

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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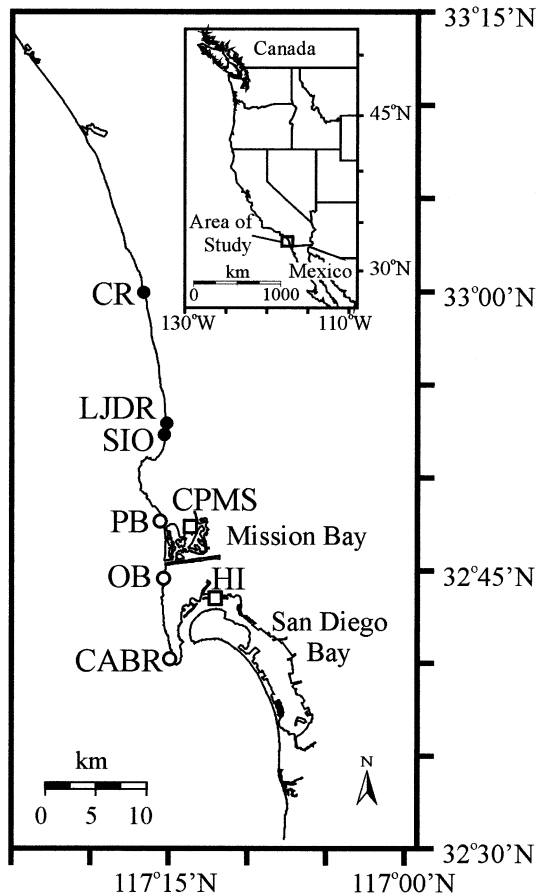


Fig. 1. Map of *Mytilus* mussel collection sites in San Diego County, California USA. Northern region (filled circles): CR = Cardiff Reef; LJDR = La Jolla Dike Rock; SIO = Scripps Institution of Oceanography Pier. Southern region (open circles): PB = Pacific Beach (Crystal) Pier; OB = Ocean Beach; CABR = 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. (2003a) 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 (Koidé 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' GGTGAA-GAGGCCTTATGAAG 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 ap-

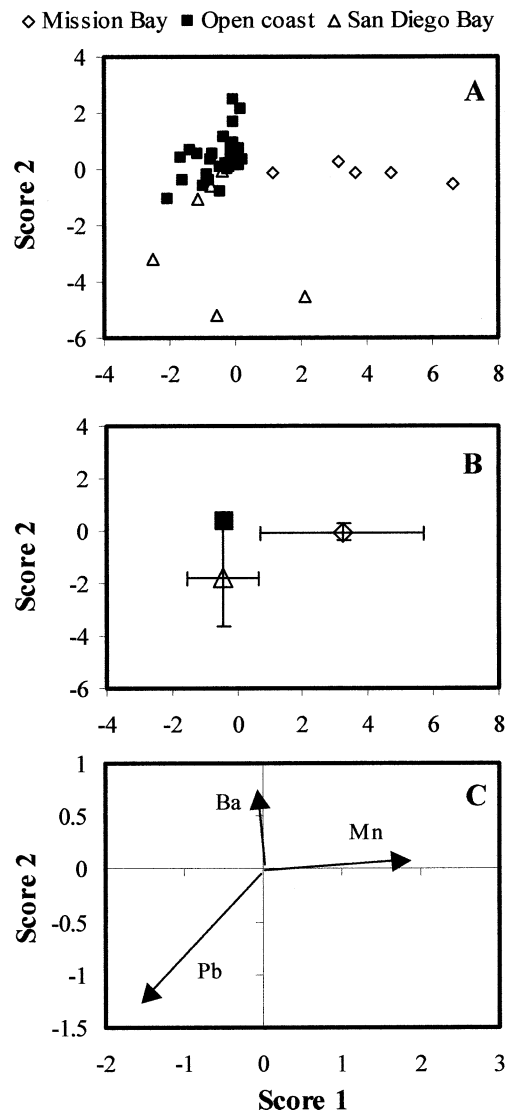


Fig. 2. Discriminant scores of element (Mn, Ba, Pb) to Ca ratios in shells of *Mytilus* mussel recruits collected between 26 December 2001 and 9 January 2002 at sites in San Diego County, grouped as Mission Bay, San Diego Bay, and Open Coast sites (Cardiff Reef, La Jolla Dike Rock, Scripps Institution 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 $\pm 95\%$ 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.

