Connectivity clues from short-term variability in settlement and geochemical tags of mytilid mussels

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Running Head: Geochemical tags in newly settled mussels

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Abstract

The use of geochemical tags in calcified structures of fish and invertebrates is an exciting tool for investigating larval population connectivity. Evaluating these tags over relatively short intervals (weeks) may detect environmental and ecological variability at a temporal scale highly relevant to larval transport and settlement. We collected newly settled mussels (*Mytilus californianus* and *M. galloprovincialis*) weekly during winter/spring of 2002 along the coast of San Diego, CA, USA, at sites on the exposed coast (SIO) and in a protected coastal bay (HI), to investigate temporal patterns of geochemical tags in mussel shells. Analyses of post-settlement shell via LA-ICP-MS revealed statistically significant temporal variability for all elements we examined (Mg, Mn, Cu, Sr, Cd, Ba, Pb and U). Despite this, our ability to distinguish multielemental signatures between sites was largely conserved. Throughout our 13-week study, SIO and HI mussels could be chemically distinguished from one another in 78-87% of all cases. Settlement varied between 2-27 settlers gram-byssus^{-1} week^{-1} at SIO and HI, and both sites were characterized by 2-3 weeks with "high" settlement. Geochemical tags recorded in early larval shell of newly settled mussels differed between "high" and "low" settlement weeks at both sites (MANOVA), driven by Mg and Sr at SIO ($p = 0.013$) and Sr, Cd, Ba and Pb at HI ($p < 0.001$). These data imply that shifts in larval sources or transport corridors were responsible for observed settlement variation, rather than increased larval production. In particular, increased settlement at HI was observed concurrent with the appearance of geochemical tags (e.g., elevated Cd) that suggest those larvae were retained in upwelled water near the mouth of the bay. Such shifts may reflect short-term changes in connectivity among sites due to altered transport corridors, and influence the demography of local populations.

1. Introduction

The bipartite life history of many marine invertebrates and fishes includes a planktonic larval phase that is capable of connecting sites within a regional metapopulation, as well as contributing significantly to spatial and temporal variability in local densities (Caley et al. 1996). Despite the achievements of researchers throughout the 19th and 20th centuries in investigating how larval ecology affects species persistence and biodiversity maintenance (Prytherch 1929, Thorson 1950), there has been, until recently, severe limitations on the ability to track the movement of very small, dilute larvae throughout their entire planktonic phase in the vast, dynamic ocean (Levin 1990). Levin (2006) noted that renewed vigor for tracking larvae has been driven by both conservation needs [e.g., connectivity occupies a central role in the design (placement of networks) and evaluation (spillover and self-recruitment rates) of marine reserves (Hastings and Botsford 2006)] and methodological advances [e.g., physical-biological models used to simulate larval dispersal (Cowen et al. 2006, Rasmussen et al. 2009)]. In particular, the discovery and exploitation of environmental (geochemical) markers deposited and then retained within calcified structures of larvae has allowed for the reconstruction of the locations where larvae developed, and therefore identification of the natal origins of settled individuals (Thorrold et al. 2002, Thorrold et al. 2007). The resulting insights have been considerable; for example, we now understand that some populations are more "self-seeding" and less demographically "open" than previously expected (e.g., Almany et al. 2007).

The use of geochemical tags, both natural and induced, to track larvae and explore connectivity remains a growth field (Campana 2005, Thorrold et al. 2007). Part of the continuing challenge of these studies derives from the time- and labor-intensive nature of this work, forcing datasets and conclusions to be based on few seasonal or annual collections of settled larvae (e.g.,

Swearer et al. 1999, Becker et al. 2007). This is somewhat problematic given high variability of larval dynamics over multiple spatial and temporal scales (Siegel et al. 2008). As a result, the resolution, especially temporally, of geochemical tagging studies may not match up well with the scales of variability that should be expected in settlement or transport pathways of larvae (sensu Gaines and Denny 1993). In deed, the endpoint of larval dispersal (i.e., settlement), and therefore connectivity itself, is known to vary over annual, seasonal, fortnightly and diel scales due to multiple factors such as behavior (Kingsford et al. 2002) upwelling relaxation (Shanks et al. 2000), the spring transition and wave climate (Shanks and Pfister 2009). Thus, studies that explore variability in geochemical tags over a range of time scales, both large and small, should add to our understanding of larval-driven population connectivity (Levin, 2006, Pineda et al. 2007). It is within this context that we explore and report on short-term (weekly) variability in multielemental signatures obtained from newly arrived settlers of two common intertidal mussels.

