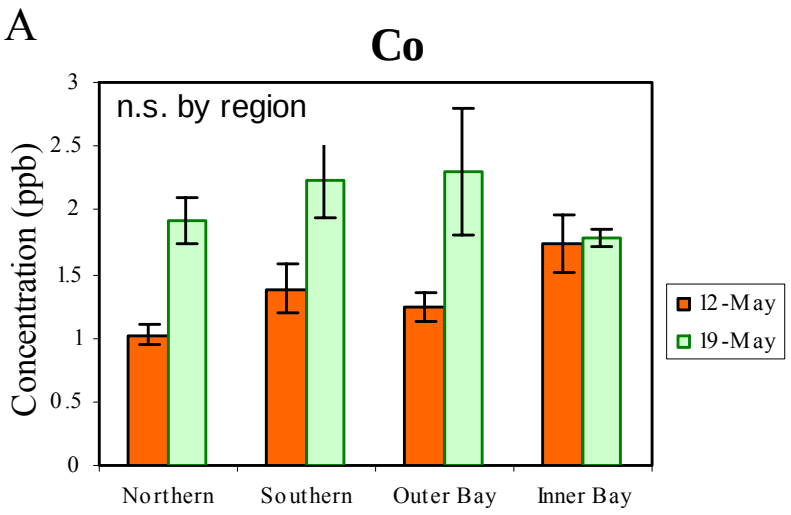
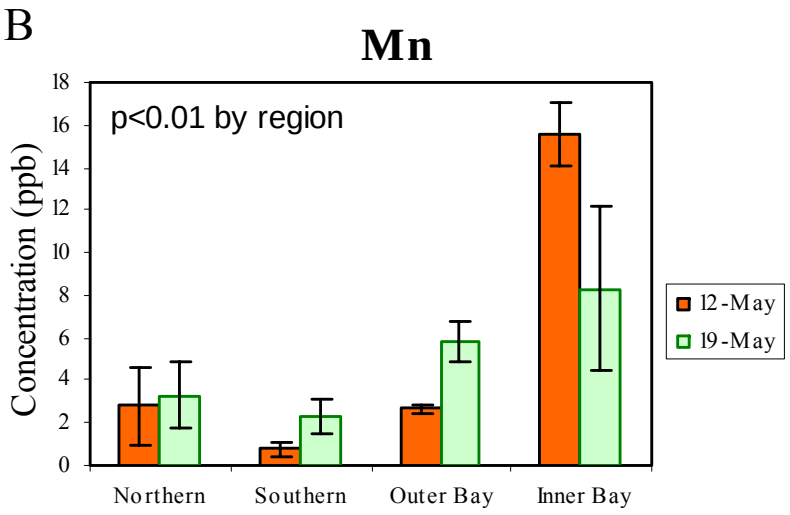


Figure 5.12. Concentrations of (A) Co, (B) Mn and (C) U in seawater collected on the first (May 12, 2003) and last day (May 19, 2003) of the in situ larval culturing experiment. Northern=Northern coastal region (AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock), Southern=Southern coastal region (SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island), Outer bay region (HI=Harbor Island), Inner bay region (CV=Chula Vista, CPMS=Crown Point Mitigation Site). Error bars are $\pm$ 1 SE.


A
Co
n.s. by region
Concentration (ppb)
3
2.5
2
1.5
1
0.5
0
Northern
Southern
Outer Bay
Inner Bay
12-May
19-May


B
Mn
p<0.01 by region
Concentration (ppb)
18
16
14
12
10
8
6
4
2
0
Northern
Southern
Outer Bay
Inner Bay
12-May
19-May

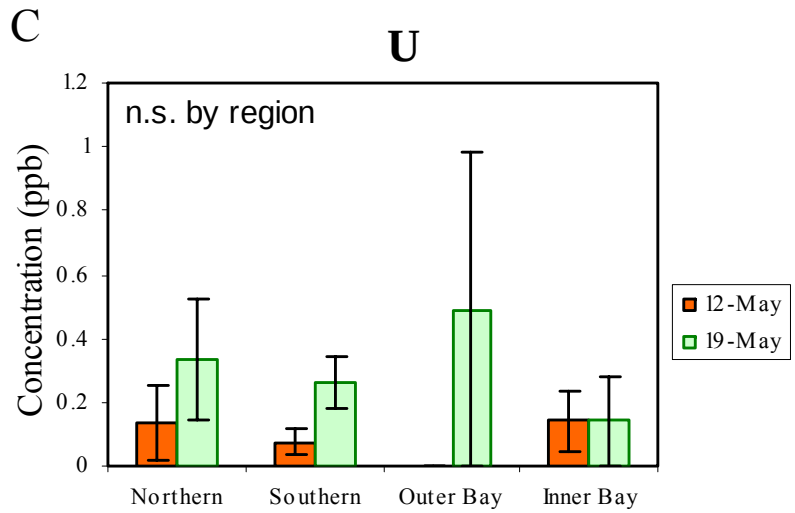

C
U
n.s. by region
Concentration (ppb)
1.2
1
0.8
0.6
0.4
0.2
0
Northern
Southern
Outer Bay
Inner Bay
12-May
19-May

Figure 5.13: Number of juvenile mussels (<3 mm length) identified to species using a PCR assay and that were collected in either algal mats or amongst adult mussel byssal threads (unequal amounts of effort) at sites in San Diego County between June 3-6, 2003. AL=Agua Hedionda Lagoon, 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 Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). Juveniles found at AL in a clump of dislodged *M. californianus* adults were likely settled outside of the lagoon at AH, and are therefore considered separately (AL/M).

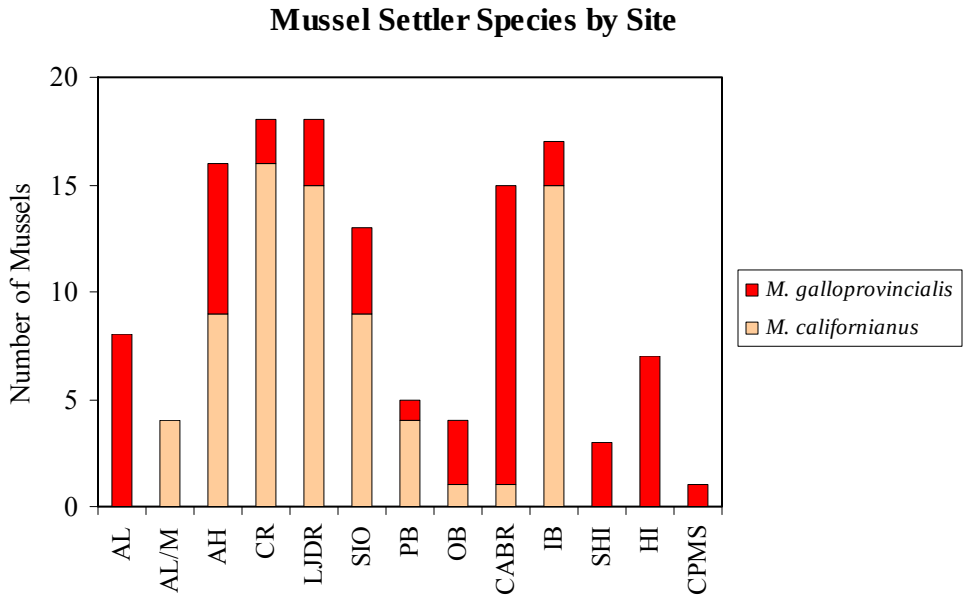


Figure 5.14: Determination of natal origin of *M. californianus* juveniles collected at sites in San Diego County using shell chemistry of *M. californianus* larvae outplanted offshore for one week (Table 5.4, Figure 5.7). Northern coastal region (Blues): AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region (Reds and Oranges): SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR II=Cabrillo National Monument (management Zone II), IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region (Green): HI=Harbor Island (San Diego Bay). Inner bay region (Pinks): CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay).