proximately $350 \mu\text{m}$, with a thickness (i.e., spot size) of $100 \mu\text{m}$. The laser was set at 55% power, with a speed of $100 \mu\text{m s}^{-1}$.

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 $600\text{-}\mu\text{m}$ 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 (μ mol mol ⁻¹) | Pb:Ca (μ mol mol ⁻¹) | U:Ca (μ mol mol ⁻¹) |
|-----------------|-----------------|----|------------------------------------|------------------------------------|------------------------------------|--|--|---|
| CPMS | 27 Dec 2001 | 6 | 49.87 \pm 10.65 | 0.66 \pm 0.18 | 2.37 \pm 0.30 | 16.15 \pm 3.34 | 36.93 \pm 8.98 | 1.45 \pm 0.32 |
| HI | 27 Dec 2001 | 8 | 46.87 \pm 9.53 | 0.44 \pm 0.22 | 2.25 \pm 0.33 | 19.03 \pm 7.11 | 101.20 \pm 37.06 | 1.41 \pm 0.24 |
| CR | 27 Dec 2001 | 7 | 33.95 \pm 3.43 | 0.02 \pm 0.00 | 2.80 \pm 0.07 | 7.61 \pm 3.19 | 4.37 \pm 1.76 | 0.82 \pm 0.17 |
| LJDR | 27 Dec 2001 | 4 | 30.98 \pm 3.19 | 0.01 \pm 0.01 | 3.29 \pm 0.20 | 6.11 \pm 3.53 | 4.60 \pm 1.32 | 0.54 \pm 0.21 |
| SIO | 01 May 2001 | 7 | 23.46 \pm 4.21 | 0.01 \pm 0.01 | 3.38 \pm 0.28 | 7.11 \pm 2.36 | 5.65 \pm 2.24 | 1.64 \pm 0.44 |
| | 08 Sep 2001 | 15 | 30.78 \pm 3.47 | 0.02 \pm 0.01 | 2.89 \pm 0.18 | 3.72 \pm 1.07 | 6.45 \pm 1.61 | 1.44 \pm 0.48 |
| | 09 Jan 2002 | 5 | 15.64 \pm 4.36 | 0.00 \pm 0.00 | 2.86 \pm 0.29 | 2.31 \pm 1.06 | 6.55 \pm 1.72 | 1.96 \pm 0.46 |
| | 26 Jan 2002 | 8 | 12.40 \pm 1.30 | 0.01 \pm 0.01 | 1.55 \pm 0.32 | 1.69 \pm 1.20 | 24.21 \pm 12.57 | 0.90 \pm 0.24 |
| | 01 Feb 2002 | 6 | 12.84 \pm 1.63 | 0.03 \pm 0.01 | 1.82 \pm 0.32 | 4.48 \pm 1.64 | 18.95 \pm 5.36 | 1.78 \pm 0.59 |
| | 08 Feb 2002 | 8 | 11.32 \pm 1.00 | 0.03 \pm 0.01 | 1.44 \pm 0.20 | 4.16 \pm 1.27 | 7.78 \pm 2.90 | 2.58 \pm 0.85 |
| | 13 Feb 2002 | 9 | 10.65 \pm 0.48 | 0.08 \pm 0.03 | 2.85 \pm 0.32 | 5.16 \pm 1.90 | 31.22 \pm 7.86 | 5.85 \pm 0.70 |
| | 21 Mar 2002 | 3 | 15.72 \pm 1.38 | 0.10 \pm 0.06 | 2.06 \pm 0.49 | 0.00 \pm 0.00 | 16.39 \pm 8.19 | 4.21 \pm 1.63 |
| PB | 26 Dec 2001 | 11 | 30.68 \pm 6.27 | 0.06 \pm 0.01 | 2.26 \pm 0.19 | 22.77 \pm 5.79 | 22.26 \pm 5.08 | 1.31 \pm 0.27 |
| OB | 26 Dec 2001 | 7 | 38.47 \pm 5.88 | 0.20 \pm 0.05 | 1.41 \pm 0.16 | 41.35 \pm 8.62 | 67.95 \pm 8.57 | 2.34 \pm 1.53 |
| CABR | 27 Dec 2001 | 7 | 18.79 \pm 3.30 | 0.02 \pm 0.01 | 1.94 \pm 0.12 | 24.83 \pm 7.90 | 12.40 \pm 6.29 | 1.93 \pm 0.56 |