In addition to changes in connectivity patterns, local environmental fluctuations at source sites or within the water masses that larvae pass through can drive variation in the geochemical tags associated with larval shells (Strasser et al. 2008), statoliths (Zacherl 2005) and otoliths (Gillanders 2002) of newly-settled individuals. Campana et al. (2000) identified three requisites for using natural geochemical tags: (1) distinct, reproducible markers among locations, (2) chemical characterization of all possible source groups, and (3) consistency of signals throughout the duration of population mixing. With these rules in mind, it is also important from a logistical standpoint to explore temporal variability over relatively short scales to determine if the first and third of these guidelines can be satisfied in geochemical tagging studies that would quantify larval connectivity. Consider, seasonal (Swearer et al. 2003) and annual (Gillanders 2002)

variability in multielemental signals of fish otoliths have been quantified as a requirement to track the nursery contribution of juvenile habitats (Gillanders 2005). Because fish occupy and then recruit from nurseries on a roughly annual basis, understanding signal variability over the scale of 0.5-1.0 years satisfies the requirements presented by Campana et al. (2000). However, planktonic larval durations can be much shorter than this (Thorson 1946), and therefore analogous short-term studies quantifying variation in multielemental signatures are needed, in addition to studies covering longer time scales (e.g., Zacherl 2005). Becker et al. (2005) reported temporally stable geochemical tags (Sr and Pb) in post-settlement shells of mytilid mussels collected from an exposed coast site over five weeks. Here, we report on an expanded mytilid dataset first used by Becker et al. (2005) to further explore how temporal variability may influence, and be useful in, geochemical tagging studies.

Mytilus californianus and *M. galloprovincialis* are widely distributed ecosystem engineers within rocky intertidal environments and have been valuable species for identifying the natal origins of individual larvae to estimate connectivity among sites along the southern California coastline (Becker et al. 2007, Rasmussen et al. 2009). These species are attractive candidates for geochemical tagging because: (1) each individual has a larval shell that incorporates trace elements and is retained after settlement, (2) they have larval durations between 1-4 weeks (Strathmann 1987, Becker et al. 2007 and references therein), which are logistically manageable in field experiments, and 3) they co-occur over regional and meter scales. *M. californianus* dominate along exposed coasts and can be found within the outer regions of bays. Conversely, *M. galloprovincialis* are most abundant within bays but also settle along the exposed coastline (Becker et al. 2005).

With the goal of exploring the magnitude and consequences of short-term (weekly) variability in the geochemical tags of mytilid mussel shells, we asked: (1) Do multielemental signatures in post-settlement shell of mussels vary appreciably over weekly time scales? If so, is this temporal variability comparable in magnitude to spatial differences in geochemical signatures that might confound tracking studies (and is there consistency in the elements that distinguish sites)? And (2) Do geochemical tags in the portion of settled mussels' shell formed during the larval phase exhibit differences based on settlement date? If so, are these changes related to shifts in natal sources or oceanographic conditions that affect local delivery rates of settlers?

2. Methods

2. 1. Field collections and sample preparation

To investigate variability in mytilid larval settlement and geochemical tags over weekly time scales, we collected newly settled mussels every week from January 25 until April 19, 2002 (13 weeks). These dates overlap with typical seasonal pulses in reproduction for these two species (Curiel-Ramirez and Caceres-Martinez 2004). Our collections occurred at 2 sites along the southern California coastline (Fig. 1): on the most-seaward pilings of the Scripps Institution of Oceanography Pier (SIO) in La Jolla, CA (N 32.87˚, W 117.25˚), and from riprap seawalls fringing Harbor Island (HI) inside San Diego Bay, CA (N 32.72˚, W 117.20˚). Thus, we sampled a population along the exposed coast and another located in a well flushed region (5 km from the bay mouth) of a 20-km long protected bay (Chadwick and Largier 1999). At both sites, collections were made at 0.3-0.7 m above the mean lower-low tide line to minimize biases related to tidal level/transport (Porri et al. 2007). To collect newly settled mussels, we pulled

clumps of adult mussels away from underlying substrate until 3 replicate 0.5-L bags were filled. Newly settled mussels measuring less than 2.5 mm ($\leq$ 2 weeks post-settlement; Coe and Fox 1942) were obtained by dissecting the byssus threads that held adult masses together and then sorting through the byssus threads (settlement habitat for mussels) under a microscope. For each replicate 0.5-L bag, we searched for newly settled mussels for 30 minutes or until 30 settlers were collected, whichever came first. We then dried and weighed the sorted byssus threads to standardize settlement rates for each site and week during our study (settlers gram-byssus^{-1} week^{-1}). We also used a subset of these newly settled mussels to investigate spatio-temporal variability in multielemental signals of shells, as well as explore patterns of larval population connectivity.

We analyzed geochemical tags in shells of 181 mussels (1.42 $\pm$ 0.53 mm; mean $\pm$ 1 SD), including 127 from SIO and 54 from HI. *M. californianus* and *M. galloprovincialis* settlers could not be identified visually. Therefore, mussel tissue samples were identified to species using a molecular approach detailed in Becker et al. (2005). In short, a Polymerase Chain Reaction (PCR) technique was employed using species-specific primers targeting the 16S r-RNA subunit and identification of each mussel was determined from the presence and length of a PCR product.