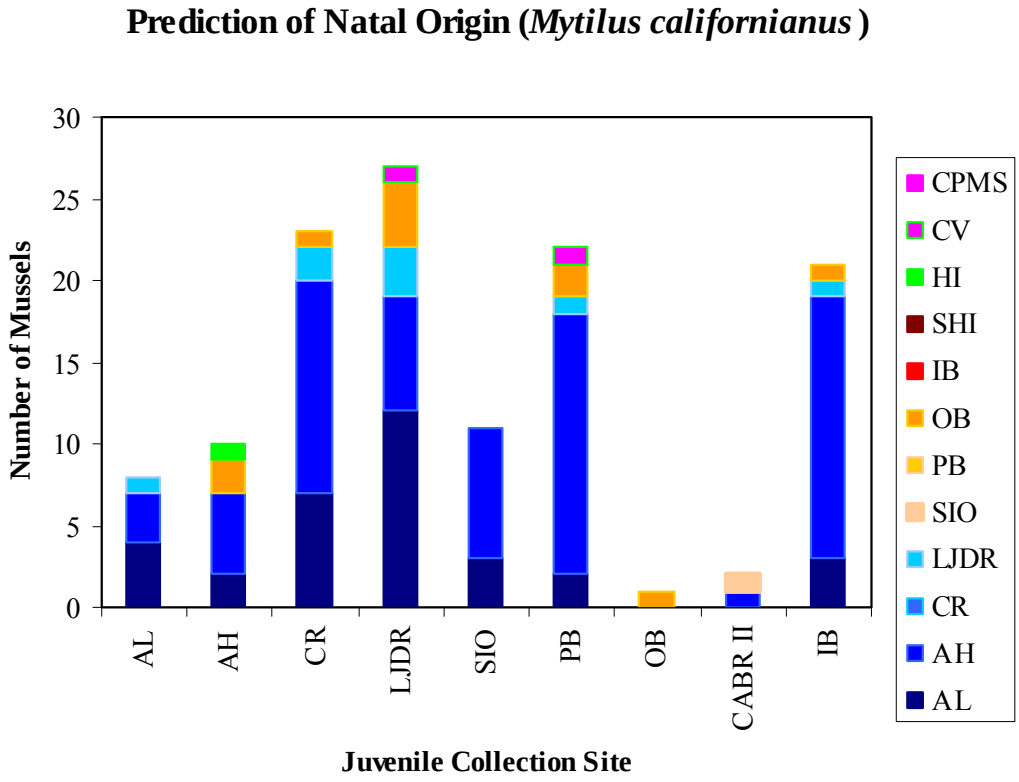


Figure 5.15: Determination of natal origin of *M. galloprovincialis* juveniles collected at sites in San Diego County using shell chemistry of *M. californianus* and *M. galloprovincialis* larvae outplanted offshore for one week (Table 5.5, Figure 5.9). Northern coastal region (Blues): AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock. Southern coastal region (Reds and Oranges): SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument (3 management zones), IB=Imperial Beach Pier, SHI=Shelter Island. Outer bay region (Green): HI=Harbor Island (San Diego Bay). Inner bay region (Pinks): CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay).

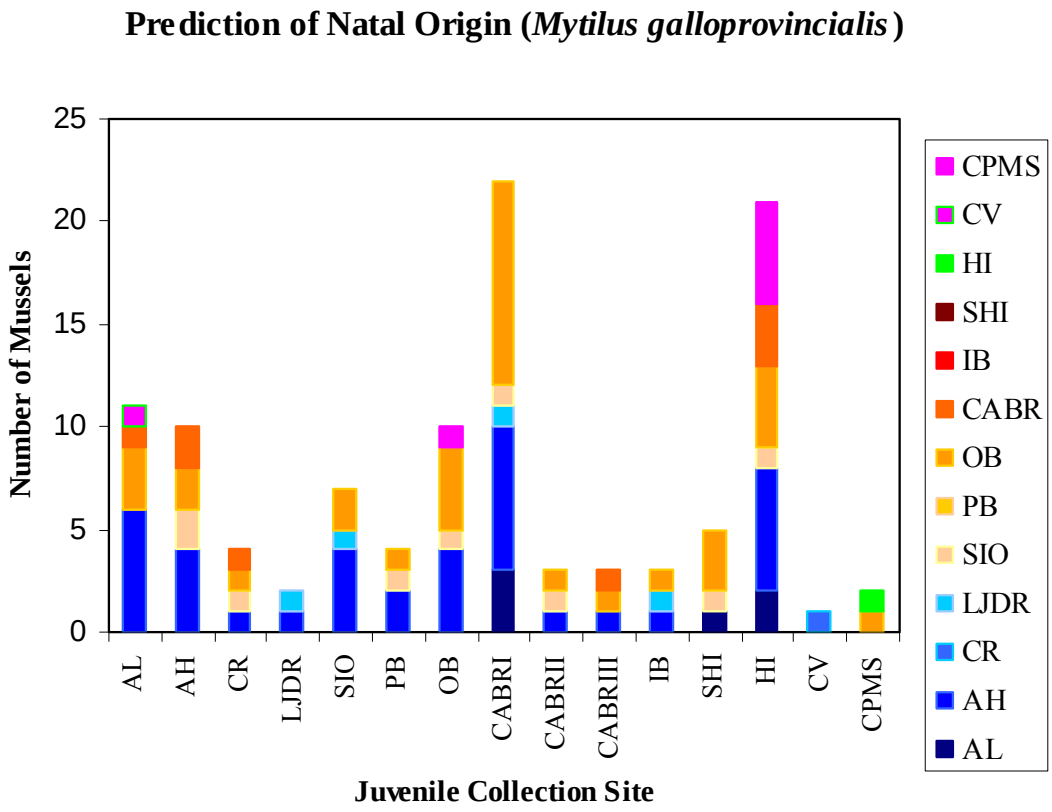
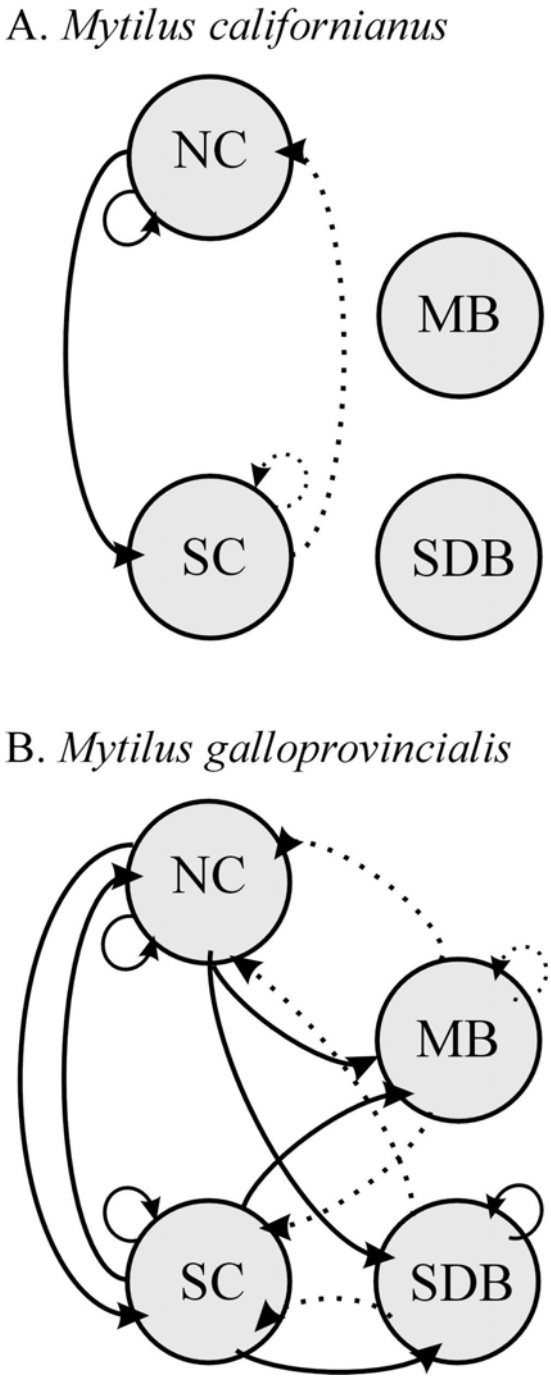


Figure 5.16: Schematic diagram of population connectivity among natal regions of (A) *M. californianus* and (B) *M. galloprovincialis* in San Diego County as determined using shell chemistry larvae outplanted offshore for one week. NC=northern coastal region, SC=southern coastal region, MB=Mission Bay, SDB=San Diego Bay.



APPENDIX 5A

Evaluating the success of in situ larval culturing of *Mytilus californianus* and *M. galloprovincialis*: outplanting and larval home effects

INTRODUCTION

Larvae of *Mytilus californianus* (California mussels) and *M. galloprovincialis* (bay mussels) were cultured in situ at 13 sites in San Diego County, CA in order to develop a trace elemental fingerprint of mussel larval shells for use as a larval tracking tool. In this appendix, the survivorship and growth rate data for this experiment are presented. In addition, the chemical analysis of two laboratory control cultures, in which both species were raised either loose in buckets or in larval homes, are compared with data from field-raised larvae. By comparing larvae of both species, control treatments, and selected sites, I explore the effects of species and larval home treatment on chemical signatures of mussel larval shells.

METHODS

M. californianus and *M. galloprovincialis* were spawned in the laboratory on May 11, 2003, and resulting embryos were quickly outplanted in larval "homes" at 13 sites (2 homes per site for *M. californianus* and 1 home per site for *M. galloprovincialis*). At the time of outplanting, some embryos were retained in the laboratory as controls. One set of each species was raised loose in buckets and the other was raised in larval homes in buckets. Larvae were allowed to grow and accumulate local chemical signatures for seven days, after which the homes were picked up and brought to the laboratory. A detailed description of these methods is given in Chapter 5.