intensity, 25 μ m s⁻¹ line speed, and 100- μ m 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- μ m line was run at 45% intensity, 50 μ m s⁻¹, and 50- μ m 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 ⁴⁸Ca, 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 natu-

rally 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.

| Actual grouping | Predicted grouping | | | Total per site | % correctly classified |
|-----------------|--------------------|-------------|---------------|----------------|------------------------|
| | Open coast | Mission Bay | San Diego Bay | | |
| 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.

| Actual site | Predicted site | | | | | | Total per site | % correct (sites) | % correct (regions) |
|-------------|-----------------|------|-----|-----------------|----|------|----------------|-------------------|---------------------|
| | Northern region | | | Southern region | | | | | |
| | CR | LJDR | SIO | PB | OB | CABR | | | |
| 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 ⁵³Cr and 11% more ⁶⁴Zn in the slide than in the mussels, these isotopes were removed from further analyses.

Statistical tests—Resulting element ratios ($X: ^{48}\text{Ca}$) 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.

| Actual grouping | Predicted grouping | | | Total per date | % correct |
|-----------------|--------------------|-------------|---------------|----------------|-----------|
| | Open coast | Mission Bay | San Diego Bay | | |
| 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 | 55 | | 6 | 61 | 89 |

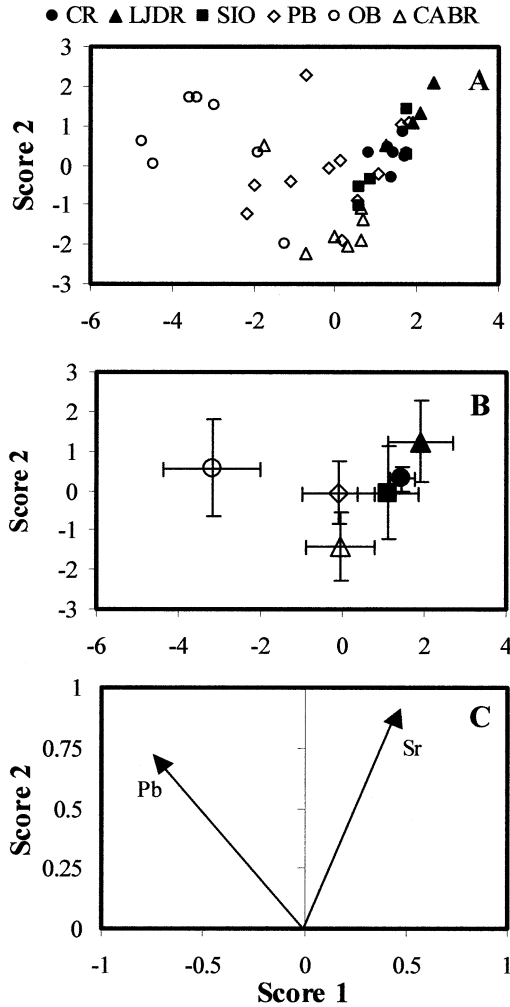


Fig. 3. Discriminant scores of element (Pb, Sr) to Ca ratios in shells of *Mytilus* mussel recruits collected between 26 December 2001 and 9 January 2002 at open coast sites in San Diego County. Sites are listed from north to south. The northern region is represented by filled shapes; the southern region is represented by open shapes. (A) Scatterplot of DFA scores; (B) same data as A plotted as averages with $\pm 95\%$ 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.