Using ceramic forceps and tungsten probes to limit potential metal contamination, mussels were split open and flesh was removed and retained for PCR. Valves were separated and we stored the "left" valve based on the position of the dorsal apex. The "right" valve was scraped of debris and transferred to a clean plastic vial (if the right valve was damaged, the "left" valve was used instead). Samples were leached overnight in 15% H_2O_2 buffered with 0.05 mol L^{-1} NaOH, and then sonicated in 3% HNO_3^- for 5 min to further remove organics. Subsequently, shells were rinsed 3 times in Mill-Q water and then mounted on petrographic slides against

double-stick tape using Milli-Q and a paintbrush. Once mussels were mounted, slides were stored in a C-100 laminar flow hood until analyses. All plastic containers, glass slides, and forceps were leached in 3% HNO_3^- and rinsed with Milli-Q before coming in contact with mussels.

2. 2. LA-ICP-MS

We analyzed the multielemental composition of mussel shells at 3 locations: on the outer margin of dissoconch shell adjacent to the dorsal apex (post-settlement shell), along the base of the prodissoconch shell perpendicular to the axis of growth ("early" larval shell), and on the prodissoconch shell immediately adjacent to the prodissoconch-dissoconch boundary ("late" larval shell) (Fig. 2). We confine this report, however, to data collected from post-settlement shell and "early" larval shell. Because dissoconch shell is deposited once mussels are settled and fixed at a site, we used these data to investigate spatial variability in environmental signals between shell formed at SIO and HI, as well as temporal variability among weeks within both sites (question #1 above). Based on our observations of laboratory-reared larvae, and growth of larvae during 7-day field outplantings (Becker et al. 2007), "early" larval shell material represents the environmental conditions experienced by individual mytilids during the first week after fertilization. Therefore, "early" larval shell provided us an opportunity to evaluate weekly variability in geochemical tags associated with the natal origin(s) or early larval transport corridor(s) of newly settled individuals (question #2 above).

Shell regions were sampled using a New Wave UP 213-nm laser ablation (LA) unit. Larval and post-settlement shells were sampled by ablating a 75-μm line with a laser output of 0.5 mJ, a scan speed of 15 μm s^{-1}, and a burn width of 20 μm. Experimental work by Strasser et al. (2007) demonstrated that larval shell of softshell clams could not be sampled via laser

ablation without also simultaneously sampling post-settlement shell. This is problematic if larval shell samples are corrupted by environmental signals from the settlement site (post-settlement shell) of specimens, potentially leading to overestimates of self-seeding. Through visual examination of ablations on pre-settlement mytilid larvae (Fig. 2) and careful attention to Mg data collected during this study (higher concentrations in post-settlement shell; Becker et al. 2007), we were confident that we could fire on mussels without burning completely through larval shell (5% of the larval shell data were thrown out due to concerns related to burn through based on the Mg check). Furthermore, we paired post-settlement and early larval shell data (X:Ca) recorded from each individual mussel for regression analyses, and found that larval shell data appeared largely independent of post-settlement shell (r^2 <0.33 for all eight elements we considered separately at each site).

Ablated shell material was transported using He gas (mixed with Ar) to a Thermoquest Finnigan Element 2 double-focusing, single-collector, magnetic-sector Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Based on previous geochemical tagging studies in this region, we sampled for the following isotopes: ^{26}Mg, ^{48}Ca, ^{55}Mn, ^{63}Cu, ^{88}Sr, ^{65}Cd, ^{135}Ba, ^{208}Pb, and ^{238}U (Fodrie and Levin 2008). Data processing to calculate elemental concentrations standardized to calcium (X:Ca), and corrections for machine drift using NIST glass (National Institute of Standards and Technology Reference Material 612; Pearce et al. 1996) followed Becker et al. (2005). Detection limits on this instrument (3 standard deviations above background counts) at the time of our analyses were: 0.02 mmol mol^{-1} (Mg:Ca), 0.002 mmol mol^{-1} (Mn:Ca), 0.001 mmol mol^{-1} (Cu:Ca), 0.01 mmol mol^{-1} (Sr:Ca), 0.004 mmol mol^{-1} (Cd:Ca), < 0.001 mmol mol^{-1} (Ba:Ca), < 0.001 mmol mol^{-1} (Pb:Ca) and < 0.001 μmol mol^{-1} (U:Ca). Based on ablations that produced one hundred million counts of ^{48}Ca, the of percentage of X:Ca

measurements that fell below the detection limits of the instrument, as well as average concentration of elements relative to detection limits were: Mg, 0% under detection limit, average counts 68 times detection limit; Mn, 29% under detection, average counts 11 times detection; Cu, 2% under detection, average counts 64 times detection; Sr, 0% under detection, average counts 274 times detection; Cd, 50% under detection, average counts 2 times detection; Ba, 0% under detection, average counts 16 times detection; Pb, 1% under detection, average counts 31 times detection; and U, 0% under detection, average counts 300 times detection.

2. 3. Data Analyses

2.3.1. Spatio-temporal patterns in multielemental signatures

We investigated spatial (pooling all weeks) and temporal (separately for each site) differences in shell chemistry using Mann-Whitney U and Kruskal-Wallis tests, respectively. Only data collected from post-settlement shell were considered in these analyses, and each X:Ca ratio was tested separately. Non-parametric tests were employed because F_{max} tests revealed significant heteroscedasticity in shell geochemistry ($\alpha = 0.05$) for the majority of elements between sites and among weeks, and log (x+1) and square-root (x+1) transformations failed to reduce differences in these variances.