Determination of larval mortality and growth rates

Once back at the lab, the homes were filtered using the existing mesh and water collected from that site, and the contents of each home was examined for mussel larval survivorship. This was done by diluting the filtered material to 50 ml with seawater from the site, and taking a well-mixed sample of 1 ml. If few or no larvae were found in the first sample, additional aliquots were taken until four or five larvae were found or four milliliters had been searched. This value was converted to number of shell survivors per milliliter, and a survival rate was estimated using the assumption that 100,000 embryos were originally added to the home. Although the number of moving shells were counted separately, these numbers are biased since the amount of mortality during the many hours the samples were in the laboratory was not estimated and is likely to be high and different for each home depending on the order of processing. Since the larvae were added as shell-less embryos, it was assumed that the larvae survived in the field for long enough to grow a shell.

At collection, a sample was taken from each home and fixed with a small amount of buffered formalin. The maximum length and width of these individuals were measured at a later date using a video/microscope system to determine shell

growth rates for field-raised larvae. The rest of the material was stored in an acid-washed, 50 ml centrifuge tube at -20°C. The control cultures were processed in a similar manner.

Analysis of larval shells

Larval shells were analyzed for eight isotopes (^{26}Mg, ^{48}Ca, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{88}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U), which were then ratioed to ^{43}Ca, a rare isotope of calcium. Discriminant function analysis was used to compare the shell chemistry (i.e., elemental fingerprint) of both species of mussel larvae, laboratory treatment (in homes or loose in buckets), and the interaction between the two. In addition, laboratory-raised individuals were compared to larvae raised at sites where both species were successfully analyzed for shell chemistry.

RESULTS

Survival of outplanted larvae

The larval survival rates (as determined by formation of larval shell) in the field, relative to laboratory samples, were quite high (1.84% survival of field-raised larvae, 8.05% of larvae raised in homes in the lab), given the large number of potential sources of mortality in situ, such as predators, shaking, infection, and lack of food. However, the survival was variable (3.64% standard deviation for all field-raised individuals) and species-dependent (2.58% and 0.35% average survival for *M. californianus* and *M. galloprovincialis*, respectively) and site (ranging from 0% to 15.95% average survival, Table 5A.1). Natural mortality rates are reported to be around 10-20%/day (20-50% survival after 7 days, Widdows 1991).

While studying the artifacts of in situ larval culturing of *Acanthaster planci*, Olson et al. (1988) determined that size of culturing chamber and amount of flushing did not affect time to settlement or normal development of larval arms, and that the differences between the outplanted and laboratory larvae was due to differential nutrition rather than mechanical shaking. They suggest that it would be important to limit the density of larvae in the chambers. In this study, extremely high concentrations of larvae were used (reported maximum natural concentrations of *Mytilus* larvae range from 1500-40,000 larvae/m^3, Bayne 1976), and I do not mean to imply that mortality rates in larval homes mimic those in nature. The goal of the outplanting was to culture as many individuals as possible to maximize the statistical power of the reference signal.

Larval shell growth rates

The outplanting experiment yielded larval shells that were mostly greater than 100 µm long that were formed entirely in the field at known locations. There were significant differences in the length of the larval shells by species and depending on whether they were raised in the laboratory or in the field (ANOVA, $p<0.05$ for treatment, species, and interactions). The size of the larval shells recovered from the field (108.5 $\pm$ 12.7 µm long x 77.6 $\pm$ 10.1 µm wide, mean $\pm$ 1 SD) were comparable in size to those raised in homes in the laboratory (110.3 $\pm$ 5.3 µm long x 79.8 $\pm$ 4.3 µm

wide, Bonferroni post-hoc analysis, $p>0.05$), and a little smaller than those raised loose in buckets (120.3 $\pm$ 9.1 µm long x 89.6 $\pm$ 8.3 µm wide, Bonferroni post-hoc analysis, $p<0.05$). Although the *M. galloprovincialis* raised in the field (107.7 $\pm$ 7.5 µm long x 74.3 $\pm$ 7.2 µm wide) were a little smaller than the *M. californianus* (108.7 $\pm$ 13.4 µm long x 78.1 $\pm$ 10.4 µm wide), the laboratory *M. galloprovincialis* raised in the laboratory (118.5 $\pm$ 10.6 µm long x 87.2 $\pm$ 10.0 µm wide) were a little bigger than the *M. californianus* (112.0 $\pm$ 5.3 µm long x 82.2 $\pm$ 4.8). There was some variability in size depending on site as well (Table 5A.2). These differences could be due to a difference in growth rate or a higher mortality rate, since shells that were alive or dead at the time of collection were not differentiated.

Shell chemistry of controls: Home and species effects

The chemistry of larval shells raised in larval homes in the laboratory was different than those that were cultured loose in buckets (Table 5A.3, Figure 5A.1). This difference was mostly due to higher Sr/Ca and lower U/Ca in the larval shells raised in homes, while all other element ratios did not differ greatly between them (i.e., all failed meet the "F-to-Remove" criteria normally used to select variables when creating a DFA).

Likewise, there were differences between *M. californianus* and *M. galloprovincialis* raised in the laboratory (DFA with 76% classification success, Table 5A.3, Figure 5A.1). The difference was more pronounced in larvae raised in homes than those raised in buckets. In this case, *M. galloprovincialis* contained more U/Ca and Cu/Ca. Although Cu/Ca was not found to be important in the total DFA (i.e., F-to-Remove < 3.5) and Sr/Ca was more influential, when species were examined separately (regardless of home), Cu/Ca was significant and Sr/Ca was not.

Although there were discernable differences in shell chemistry due to the larval home treatment and species, it is important to place these differences in context with differences in the field. When compared to sites where both species were analyzed (AL, CPMS, PB, SIO), the shell chemistry of individuals raised in either laboratory treatment were similar (Table 5A.4, Figure 5A.2) and tended to be higher in Sr/Ca and lower in Co/Ca, U/Ca and Ba/Ca (other elements, although included in the DFA for comparability, did not meet the F-to-Remove criterion).

There were differences in shell chemistry between the two species when considering interactions with treatment. *M. californianus* raised in the laboratory was never misclassified as coming from a field site, but nine out of 33 lab-raised *M. galloprovincialis* were misclassified as coming from PB or SIO, with no difference between those raised in homes or loose in buckets (Table 5A.4, Figure 5A.2). Unfortunately, the sample sizes of larvae raised in some of the field sites where both species were successfully analyzed are quite small and were from sites (SIO, PB) that have acted as transition zones between regions (Becker et al. 2005); therefore, classification successes by site were low. However, some patterns did emerge. When AL, SIO, and PB are considered as the same open coast "natal region", classification success was relatively high and did not seem to have a clear relationship with species. However, the shell chemistry of CPMS larvae was quite different depending on

species. This difference was attributed to very high Co/Ca levels in *M. californianus* from this site.

When this analysis is repeated without Co/Ca considered within the DFA, the differences between the species at CPMS were somewhat lessened (Table 5A.5, Figure 5A.3). Unfortunately, without this element ratio, the differences between the laboratory and the SIO site were diminished, leading to lower classification successes for both. However, since the laboratory larvae were raised with seawater from the SIO pier, this similarity is not surprising. In addition, larvae from a number of sites were mistakenly classified as coming from CPMS.

CONCLUSIONS

In this experiment, I successfully raised larvae in situ and generated over 100 μm long larval shells to serve as a reference material for larval tracking purposes. Mortality of larvae within homes was high (approximately 98%) but similar to lab controls. Future studies should consider using a lower concentration of larvae within the homes (or using larger homes) to improve the culturing conditions.

There was an effect on shell chemistry of raising larvae in homes, but it was negligible compared to the signals from the field sites. The species effect was greater, although removing Co/Ca from the analysis improved the differences between the species. In order to classify natal origin of *M. californianus* juveniles, there was a large enough sample size to use only *M. californianus* larvae. Since fewer *M. galloprovincialis* larvae were analyzed, chemical signatures of larvae of both species were combined to determine natal origins of *M. galloprovincialis* juveniles. For this two-species analysis, Co/Ca was not included.

Table 5A.1: Number of larval shells found after 1 week of outplanting in sites around San Diego County. Individuals were outplanted as shell-less embryos. C=*Mytilus californianus*, G=*Mytilus galloprovincialis*. AH=Agua Hedionda, AL=Agua Hedionda Lagoon, CABR=Cabrillo National Monument, CPMS=Crown Point Mitigation Site (Mission Bay), CR=Cardiff Reef, CV=Chula Vista (San Diego Bay), HI=Harbor Island (San Diego Bay), IB=Imperial Beach Pier, LJDR=La Jolla Dike Rock, OB=Ocean Beach Pier, PB=Pacific Beach (Crystal) Pier, SHI=Shelter Island (San Diego Bay), SIO=Scripps Institution of Oceanography Pier, Lab-Home=Control grown in larval home in laboratory at SIO.