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

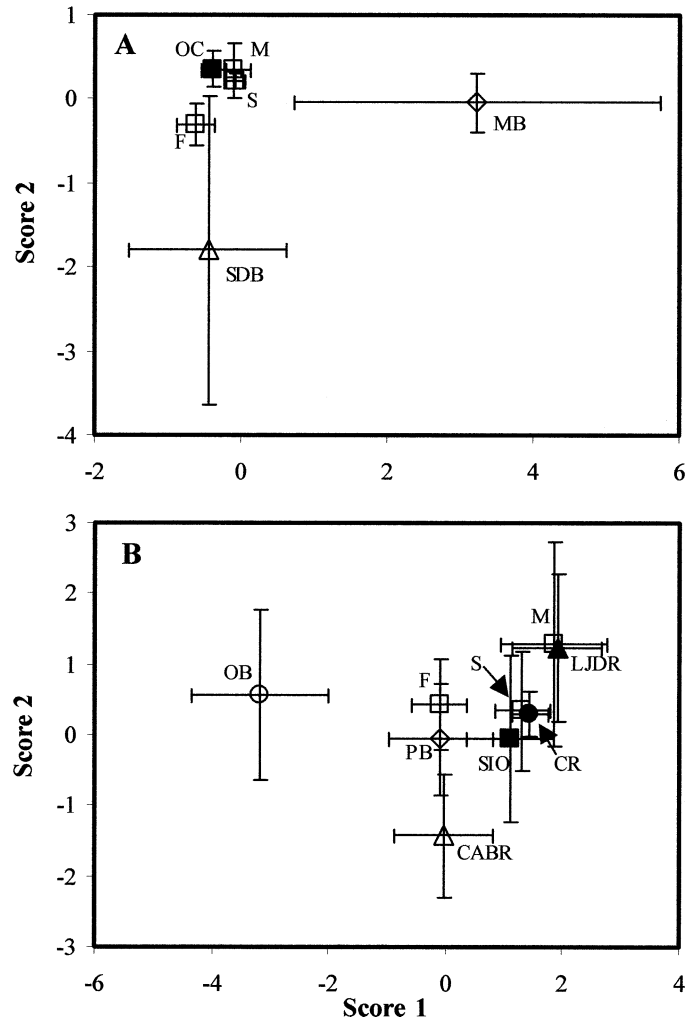


Fig. 4. Discriminant scores of element (Pb, Sr) to Ca ratios in shells of *Mytilus* mussel recruits collected from Scripps Institution of Oceanography Pier in May 2001, September 2001, and February 2002 compared with those collected at various sites between 26 December 2001 and 9 January 2002 in San 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 $\pm 95\%$ 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.

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.

| Actual grouping | Predicted grouping | | | | | | Total per date | % correct (regions) |
|-----------------|--------------------|------|-----|-----------------|----|------|----------------|---------------------|
| | Northern region | | | Southern region | | | | |
| | CR | LJDR | SIO | PB | OB | CABR | | |
| 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% HNO₃ 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- μ m 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,