We then used Discriminant Function Analyses (DFA) to determine if SIO and HI could be characterized throughout a 13-week period by distinct, multielemental signatures in post-settlement shell (Systat 9, © SPSS). All DFAs were conducted in a stepwise manner, by running the analysis on all element ratios, then dropping the least significant variable as determined by a F-to-remove statistic. This process was repeated until the F-to-remove statistic of all included element ratios was > 4. Based upon our visual inspections, there was an apparent change in shell chemistry in the mussels collected from HI after week 8 (the middle of March; Fig. 3). In particular, Mn, Ba and Pb all showed qualitative changes in chemical distinctness between SIO

and HI following mid-March (Fig. 3). Subsequent investigations using MDS and SIMPER analyses (Primer 5.2.2) confirmed that shell chemistry was notably different at HI between the first 8 sampling weeks versus last 5 sampling weeks (unpublished data). Therefore, we generated 3 separate DFAs to compare sites: a DFA generated with data from all weeks included, a DFA with only data from the first 8 weeks, and a DFA with only data from the last 5 weeks. Cross-validation of each DFA model was achieved by re-classifying each sample using a jackknife method, and comparing observed classification successes to the average of six replicate trials in which the collection site of individual mussel settlers were randomly assigned (White and Ruttenberg 2007).

2.3.2. Temporal patterns in geochemical tags of early larval shell

We also employed DFA to evaluate the coherence (spatio-temporal) of geochemical tags in early larval shell among settled mussels at SIO or HI during our 13-week study. As before, this DFA was run in a stepwise manner, dropping element ratios until all F-to-remove values were greater than 4, cross-validating the model using the jackknife method, and comparing our observed classification success against the average of six replicate trials with mussel collection sites randomly reassigned. Because we were only interested in gauging the within- and between-site similarities of geochemical tags within early larval shell, rather than attempting to explicitly define the natal origins of settled larvae, we did not employ additional statistical approaches on these data such as Markov Chain Monte Carlo methods (White et al. 2008).

Settlement rates of mytilid mussels at SIO and HI were defined by a few weeks with strong pulses of newly arrived larvae interspersed among weeks with "low" background settlement levels. We differentiated "high" settlement phases as weeks with settlement greater than three standard deviations above mean settlement at that site (after removing the week in

question from the calculation of mean settlement). As a result, the first, third and ninth weeks at SIO were deemed "high" settlement phases, while at HI the tenth and twelfth weeks were considered "high" settlement phases (Fig. 4).

To test if there were distinct natal or transport signatures in early larval shell for settlers between settlement phases, we used separate MANOVA (StatView 5.0.1, © SAS) analyses for each site to compare early larval shell chemistry of individual mussels collected between "low" or "high" settlement phases (with all weeks pooled between phases). For each site, only elements that remained in an exploratory DFA to compare "high" and "low" settlement phases were included in the MANOVA. If early larval shell chemistry was not different between "high" and "low" settlement phases at a site, this would suggest that changes in settlement rates were the result of increased larval production or survivorship. If early larval shell chemistry was different between "high" and "low" settlement phases, this would indicate that changes in larval sources, or the water masses through which larvae passed during early development (perhaps interacting with larval production or survivorship), played some role in regulating the observed settlement rates at SIO or HI.

An alternative hypothesis for why we might observe significant differences in early larval shell chemistry between "high" and "low" settlement phases would be that there are changes in environmental conditions among all weeks, rather than anything specifically related to the observed settlement patterns. To evaluate this hypothesis, we randomly selected three (SIO) or two (HI) weeks and compared the geochemical tags in early larval shell of settlers collected during those randomly selected weeks to settlers from all other weeks. This was repeated six times, and we then compared the results of each MANOVA result ("high/low" and the six "random/all other week" tests) to provide a more complete context for our statistical inferences.

As an additional output of MANOVA testing, element-by-element ANOVAs comparing settlement phases were run. Data transformations were not required to reduce differences in variances between groups. Because each statistical test we conducted applied to separate and easily distinguishable hypotheses, we made no corrections to experiment-wise alpha for either the parametric or non-parametric tests we conducted (Moran 2003).

3. Results

3.1. Weekly settlement

Settlement ranged between 2-22 settlers gram-byssus^{-1} week^{-1} at SIO and 2-27 settlers gram-byssus^{-1} week^{-1} at HI (Fig. 4). As noted above, the first, third and ninth collection weeks at SIO were qualified as relatively "high" settlement phases, while the tenth and twelfth weeks were considered "high" settlement phases at HI. Genetic identification of the specimens analyzed via LA-ICP-MS revealed that all of the settlers at HI and 9% (n = 11) of settlers at SIO were *M. galloprovincialis*. The remaining 91% (n = 109) of settlers at SIO were *M. californianus*.