Home #	Site	Species	#/ml	% survival
28	AH	C	0.5	0.02
39	AH	C	122.0	6.10
11	AH	G	0.3	0.01
9	AL	C	1.5	0.08
42	AL	C	136.0	6.80
18	AL	G	27.0	1.35
23	CABR	C	0.7	0.03
26	CABR	C	5.0	0.25
16	CABR	G	2.3	0.12
37	CPMS	C	1.0	0.05
13	CPMS	C	1.5	0.08
44	CPMS	G	2.0	0.10
45	CR	C	5.0	0.25
19	CR	C	6.0	0.30
17	CR	G	1.3	0.07
4	CV	C	38.0	1.90
22	CV	C	168.0	8.40
2	CV	G	6.0	0.30
31	HI	C	6.0	0.30
36	HI	C	56.0	2.80
34	HI	G	2.5	0.13
14	IB	C	2.5	0.13
41	IB	C	25.0	1.25
25	IB	G	0.0	0.00
43	LJDR	C	8.0	0.40
40	LJDR	C	319.0	15.95
35	LJDR	G	7.0	0.35
30	OB	C	114.0	5.70
15	OB	C	261.0	13.05
29	OB	G	0.5	0.02
38	PB	C	0.0	0.00
7	PB	C	1.0	0.05
6	PB	G	3.0	0.15
3	SHI	C	10.0	0.50
32	SHI	C	43.0	2.15
33	SHI	G	26.0	1.30
20	SIO	C	0.8	0.04
8	SIO	C	12.0	0.60
24	SIO	G	12.0	0.60
47	Lab-Home	C	270.0	13.50
46	Lab-Home	G	52.0	2.60

Table 5A.2: Average length and width of larvae raised in situ at sites around San Diego County and in the laboratory for seven days. Individuals were outplanted as shell-less embryos. Homes listed in Table 5A.1 but not listed here did not have enough sample to be counted. C=*Mytilus californianus*, G=*Mytilus galloprovincialis*. AH=Agua Hedionda, AL=Agua Hedionda Lagoon, CABR=Cabrillo National Monument, CPMS=Crown Point Mitigation Site (Mission Bay), CR=Cardiff Reef, CV=Chula Vista (San Diego Bay), HI=Harbor Island (San Diego Bay), IB=Imperial Beach Pier, LJDR=La Jolla Dike Rock, OB=Ocean Beach Pier, PB=Pacific Beach (Crystal) Pier, SHI=Shelter Island (San Diego Bay), SIO=Scripps Institution of Oceanography Pier, Lab-Bucket=Control grown in laboratory loose in bucket without larval home, Lab-Home=Control grown in larval home in laboratory at SIO.

Home #	Site	Species	Number Measured	Avg. Length (µm)	SD Length (µm)	Avg. Width (µm)	SD Width (µm)
28	AH	C	1	98.6	n/a	73.0	n/a
39	AH	C	20	108.3	9.7	75.1	8.0
9	AL	C	1	101.2	n/a	75.6	n/a
42	AL	C	20	102.5	9.3	74.1	6.5
18	AL	G	15	106.9	4.7	74.3	4.5
23	CABR	C	1	97.9	n/a	73.7	n/a
13	CPMS	C	3	105.6	5.1	82.2	7.8
37	CPMS	C	2	103.1	13.9	73.6	7.1
19	CR	C	2	96.4	9.3	63.1	10.5
45	CR	C	2	103.1	18.0	77.8	11.8
4	CV	C	9	107.4	14.0	76.2	7.4
22	CV	C	7	114.6	2.3	82.2	6.7
2	CV	G	3	101.3	1.8	70.3	1.7
31	HI	C	2	86.4	14.4	62.7	4.9
36	HI	C	20	106.1	7.4	75.4	5.3
34	HI	G	1	104.5	n/a	73.6	n/a
41	IB	C	6	93.4	7.6	64.9	7.8
40	LJDR	C	20	128.9	6.6	94.6	5.2
43	LJDR	C	2	85.0	6.4	57.9	0.6
35	LJDR	G	3	114.9	8.0	83.6	11.9
15	OB	C	20	115.7	12.5	82.7	9.1
30	OB	C	20	115.5	9.0	84.2	7.0
7	PB	C	1	89.7	n/a	57.8	n/a
3	SHI	C	3	108.8	7.3	78.3	4.5
32	SHI	C	6	95.7	3.2	70.0	4.4
33	SHI	C	9	97.1	8.7	70.9	6.2
24	SIO	G	8	109.2	11.0	72.4	9.2
C	Lab-Bucket	C	20	113.7	5.5	83.5	5.7
G	Lab-Bucket	G	20	126.9	7.0	95.8	5.3
47	Lab-Home	C	20	110.3	4.7	80.8	3.3
46	Lab-Home	G	20	110.2	5.9	78.7	5.0

Table 5A.3: Jackknifed classification success of a DFA comparing shell chemistry of lab-cultured mussel larvae, comparing treatments and species. Upper grouping compares shell chemistry of both species of larvae raised in the lab, either loose in buckets (Bucket) or in a larval home in a bucket (Home). Lower grouping compares *M. californianus* (C) and *M. galloprovincialis* (G), regardless of laboratory treatment. DFA scores and element ratios used are shown in Figure 5A.1.

		Predicted Treatment		
		Bucket	Home	% Correct
Actual Treatment	Bucket	23	8	74
	Home	11	21	66
	Total			70
		C	G	% Correct
	C	26	4	87
	G	11	22	67
	Total			76

Table 5A.4: Jackknifed classification success of a DFA using all available element ratios comparing shell chemistry of lab-cultured mussel larvae with larvae raised in larval homes in situ at sites in San Diego County. DFA was conducted without grouping by species, which were used to group later. Classification success grouped by site and by Laboratory or Region (Lab, Open Coast, or Bay, as delimited by dotted line). Bucket=laboratory cultures loose in buckets, Home=laboratory cultures raised in larval homes, AL=Agua Hedionda Lagoon, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach Pier, CPMS=Crown Point Mitigation Site. C=*M. californianus* and G=*M. galloprovincialis*. DFA scores and element ratios used are shown in Figure 5A.2.

		Predicted Site							Classification Success (%)	
	Species/Site	Bucket	Home	AL	SIO	PB	CPMS	Sum	Site	Lab/ Region
Actual Site/Species	Bucket/C	12	3					15	80	100
	Bucket/G	9	3		2	2		16	56	75
	Lab/C		15					15	100	100
	Lab/G	3	9		2	3		17	53	71
	AL/C	2		4	4	7		17	24	88
	AL/G			5		1		6	83	100
	SIO/C		1		2			3	67	67
	SIO/G	3	1		8	2	1	15	53	67
	PB/C		1		1	1		3	33	67
	PB/G			3		1		4	25	100
	CPMS/C						6	6	100	100
	CPMS/G		1		4			5	0	0

Table 5A.5: Jackknifed classification success of a DFA using all available element ratios except Co/Ca comparing shell chemistry of lab-cultured mussel larvae with larvae raised in larval homes in situ at sites in San Diego County. DFA was conducted without grouping by species, which were used to group later. Classification success grouped by site and by Laboratory or Region (Lab, Open Coast, or Bay, as delimited by dotted line). Bucket=laboratory cultures loose in buckets, Home=laboratory cultures raised in larval homes, AL=Agua Hedionda Lagoon, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach Pier, CPMS=Crown Point Mitigation Site. C=*M. californianus* and G=*M. galloprovincialis*. DFA scores and element ratios used are shown in Figure 5A.3. This analysis is identical to that described in Table 5A.4 and Figure 5A.2, but without using Co/Ca as a predicting variable.

		Predicted Site							Classification Success (%)	
	Site/Species	Bucket	Lab	AL	SIO	PB	CPMS	Sum	Site	Lab/Region
Actual Site/Species	Bucket/C	11	3				1	15	73	93
	Bucket/G	9	4		1	1	1	16	56	81
	Lab/C		15					15	100	100
	Lab/G	3	9		2	3		17	53	71
	AL/C	2		4	3	7	1	17	24	82
	AL/G			4		2		6	67	100
	SIO/C				1		2	3	33	33
	SIO/G	5	1		5	2	2	15	33	47
	PB/C		1		1	1		3	33	67
	PB/G			2		2		4	50	100
	CPMS/C						6	6	100	100
	CPMS/G				3		2	5	40	40

Figure 5A.1: DFA comparing shell chemistry of lab-cultured mussel larvae, comparing treatments and species. In upper panel, individuals are grouped according to treatment, either loose in buckets (Bucket) or in a larval home in a bucket (Home) and according to species, *M. californianus* (C) and *M. galloprovincialis* (G). Lower panel shows canonical discriminant functions (standardized by within variances) of used element ratios. Only Sr/Ca and Ba/Ca met the F-to-Remove ratio criterion for inclusion in the DFA, but all element ratios were used so that this DFA could be compared to other analyses in this paper. The first two DFA scores account for 91.5% of the total dispersion in the data. Corresponding DFA classification successes are shown in Table 5A.3.