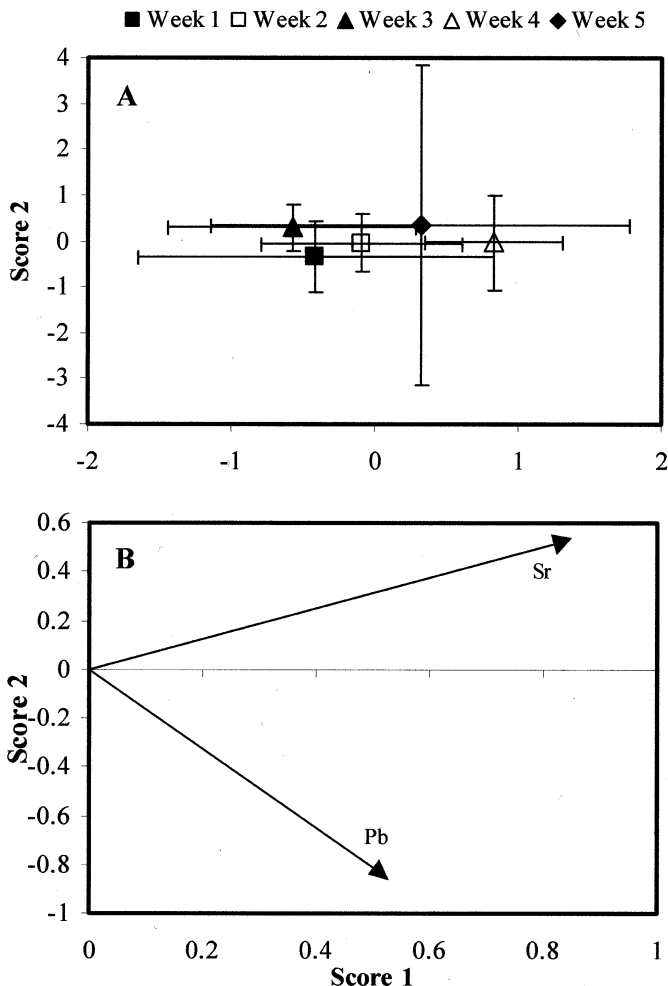


Fig. 5. Discriminant scores of element (Pb, Sr) ratios to Ca in shells of mussel recruits collected once per week between 26 January and 21 February 2002 at Scripps Pier, grouped as weeks. (A) Discriminant function analysis (DFA) scores, plotted as averages with \pm 95% confidence intervals; (B) discriminant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