3.2. *Spatio-temporal patterns in multielemental signatures*

There were significant differences ($\alpha = 0.05$) in the elemental signatures of post-settlement shell (X:Ca) between SIO and HI for Mg, Mn, Sr, Cd, Ba, Pb and U (Table 1, Fig. 3). These differences were most apparent during the first 8 weeks of the study (Jan 26 – March 15). During this interval Mg was, on average, elevated in mussel shell at HI over SIO by a factor of 2; Mn concentrations ranged between 2-20 fold greater at HI than at SIO; average Cu concentrations were nearly 3-times higher in mussel shells collected at HI; Ba was 2-6 fold higher at HI than at SIO; Cd was up to10-times more elevated in HI shells (when measures were above detection limits); and Pb was more abundant in shells from HI (Fig. 3). Conversely, Sr

concentrations were typically higher in the post-settlement shells of mussels collected at SIO. During the last 5 weeks we collected mussels, these X:Ca differences between sites tended to decrease, or even exhibit a phase change in the case of Cu and Sr (Fig. 3). At SIO, significant ($p < 0.05$) temporal variability was observed for all elements except Pb (Table 1). At HI, Mn, Cd, Ba, Pb and U concentrations varied significantly ($p < 0.05$) in post-settlement shell among weeks (Table 1).

During the winter and spring of 2002, the multielemental signatures of post-settlement mussel shells collected at SIO and HI could be distinguished from one another using DFA with 80% accuracy (compared to only 55% during random assignment trials). Notably, all 11 of the *M. galloprovincialis* settlers at SIO were correctly identified to their collection site, indicating that the discrimination between SIO and HI was a true site distinction rather than just a species comparison (i.e., that spatial gradients in geochemical tags contributed more toward our results than did potential [expected] species differences). DFA accuracy was 87% for mussels collected during the first eight sampling weeks (compared to 52% random) and 78% for mussels collected during the last five weeks (compared to 53% random) (Table 2). Regardless of the sampling interval, classification success was higher at SIO than at HI by 7-30%. Although DFA accuracy was conserved across the three sampling intervals, the elements that drove DFA algorithms varied notably. For the entire 13-week study, Ba, U, Pb and Cd (in decreasing relative importance) drove differences between SIO and HI. Ba, Pb and U were used to discriminate sites during the first 8 weeks, while Cd, Sr, Mg and U were used in the DFA during the last 5 weeks (in decreasing relative importance).

3.2. Temporal patterns in geochemical tags of early larval shell

We were able to extract early larval shell geochemical data from 151 individual mussels, and observed distinct early larval tags between the settlers at SIO and HI based on DFA. The mean (± 1 standard error) score of the lone DFA algorithm used to distinguish individuals between sites was -1.099±0.156 (SE) for settlers collected at HI, while the mean score for individuals collected at SIO was 0.431±0.096 (SE). Settlers at HI were defined by early larval shell typically more enriched with Ba, while settlers at SIO generally exhibited higher concentrations of Mg and U. Overall, geochemical tags in early larval shells collected between the two sites could be distinguished using a jackknife approach in 83% of cases as "SIO type" or "HI type", compared to only 57% in trials with collection site randomized among specimens (Table 2).

Within each site, the geochemical tags in early larval shells were also distinct between "high" and "low" settlement phases (Table 3). Mg and Sr were included in MANOVA analyses for SIO and revealed significant differences between settlement phases ($p = 0.013$). Both Mg ($p = 0.018$) and Sr ($p = 0.119$) were enriched in the early larval shells of settlers during "high" settlement phases (Fig. 5). Conversely, comparisons between geochemical tags of early larval shell from settlers at SIO during 3 randomly selected weeks and all others revealed non-significant results ($n = 6$ random trials, average $p = 0.393$, all $p > 0.2$). At HI, Sr, Cd, Ba, and Pb were included in the MANOVA and indicated a significant difference in the geochemical tags of early larval shell between "high" and "low" settlement phases ($p < 0.001$). Sr ($p = 0.049$) and Cd ($p < 0.001$) were enriched in early larval shells of "high" phase settlers, while both Ba ($p = 0.011$) and Pb ($p = 0.029$) concentrations were lower in those individuals (Fig. 5). Comparisons of geochemical tags between settlers collected in 2 randomly selected weeks versus all other weeks at HI were not significant ($n = 6$ random trials, average $p = 0.494$, all $p > 0.2$).

4. Discussion

We investigated how temporal variability in the geochemical tags of mussel shells may influence and aid estimates of larval connectivity. For logistical reasons, we tested if temporal variability in shell chemistry at our two collection sites could obscure site-specific signatures over a time scale relevant for exploring larval connectivity (~ weekly). Early in our study, differences in post-settlement shell geochemistry reflected known environmental gradients between our one bay and one open coast site. Previous studies in this region on crab (DiBacco and Levin 2000), mussels (Becker et al. 2005, Becker et al. 2007) and fish (Fodrie and Herzka 2008, Fodrie and Levin 2008) have all reported elevated concentrations of Mn (redox cycles in muddy sediments), Cu (boat paints), Ba (salinity fractionation) and Pb (pollution) in the hard parts of organisms developing within San Diego Bay relative to the exposed coast. Previously, Becker et al. (2005) reported limited variability among weeks in multielemental signatures of post-settlement shell at SIO during January 09 – February 12, 2002. Our expanded analyses of mytilid mussels from January 25 – April 19, 2002, confirmed the findings of Becker et al. (2005), but also suggested that all elements we examined in post-settlement shell (particularly Mn, Cu, Cd, Ba, and Pb) could be relatively distinct between our two collection sites over several weeks (January 25 – March 15), and then at one of the sites we studied (HI), quickly shift to lower concentrations for several more weeks (March 15 – April 20). Generally, this change after mid-March resulted in multielemental signatures at HI during the last five weeks that were quantitatively more similar to those observed at SIO throughout the 13-week study.