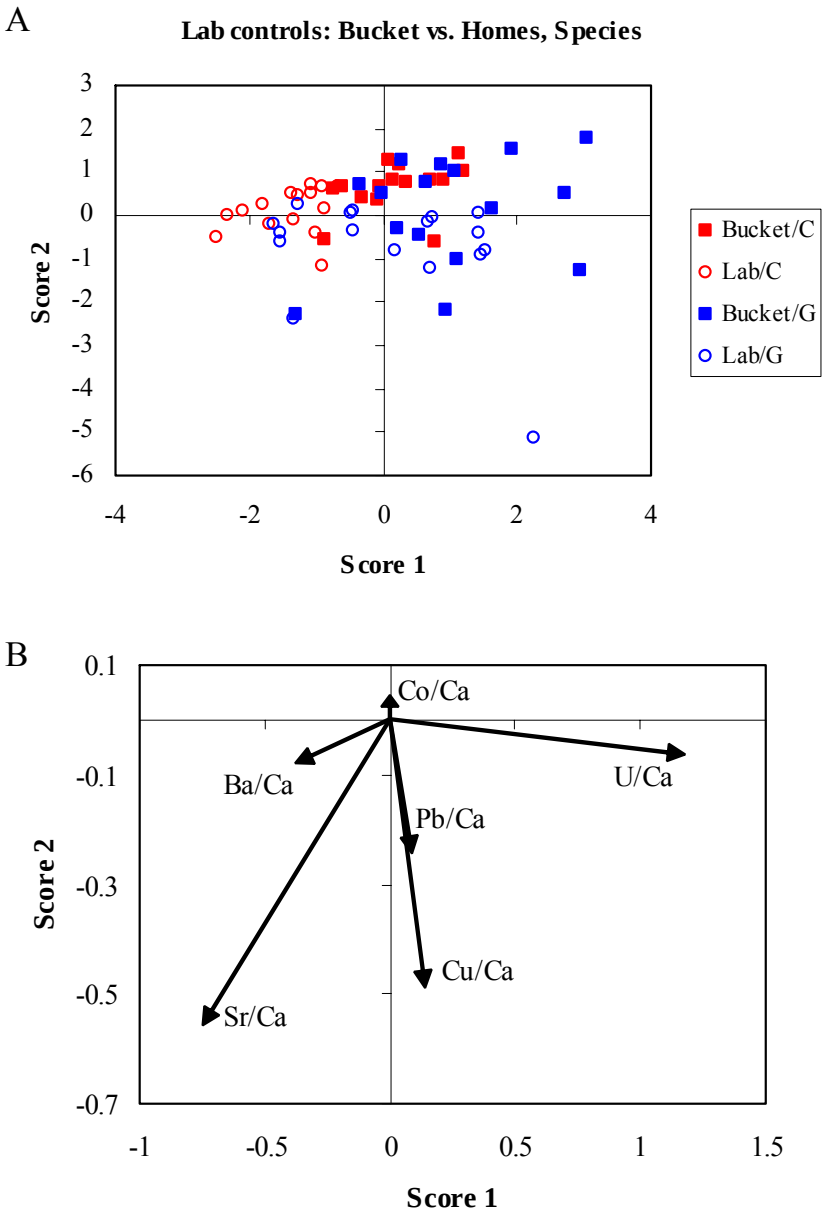


Figure 5A.2: DFA using all available element ratios comparing shell chemistry of lab-cultured mussel larvae with larvae raised in larval homes in situ at sites in San Diego County. DFA was conducted without grouping by species, which were used to group later. Upper panel shows individual scores of mussel shell chemistry, grouped by site or laboratory treatment, as well as species. Red icons are laboratory control treatments, blue icons are open coast sites, and pick icons are bay sites. Bucket=laboratory cultures loose in buckets, Home=laboratory cultures raised in larval homes, AL=Agua Hedionda Lagoon, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach Pier, CPMS=Crown Point Mitigation Site. C=*M. californianus* and G=*M. galloprovincialis*. Lower panel shows canonical discriminant functions (standardized by within variances) of used element ratios. Only Co/Ca, Sr/Ca, and Pb/Ca met the F-to-Remove ratio criterion for inclusion in the DFA, but all element ratios were used so that this DFA could be compared to other analyses in this paper. The first two DFA scores account for 92.0% of the total dispersion in the data. Corresponding DFA classification successes are shown in Table 5A.4.

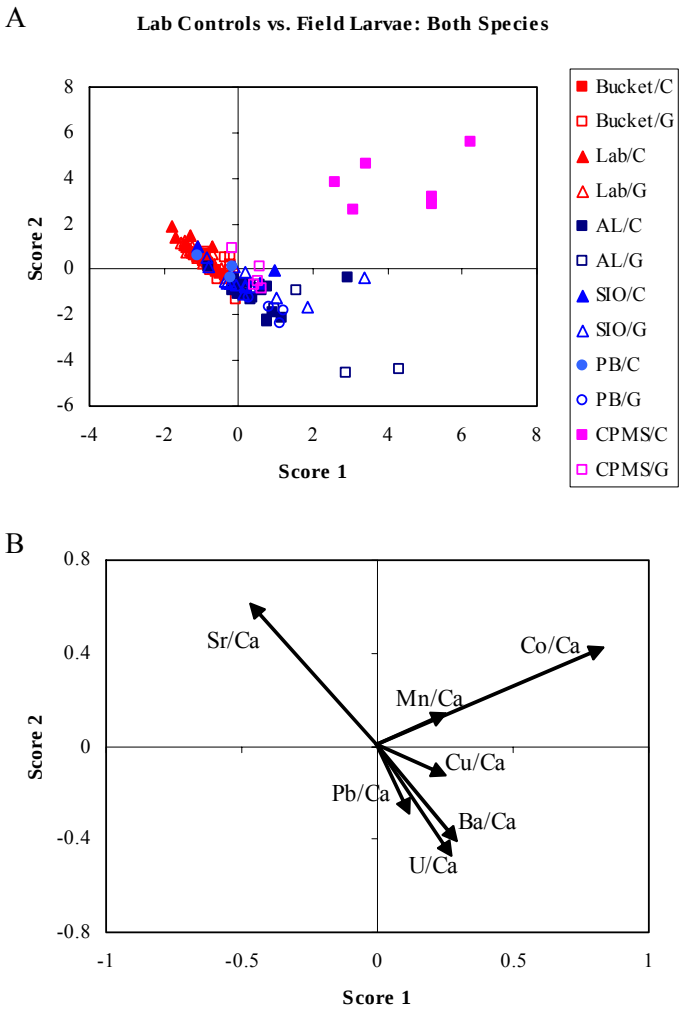
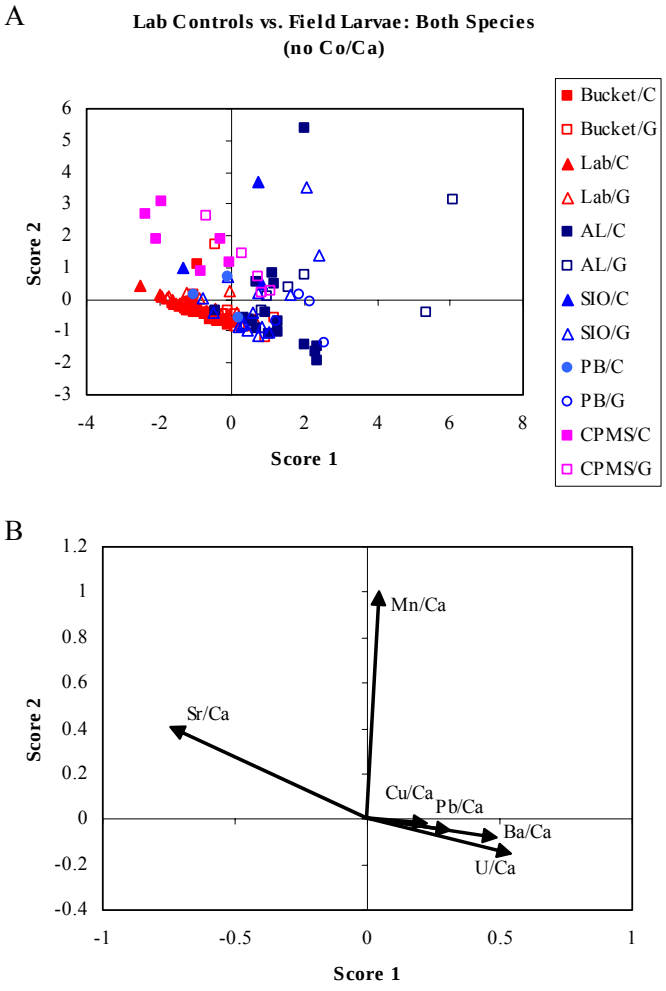