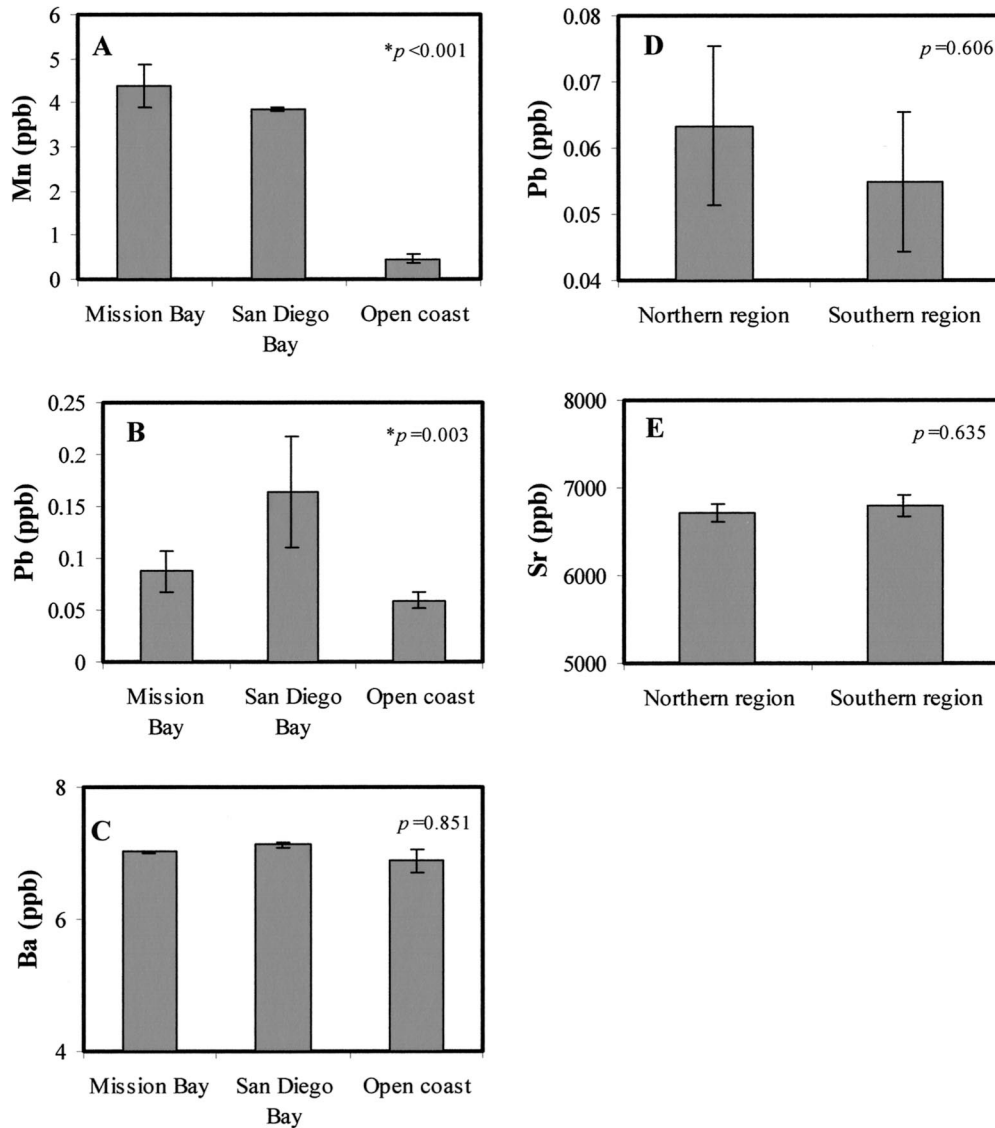


Fig. 6. Concentrations of elements (parts per billion) in seawater collected 25–26 January 2002 from the mussel collection sites in this study. Error bars represent ± 1 standard error, p values are from ANOVA analysis, values with an asterisk are significant at $p < 0.05$. Panels (A), Mn, (B), Pb, and (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

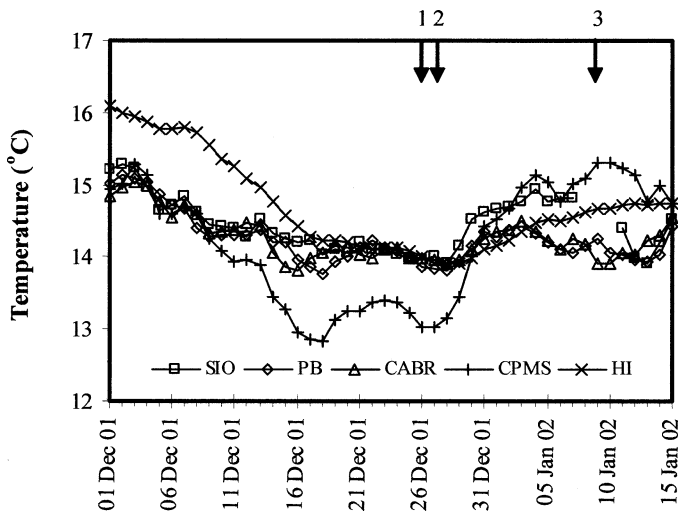


Fig. 7. Temperature data for San Diego County. Sampling times for this study are noted with numbered arrows. Daily averages of sea surface temperature immediately offshore of five sites, collected using a temperature logger located on a surface float; 1 = 26 December 2001; 2 = 27 December 2001; 3 = 9 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. 2003a), 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, Stuesson (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. 2003b). 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 si-

multaneously 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 2003a), and shells (this study, Zacherl 2003b) are likely to yield signals that can be used for larval tracking and connectivity studies in many invertebrate species around the world.

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