Often, changes in bay-ocean exchange due to either wind or tidal forcing, or changes in the amount of fresh water runoff (i.e., rainfall) are invoked to explain temporal variability in

shell/otolith geochemistry within estuarine systems (Gillanders and Kingsford 1996). However, *post hoc* examination of wind data from the Coastal Data Information Program station 73 at SIO (http://cdip.ucsd.edu), tide data from the National Oceanic and Atmospheric Administration buoy station 9410230 in La Jolla, CA (http://tidesandcurrents.noaa.gov), and rainfall data at Lindbergh field in San Diego, CA (http://cdec.water.ca.gov), during January-April of 2002 reveal no clear explanation for the shift in multielemental signatures in post-settlement shell at HI away from a "bay-type" signature following March 15. Throughout our study, winds were typically mild (<4 m s^{-1}), spring and neap tides were experienced during each month, and precipitation (i.e., runoff) was actually higher during March-April (19.6 mm) than during January-February (12.5 mm).

Despite the temporal variability we recorded, our ability to generate distinct chemical tags from post-settlement shell between SIO, on the open coast, and HI, within a protected bay, was largely unhampered. Even during the last five weeks of our study, when signals at SIO and HI appeared to converge, multivariate analyses (DFA) were able to tease apart unique multielemental signatures in post-settlement shell and allow for the correct identification of collection site for individual mussels 78% of the time (compared to 80% and 87% for the entire 13 weeks and first 8 weeks, respectively). Thus, despite some variability among weeks, our data suggest that it is possible at the scale of a single bay site versus a single exposed coast site to satisfy at least two of the requirements Campana et al. (2000) listed for employing geochemical tags to track larvae: (1) distinct, reproducible markers among locations, and (2) chemical characterization of all sources.

Importantly, our data indicate that species effects did not play a major role in our findings even though 100% of the settlers at HI were *M. galloprovincialis*, while 90% of the settlers at

SIO were *M. californianus*. All 11 *M. galloprovincialis* we analyzed via LA-ICP-MS that settled at SIO were correctly identified to their collection sites based on post-settlement shell geochemistry. Thus, multielemental discrimination between SIO and HI was a true site distinction rather than just a species comparison. This is not to say that species difference do not exist for certain X:Ca shell concentrations or multi-elemental geochemical tags, but that in this study system those differences were relatively minor when compared to spatial gradients in shell chemistry.

Our data also identify points of caution regarding temporal variability in multielemental signatures. We found that different elements defined the geochemical tags at SIO and HI shell during the first 8 weeks (Ba, PB and U) and final 5 weeks (Cd, Sr, Mg and U). This indicates, to a manageable degree, that the third requirement advised by Campana et al. (2000) is more difficult to meet: temporal consistency of chemical signals. Clearly, it is important to quantify site-specific, reference signatures indicative of natal origins at the time larval structures are forming and over a time scale appropriate for a typical planktonic larval stage (i.e., days-weeks). For instance, using a geochemical atlas generated in late March to determine the natal origin of larvae developing during early March (or vice versa) during 2002 could have generated misleading results (albeit based on post-settlement shell data). We also recognize that we only collected settlers at two sites, and this limits our ability to negate temporal variability as a concern for geochemical tagging studies. For instance, two sites within San Diego Bay might become completely indistinguishable, or even mistaken for one another, given the magnitude of geochemical variation we observed at HI. Ultimately, however, we expect there are identifiable "regions" (25-100 km) over which relatively stable, characteristic elemental signals can be used to explore larval connectivity (e.g., Becker et al. 2005, Zacherl 2005, Carson et al. 2008).

Throughout our 13-week study, the early larval chemical signatures of newly settled mussels collected at either SIO (mainly *M. californianus*) or HI (*M. galloprovincialis*) were distinguishable from each other as "SIO type" or "HI type" (83% overall classification success). Without identifying the natal origin(s) of these larvae, we could hypothesize that most (91%) of settlers at HI had a distinct natal source from that of most (81%) settlers at SIO (Table 2). Specifically, we found that early larval shells of setters at HI were relatively enriched with Ba (indicative of bay environments; DiBacco and Levin 2000, Becker et al. 2005) while Mg was more enriched in the early larval shells of SIO settlers (indicative of exposed environments; DiBacco and Levin 2000, Fodrie and Herzka 2008). These results suggest high self-seeding rates at a coarse habitat level for the HI and SIO populations. This is predictable given the distribution of *M. californianus* and *M. galloprovincialis*, although DiBacco and Levin (2000) did find considerable exchange of crab zoea between San Diego Bay and the exposed coast, while Becker et al. (2007) reported divergent scenarios for *M. californianus* (little exchange) and *M. galloprovincialis* (moderate exchange).