Figure 5A.3: DFA using all available element ratios except Co/Ca comparing shell chemistry of lab-cultured mussel larvae with larvae raised in larval homes in situ at sites in San Diego County. DFA was conducted without grouping by species, which were used to group later. Upper panel shows individual scores of mussel shell chemistry, grouped by site or laboratory treatment, as well as species. Red icons are laboratory control treatments, blue icons are open coast sites, and pick icons are bay sites. Bucket=laboratory cultures loose in buckets, Home=laboratory cultures raised in larval homes, AL=Agua Hedionda Lagoon, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach Pier, CPMS=Crown Point Mitigation Site. C=*M. californianus* and G=*M. galloprovincialis*. Lower panel shows canonical discriminant functions (standardized by within variances) of used element ratios. Only Mn/Ca, Sr/Ca, and U/Ca met the F-to-Remove ratio criterion for inclusion in the DFA, but all element ratios were used so that this DFA could be compared to other analyses in this paper. The first two DFA scores account for 88.6% of the total dispersion in the data. Corresponding DFA classification successes are shown in Table 5A.5. This analysis is identical to that described in Table 5A.4 and Figure 5A.2, but without using Co/Ca as a predicting variable.



APPENDIX 5B

Evaluating the settlement of *Mytilus californianus* and *M. galloprovincialis* into two different substrates (red algal turf and adult byssal threads) at thirteen sites within San Diego County

INTRODUCTION

Juvenile mussels were collected from sites in San Diego County in order to determine their natal origins using shell chemistry. As part of that study, the settlement rates of two species (California mussels, *Mytilus californianus* and bay mussels, *M. galloprovincialis*) in different locations (including coastal and bay environments) and into different settlement substrates (adult mussels and red algal turf), are contrasted.

M. californianus are mostly found in wave-exposed coastal areas, and are generally not tolerant of conditions in bays and harbors (Harger 1968). *M. galloprovincialis* are found in calm water as well as on the open coast, where it competes with the larger congener. *M. californianus* is generally the better competitor, as it is bigger, more robust, and more tolerant of high wave action (Suchanek 1981). Due to past observations, it was predict that more *M. californianus* settlers would be found at open coast sites and more *M. galloprovincialis* in the embayments.

Many studies of mussel recruitment use artificial settling substrates, such as "Tuffy" plastic scrubbers, to standardize between their sites. However, there is indication that *M. californianus* and *M. galloprovincialis* have preferential settlement behaviors that could lead to bias depending on the settlement substrate used. In laboratory preferential settlement studies, Petersen (1984) found that *M*. "*edulis*" (probably misidentified *M. trossulus*, Mcdonald and Koehn 1988) avoided settling on *M. californianus* (although they occasionally did), and favored conspecifics or red algae for settlement. *M. californianus* preferred to settle on *M. "edulis"*. He hypothesized that this result was related to the competition between the two species, of which *M. californianus* is the stronger competitor (Suchanek 1981, although see Cáceres-Martínez et al. 1994 for alternative explanations). I therefore hypothesize that more *M. californianus* settle on adult mussels and more *M. galloprovincialis* settling in turf-forming algae.

METHOD

Collection and sorting of mussel early settlers

Juveniles were obtained from the intertidal zone by collecting materials that mussels are known to settle into (adult mussels and turf-forming algae) from 13 sites in San Diego County, California during the period of June 3-6, 2003. Whenever possible, both adult mussels and red algal turf were collected to maximize the number

of settlers; however at seven sites only one was available or legally collectable (see Table 5.2 and Figure 5.3 for site descriptions).

Samples were frozen (-20^{o}C) in zip lock bags within two hours of collection and were sorted at a later date. The material was examined under a dissecting microscope in Milli Q water in an acid-washed plastic dish and mussel juveniles (less than 3 mm maximum length) were removed using acid-dipped ceramic-tipped forceps (Fine Science Tools). Approximate settlement levels (defined as number of mussels found at time of collection less than 3 mm maximum length) were determined in both settling substrates by standardizing for search effort. For algae samples, 15 g of algae and sand were completely sorted. Adult mussel samples were stripped of all byssal threads and fouling materials, which were then divided in half and sorted in random order for up to one hour. The byssal threads were then dried and weighed. In both cases, individuals were sorted into approximate size classes, and stored in acid-washed vials at -20^{o}C.

If there were less than approximately 20 mussels found after the allotted amount of algae or search time, the remaining substrate was sorted to supplement the sample size for the chemical analyses (Chapter 5). These additional mussels were not included in comparisons of total settlement rates but were identified to species.

Species identification of early mussel settlers

A PCR-based assay, as described in Becker et al. (2005), was utilized to determine the species of mussel in 99 individuals. It should be noted that this assay does not discriminate between *M. galloprovincialis* and the similar *M. trossulus*; it is possible that some of the San Diego Bay mussels were *M. trossulus* or hybrids between the two species (Suchanek et al. 1997).

RESULTS

Settlement rates of juveniles

The estimated settlement of all mytilid juveniles was site- and settlement substrate-dependent (Table 5B.1, Figure 5B.1). The number of mussels settling in algae was highest in northern open coast sites and declined to the south and in the bays (Figure 5B.1A). Although AL is an enclosed bay, the amount of settlement there was higher than the other bay sites. The mussel settlement in the three management zones of CABR also reflected this north to south pattern, with CABR III, the southernmost area of the park closed to all visitors experiencing the least settlement of any site except CV. The number of juveniles settling in byssal threads was also higher in open coast sites than bay sites, with AL experiencing more settlement than most bay sites (Figure 5B.1B). In addition, HI received as much settlement as CR and SIO, two northern open coast sites. The north to south pattern was not evident in byssal thread settler estimates; for example, IB pier, the most southerly site, had the second highest number of settlers per gram of byssal thread.

Species identification of juveniles

There were clear geographic patterns in the number of identified *M. californianus* and *M. galloprovincialis* settlers (Table 5B.2, Figure 5B.2). Of the mussels identified with PCR, no *M. californianus* were found in Mission or San Diego Bays. In Agua Hedionda Lagoon, all of the mussels that settled in attached algae were *M. galloprovincialis*, while all of the mussels found in a clump of *M. californianus*, likely dislodged from the outer mouth of the lagoon, were also *M. californianus*.

Samples from all open coast sites contained a mixture of both species (Table 5B.2, Figure 5B.2). At most sites, more *M. californianus* were identified than *M. galloprovincialis*. However, at two southern open coast, southern sites, CABR and OB settlers, were mainly *M. galloprovincialis* (93% and 75%, respectively). The most southerly site (IB), unlike its nearest neighbors, was dominated by *M. californianus* (88%).

Differences in settlement by the two species were related to the substrate of settlement. Among open coast sites where both adult mussels and turf algae were examined, more *M. californianus* were found in the mussels while more *M. galloprovincialis* were found in the algae.

CONCLUSIONS

The settler distribution of the two mussel species was not random with respect to site or substrate. Although both species settled on the open coast, only *M. galloprovincialis* was found in the bays. Johnson (2003) found that despite similar larval distributions throughout her study area, bay mussel settlers (*M. galloprovincialis* and *M. trossulus*) were found in wave-swept and protected areas, while *M. californianus* settlers were only found on the open coast. These results corroborate the previous observations, and indicate that the lack of *M. californianus* in bays is due to pre-settlement processes (including settlement preferences); post-settlement mortality is likely the reason why fewer *M. galloprovincialis* are found on the open coast.

More *M. californianus* was found in the northern coastal region than in the other parts of the study area. This could be related to fact that this region serves as the main source for new *M. californianus* in the region (see Chapter 5). Two of the southern sites (OB and CABR), that do not support large populations of *M. galloprovincialis*, had proportionally higher settlement of this species. Further south at IB, most of the mussels were *M. californianus*. Roughan et al. (in press) documented the presence of a current-driven upwelling area in the lee of Point Loma that existed the month before this study and is generally persistent. They note that the month before the outplanting began, there was an additional water mass that bypassed this region in a current to the west. It is not known if the hydrography of the Point Loma area, including an area of upwelling, could have led to the exclusion of larvae to this area (e.g., Wing et al. 1995, Wing et al. 1998). This difference between sites in the southern region needs further study.