Without a detailed chemical atlas of potential source populations (i.e., we only sampled two sites), we hesitate to go further and quantitatively estimate exchange rates between and among bay and exposed coast populations. We also have reasons to qualify our classification of higher Ba and lower Mg in early larval shell as a signature indicative of bay environments, as these expectations are largely drawn from data we extracted from post-settlement shell (although confirmed in other studies). Becker et al. (2007) discussed the differences in mineralogy between post-settlement (aragonite/calcite mix) and larval (mostly aragonite) shells of mussels that affected Sr and Mg uptake rates, and subsequently relied on larval outplanting as the best approach for generating a chemical atlas of potential source populations for larval tracking.

The geochemical tags in early larval shell of mussels during "high" and "low" settlement phases were distinct at both SIO and HI. These data may suggest that changes in reproductive output or larval survival alone did not drive the observed variability in settlement rates. Rather, we hypothesize that newly-settled mussels carried a chemical marker that suggested changes in (1) larval sources or (2) the water masses in which developing larvae passed through (as we sampled approximately 1 week of shell growth during our ablations; Fig. 2), also contributed to settlement variability.

The data from HI were particularly intriguing. At HI, source signatures in larval shells of *M. galloprovincialis* appeared more influenced by exposed coast conditions during "high" settlement phases than during "low" settlement phases (i.e., higher Sr, lower Ba and lower Pb). Perhaps most tellingly at HI, Cd concentrations in larval shell were ~100 times more enriched during "high" settlement weeks than during "low" settlement weeks. Cadmium has previously been shown to be a clear indicator of upwelling in the waters adjacent to San Diego Bay (seawater concentrations elevated by 50-fold relative to non-upwelling conditions; Segovia-Zavala et al. 1998), and is dependably recorded in *M. californianus* as an indicator of upwelling along the West Coast (Lares and Orians 1997). Recently, Levin (2006) noted that "*evaluat[ing]… larval movements through upwelling zones, oxygen minima, turbidity plumes, warm or cold eddies, or salinity fronts*" is among five important directions in which geochemical tags should be applied. With this in mind, we consider briefly how our larval shell data might lead to future, more rigorous studies that evaluate the role upwelling plays in determining transport corridors and realized larval population connectivity for mytilid mussels in this region.

In particular, we hypothesize that changes in local oceanographic conditions near San Diego Bay (i.e., upwelling) affected settlement rates of *M. galloprovincialis* at HI based on our

analyses of early larval shell. Upwelling (Pineda 1991) and retention zones in the lees of headlands (Mace and Morgan 2006) have strong effects on dispersal and settlement of larvae for many nearshore species. Roughan et al. (2005) reported isolated upwelling during early April, 2003, in the lee of Point Loma, immediately adjacent to the mouth of San Diego Bay (Fig. 1), following the offshore divergence of the dominant southerly flow as it passed this headland. It is plausible, although ultimately untested, that similar oceanographic conditions occurred intermittently during our study, and that some larvae were entrained in upwelled water in the lee of Point Loma. This is supported by the change in post-settlement shell chemistry at HI following week 8, assuming some of the upwelled coastal water entered San Diego Bay. If this water mass retained *M. galloprovincialis* larvae near San Diego Bay and decreased offshore wastage, or increased survivorship because of (a) enhanced feeding opportunities for larvae, or (b) reduced predation pressure relative to within the Bay (DiBacco and Levin 2008), this could explain the settlement peaks we recorded that were associated with a geochemical tag indicative of upwelling (elevated Cd). Although upwelled water would eventually advect offshore (Roughan et al. 2005), upwelling is not necessarily a barrier to nearshore retention for larval bivalves (Shanks and Brink 2005, Shanks and Shearman 2009), particularly in this system where upwelling occurs over just a few kilometers (Roughan et al. 2005).

Variability in pre-recruitment dynamics (dispersal pathways) is known to drive large fluctuations in population size and age structure for many marine species. For instance, Gaines and Bertness (1992) found that shifting transport corridors (retention versus export) near Narragansett Bay, Rhode Island, was the mechanism behind variable recruitment. Specifically, high settlement occurred when flushing time (forced by riverine input) of the bay was more than 25 days and larval retention was high, and this only occurred in 3 of 9 years during their study.