As predicted, more *M. galloprovincialis* were found in turf-forming algae and more *M. californianus* in adult mussel byssal threads. This result could be useful in

the planning of future studies; the use of artificial substrates to compare the settlement rates of the two species might lead to biased results. It was difficult to assess the relative density of settlers on algae and byssal threads, since weights of these two materials were not comparable to account for effort. However, from these results it appeared as if adult mussel byssal threads hosted more settled juveniles. More mussels could be found in a smaller amount of byssal thread material than algal material, and it was generally easier to sort. In addition, subtle differences in algal species composition could bias mussel settlement. The advantages of using algae were that it is easier to standardize by weight (e.g., settlement rate might vary with size of adult mussels, which might not be reflected in the amount of byssal material sorted), was often easier to collect (especially from areas that regulate the removal of animals), and is less destructive to the habitat.

Table 5B.1: Number of juvenile mussels (<3 mm length) collected in either algal mats (Algae) or amongst adult mussel byssal threads (Mussels) at sites in San Diego County between June 3-6, 2003. The total number of mussels found are expressed as total per dry weight of settlement material (either algae or byssal threads) and as total per hour searched (for adult mussel samples only). AL=Agua Hedionda Lagoon, 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 Pier, CABR=Cabrillo National Monument (three management zones), IB=Imperial Beach Pier, SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay).

Site	Settlement Substrate	Total Mussels Found	Mussels/g	Mussels/h
AL	Algae	17	2.76	
AH	Algae	68	12.08	
CR	Algae	48	5.45	
SIO	Algae	10	3.10	
CABR I	Algae	27	2.15	
CABR II	Algae	9	1.37	
CABR III	Algae	5	0.74	
SHI	Algae	9	1.23	
HI	Algae	5	1.87	
CV	Algae	0	0.00	
AL	Mussels	34	13.44	20.34
AH	Mussels	18	47.37	15
CR	Mussels	38	20.54	21
SIO	Mussels	42	21.54	27.12
PB	Mussels	81	27.00	40.5
IB	Mussels	58	27.62	28.98
SHI	Mussels	8	4.44	4.02
HI	Mussels	30	23.26	16.98
CV	Mussels	3	1.01	1.5
CPMS	Mussels	4	1.10	1.98

Table 5B.2: Number of juvenile mussels (<3 mm length) identified to species collected in either algal mats (Algae) or amongst adult mussel byssal threads (Mussels) at sites in San Diego County between June 3-6, 2003. "PCR" and "PCR-no chemistry" were both identified using the same molecular genetic assay, but the latter samples were not analyzed chemically and were not included in the rest of the analyses in this study. The mussels under the "DFA Angle" were identified using a Discriminant Function Analysis that used the "PCR" mussels as known samples and a variety of factors (site, length/width ratio, hinge angle, and shell chemistry) as predictive variables. Those in the "DFA-no angle" column were not measurable for all of the variables used in the former DFA and were identified using site, settlement substrate, and shell chemistry. The percentage of *M. californianus* found at each site is calculated using PCR-identified mussels only. AL=Agua Hedionda Lagoon, 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 Pier, CABR=Cabrillo National Monument (three management zones), IB=Imperial Beach Pier, SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). These data do not reflect the same search effort for each site.

Site	Settlement Substrate	*Mytilus californianus* DFA Angle	DFA No Angle	PCR	PCR-no chemistry	Total	*Mytilus galloprovincialis* DFA Angle	DFA No Angle	PCR	PCR-no chemistry	Total	PCR-identified only % *M. californianus*
AL	Algae		1			1	3	3	5	3	14	0
AL	Mussels	4		4		8						100
AH	Algae	4		1	4	9	3		3	1	7	56
AH	Mussels	2		3	1	6	2		2	1	5	57
CR	Algae	2		3	2	7			1	1	2	71
CR	Mussels	8		10	1	19	3				3	100
LJDR	Algae	10	2	15		27			2	1	3	83
SIO	Algae						1		1		2	0
SIO	Mussels	4	1	7	2	14	3		2	1	6	75
PB	Mussels	18		4		22	3		1		4	80
OB	Mussels			1		1	7		3		10	25
CABRI	Algae						15		7	1	23	0
CABRII	Algae	1		1		2	2		2	1	5	25
CABRIII	Algae								3		3	0
IB	Mussels	8		13	2	23	1		2		3	88
Shelt	Algae						3	1	1		5	0
Shelt	Mussels						1	1	1	1	4	0
HI	Algae						1	1	1		3	0
HI	Mussels						11	3	5	1	20	0
CV	Mussels						1				1	n/a
CPMS	Mussels						2			1	3	0

Figure 5B.1: Number of juvenile mytilid mussels (<3 mm length) settling at sites in San Diego County on June 3-6, 2003. Two different settlement substrates were examined using different methods of standardizing for effort: (A) red algal turf was standardized by weight in grams and (B) adult mussel byssal threads were standardized by hours searched. AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). ND=not determined.

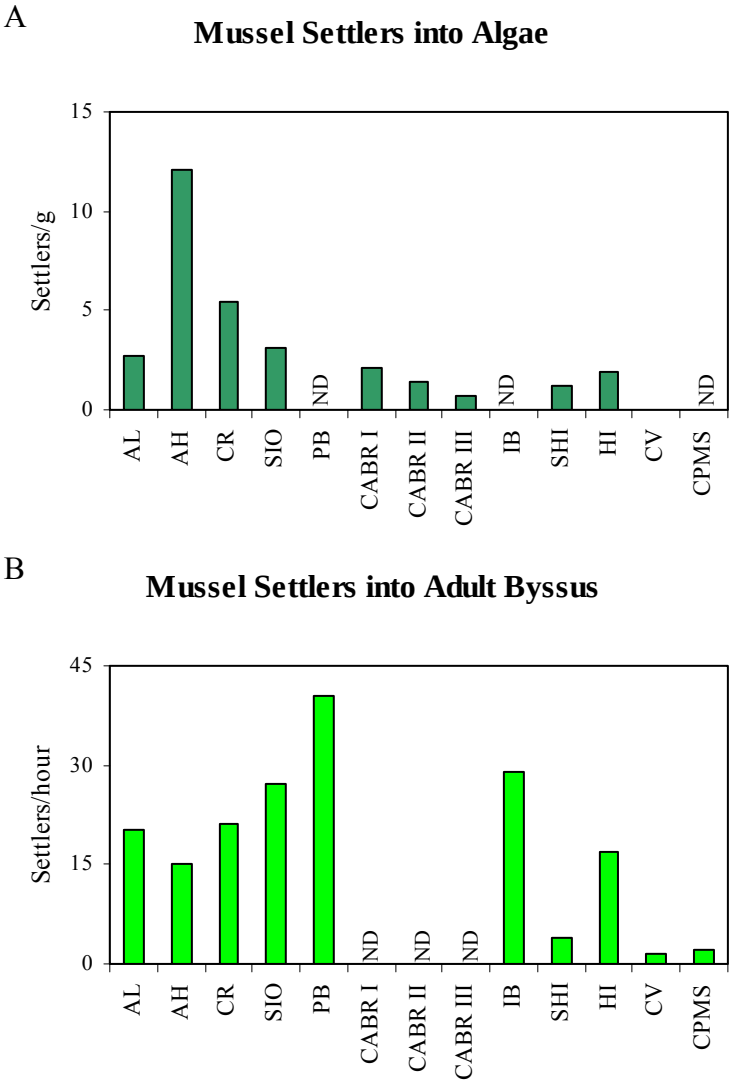
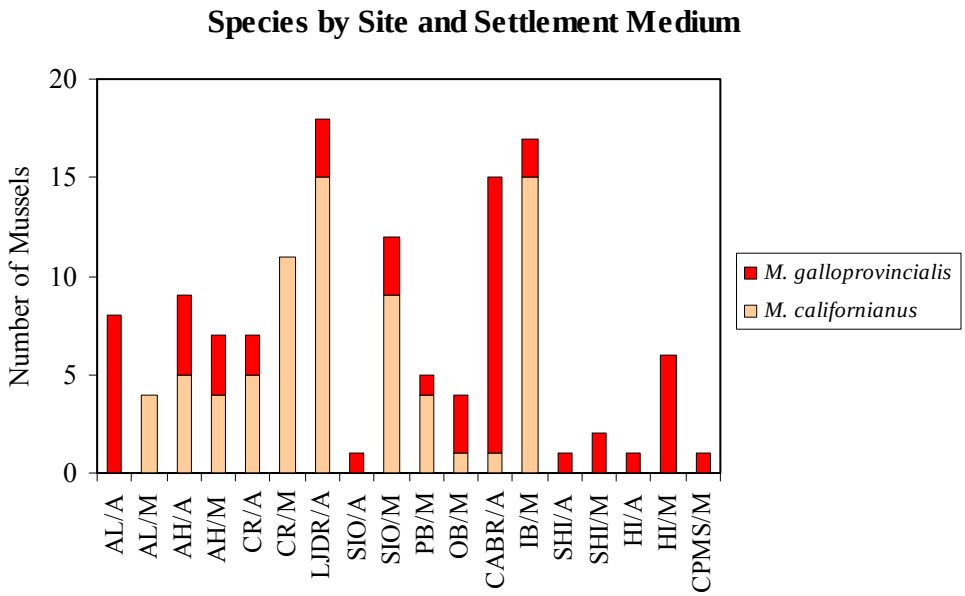


Figure 5B.2: Number of juvenile mussels (<3 mm length) identified to species using a PCR assay and that were collected in either algal mats (A) or amongst adult mussel byssal threads (M) at sites in San Diego County on June 3-6, 2003. AL=Agua Hedionda Lagoon, AH=Agua Hedionda, CR=Cardiff Reef, LJDR=La Jolla Dike Rock, SIO=Scripps Institution of Oceanography Pier, PB=Pacific Beach (Crystal) Pier, OB=Ocean Beach Pier, CABR=Cabrillo National Monument, IB=Imperial Beach Pier, SHI=Shelter Island (San Diego Bay), HI=Harbor Island (San Diego Bay), CV=Chula Vista (San Diego Bay), CPMS=Crown Point Mitigation Site (Mission Bay). These data do not reflect the same search effort for each site.



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CHAPTER VI

CONCLUSIONS

This research contributes to marine ecology and conservation in both an applied and a theoretical sense. The list of possible mechanisms leading to the decline of mussel population in Cabrillo National Monument have been narrowed down. Scales of mytilid mussel population dynamics and connectivity in southern California have been defined, both of which allow for formation of targeted hypotheses to further explore the abundance and distribution of intertidal organisms. Additionally, this work has broad implications for the understanding of larval connectivity of marine invertebrate populations.

CABRILLO NATIONAL MONUMENT

The mechanisms underlying a sustained decline in mussel cover at Cabrillo National Monument (CABR) are likely to be acting on a local (km) rather than a regional scale (100s of km), as determined by comparing mussel cover trends from CABR to that in sites throughout 500 km of shoreline in central and southern California (Chapter 3).

Over the past few years there have been low levels of settlement (defined as number of individuals <2-3 mm) in CABR compared to other sites in San Diego County (Chapter 2, Appendix 5B). It is not known if there is some local force that hampers larval delivery, inhibits settlement, or that leads to early mortality of settlers

in this area; in future studies, the survival of early life-history stages should be quantified and compared in order to isolate whether the CABR recruitment failure is due to pre- or post-settlement processes. It is possible that the large kelp forest offshore of Point Loma or the persistent upwelling in this area (Roughan et al. in press) act as a physical barrier to the delivery of larvae along the CABR shoreline. In addition, the lack of adult conspecifics or congeners in the Park might reduce the establishment of new mussel beds there due to lack of settlement cues (*Mytilus californianus*, the dominant adult mussel at CABR preferentially recruits into adult congenerics, Petersen 1984), or vulnerability of juveniles mussels without the protection of adult mussel beds. Since most new settlers of *M. californianus* collected throughout San Diego County (including sites to the north and south of CABR) in June of 2003 originated in the northern part of the study area (Chapter 5), it is unlikely that the lack of new recruits are directly related to the lack of spawning stock within the Park. In addition, active restoration of adult *M. californianus* within the Park, although potentially beneficial to improve settlement conditions for larvae arriving from the north, is unlikely to directly lead to an increase in local larval supply.

During June 2003, the majority of mussel settlers to CABR were *M. galloprovincialis*, rather than *M. californianus* (as determined by PCR assay, Chapter 5 and Appendix 5A), the species that dominates this area as an adult. *M. galloprovincialis* is capable of living in an open coast environment, but is an inferior competitor in this type of habitat (Suchanek 1981). Perhaps the San Diego Bay water

influence in the southern part of the Park has led to preferential settlement of *M. galloprovincialis* in this area, and these individuals do not survive to adulthood due to competition with or predation from open coast species. The natal origins of *M. galloprovincialis* settlers in San Diego County in June 2003 were quite diverse (Chapter 5), potentially allowing for connectivity between healthy populations of this species with those at CABR. Population models to explore the amount of settlement required for rebuilding populations of both mytilid species within the Park will help determine if current levels of settlement could directly lead to the lack of observed recovery.

Although there are indications that there are very low levels of settlement of mytilid mussels within the Park, there is some preliminary evidence that adult growth is also compromised on Point Loma (Chapter 2). This could be due to the influence of polluted seawater from San Diego Bay (as determined using PCBs as a tracer), although there does not appear to be a correlation between low growth and high PCB tissue levels. Other water quality parameters, such as amount of available food (ChlA) or other types of pollution (such as another contaminant that is more bioavailable at the mouth of the Bay than within it, e.g., Deheyn and Latz 2005) should be measured in more detail in this area.

SCALE OF MUSSEL POPULATION DYNAMICS AND CONNECTIVITY

This work explored the scaling of central and southern California mussel population dynamics (Chapter 3) and larval connectivity (Chapter 5). In most years,

mussel cover varied coherently only at very small scales (<1 km, as determined by spatial autocorrelation analysis), and was likely driven by local-scale processes such as predation, competition, erosion, or human harvesting. However, in some years (e.g., 1997), mussel cover in areas of coastline declined concurrently on much larger scales (100-200 km); this pattern could have been caused by storms during this time period (which was during an El Niño) that might act as crucial disturbance agents for these populations. This work demonstrates the importance of exploring population dynamics over multiple years, since the patterns observed varied greatly among six month periods.

The scale of mussel coherence during periods of increase also varied among different years (Chapter 3). For example, in 1998 mussel populations were generally increasing but not in a coherent way. In contrast, during a period of greater increases in mussel cover in 2003, populations in 150-km patches appeared to increase concurrently. This could imply that occasional, widespread recruitment pulses (either due to high population connectivity or conditions favorable to settlement and growth) are important in structuring adult populations, but in most years a low level of connectivity exists.

The actual population connectivity between mussel populations was determined for a single time period (spring 2003) in a 75-km study region using in situ larval culturing and trace elemental fingerprinting (Chapter 5). The majority of *M. californianus* settlers throughout San Diego County appeared to originate from a 30-km region in the north. On the other hand, *M. galloprovincialis* settlers appeared to

have a greater diversity of origins. Self-seeding apparently occurs on at least regional scales (~30 km) and possibly on smaller, site scales (<5 km) in coastal populations with dispersive larvae. Some exchange among most regions was found. This would lead to homogenization of genetic differences, even though the demographic consequences of self-seeding could be great.

The different connectivity patterns found for *M. californianus* and *M. galloprovincialis* raise a number of interesting questions about how biological processes interact with physical oceanography to cause different larval trajectories. Additional studies on life-history parameters of mussels in local waters (pelagic larval duration, delay of metamorphosis, initiation of spawning, vertical positioning of larvae) will improve our understanding of how these factors affect larval transport in mussels and other marine invertebrates with similar traits. The distribution of adult spawning stock of these two mytilid species is likely to have a significant influence on observed connectivity patterns, and would be relatively easy to determine in the near future. The relative importance of these different factors could be modeled in order to focus future biological studies. The hydrography and predicted transport trajectories among sites in this region are being explored in a multi-disciplinary effort to integrate this elemental fingerprinting study with oceanographic and metapopulation models. Ultimately many of these variables will likely be incorporated to help explain some of these connectivity patterns.

The high level of seasonal and annual variability in the spatial scale of mussel increases observed in Chapter 3 underscores the importance of repeating studies that

determine population connectivity (Chapter 5), in order to resolve demographically important exchange between populations through time. It is likely that connectivity patterns will not be static through time.

GENERAL IMPLICATIONS OF WORK

This is the first time that trace elemental fingerprinting was successfully applied to determine natal origins of settled marine invertebrates. This technique has great potential for use with invertebrate species that retain larval structures after settlement in sites throughout the world. In addition, the use of in situ larval culturing expands the application of this method to create reference elemental signatures for tracking species that do not locally retain their embryos for some period of time (e.g., using some form of benthic encapsulation). There are a vast number of ecological and evolutionary questions that can be best addressed using this sort of direct larval tracking method. Marine reserve design and management will also benefit greatly from an understanding of realized dispersal and connectivity among protected and non-protected sites.

Determining the degree of connectivity among marine populations is currently one of the "greatest challenges" in marine ecology (Swearer et al. 2002), and evidence of larval retention in mostly continuous coastal populations has been difficult to obtain (Sponaugle et al. 2002). Mytilid mussels have long been considered to be examples of species with highly dispersive larvae (Bayne 1976, Widdows 1991). In this study, I found evidence of small scale (10s of km) self-

recruitment within mussel populations that were not isolated from each other or located on offshore islands. As direct determination of natal origins is applied to an increasing number of species, our general hypotheses about how marine populations are connected are likely to be tested and refined.

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