Similarly, Kraus and Secor (2005) demonstrated that during most years, recruitment pulses of white perch in Chesapeake Bay were mainly from freshwater nurseries. However, in years that produced the dominant year-classes of the population, recruitment pulses came mostly from brackish nurseries. Locally, Rasmussen et al.(2009) showed that relatively small changes in the wind field along the San Diego coast (and more specifically, uncertainty in the dynamics of wind-driven circulation near a geomorphologically complex shoreline) could significantly affect measures of regional-scale connectivity for a passive tracer. Using a bio-physical model of "fish" larval dispersal along an idealized coastline, Siegel et al. (2008) demonstrated that episodic events driven by interactions between larval life histories and complex coastal circulation would result in unpredictable settlement even in the most homogeneous environments. Therefore, it follows that larval connectivity would be inherently stochastic and highly temporally variable. Taken together, these data on fish and invertebrates, in combination with our data, highlight the importance of incorporating measures of variability in estimates of population connectivity, as larval ecology cannot be well described by mean conditions (Siegel et al. 2008). Thus, we conclude that investigating variability in the geochemical tags of larval hard parts over a range of scales [from diel (i.e., internal bore warm fronts) to decadal (oceanographic/reproductive cycles related to El Niño Southern Oscillation)] remains an exciting avenue in the development of methods for exploring larval ecology and population connectivity (Pineda et al. 2007, Thorrold et al. 2007).

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Table 1. Summary table of X:Ca ratios in mytilid mussel post-settlement and larval shells collected from the Scripps Institution of Oceanography Pier (SIO) and Harbor Island riprap seawall within San Diego Bay (HI). Included are the effects of site (weeks pooled) and time (among weeks within a site) on post-settlement shell chemistry based on non-parametric testing.

	Mg:Ca	Mn:Ca	Cu:Ca	Sr:Ca	Cd:Ca	Ba:Ca	Pb:Ca	U:Ca
Post-Settlement Shell Concentration (mmol mol^{-1})								
SIO (n = 120)	1.423 ± 0.058	0.056 ± 0.009	0.069 ± 0.005	2.238 ± 0.071	0.006 ± 0.001	0.010 ± 0.001	0.016 ± 0.002	0.003 ± 0.001
HI (n = 51)	1.670 ± 0.090	0.421 ± 0.132	0.079 ± 0.009	1.890 ± 0.100	0.012 ± 0.005	0.023 ± 0.001	0.096 ± 0.026	0.001 ± 0.001
Site Comparison (Mann-Whitney U)								
U	2486	992	3142	2652	1722	1876	1390	2777
z-value	-2.924	-7.556	-0.890	-2.409	-5.293	-4.815	-6.322	-2.022
p-value	0.004	<0.001	0.374	0.016	<0.001	<0.001	<0.001	0.043
Temporal Comparison (Kruskal-Wallis)								
SIO								
df	12	12	12	12	12	12	12	12
H	36.517	25.384	43.486	23.449	31.310	31.283	15.980	59.535
p-value	<0.001	0.013	<0.001	0.024	0.002	0.002	0.192	<0.001
HI								
df	12	12	12	12	12	12	12	12
H	17.780	26.506	19.168	11.709	25.555	22.231	29.436	25.833
p-value	0.123	0.009	0.085	0.469	0.012	0.035	0.003	0.011
Larval Shell Concentration (mmol mol^{-1})								
SIO (n = 108)	0.267 ± 0.025	0.102 ± 0.022	0.016 ± 0.002	3.319 ± 0.080	0.002 ± 0.001	0.010 ± 0.001	0.037 ± 0.005	0.003 ± 0.001
HI (n = 43)	0.292 ± 0.051	0.400 ± 0.133	0.036 ± 0.010	2.745 ± 0.146	0.122 ± 0.116	0.016 ± 0.002	0.094 ± 0.024	0.001 ± 0.001

Table 2. Classification success (jackknifed) of DFA algorithms used to distinguish: 1) multielemental signals in post-settlement shell between mussels collected at Harbor Island (HI) within San Diego Bay and at Scripps Pier (SIO) along the open coast, or 2) geochemical tags in early larval shell of settled mytilid mussels specimens collected at HI and SIO (used to infer larval dispersal). Rows list the collection site of specimens, while columns register the predicted collection site (for post settlement shell) or natal signature (for early larval shell) of individuals based on shell chemistry entered in to a DFA model. For post-settlement shell, classification successes are presented for the entire sampling period, during only the first 8 weeks of sampling and during only the last 5 weeks of sampling.

Post-Settlement Shell: All Weeks				
	Predicted		Classification Success %	
	HI	SIO	Correct	Random
Actual				
HI	30	21	59	43
SIO	14	106	89	65
Total	44	127	80	55

Post-Settlement Shell: First 8 Weeks				
	Predicted		Classification Success %	
	HI	SIO	Correct	Random
Actual				
HI	20	8	71	49
SIO	7	76	93	53
Total	27	84	87	52

Post-Settlement Shell: Last 5 Weeks				
	Predicted		Classification Success %	
	HI	SIO	Correct	Random
Actual				
HI	17	6	74	52
SIO	7	30	81	54
Total	24	36	78	53

Early Larval Shell: Natal Origins				
	Natal Origin		Larval Trajectory %	
	HI "type"	SIO "type"	Local "type"	Random
Settlement Site				
HI	39	4	91	48
SIO	21	87	81	60
Total	60	91	83	57

Table 3. Effect of settlement phase ("low" versus "high") on the geochemical tags within early larval shell of settled mytilid mussels at the Scripps Pier (SIO; 2 elements) and at Harbor Island within San Diego Bay (HI; 4 elements) based on MANOVA. Also included are the average MANOVA results for 6 trials in which settlers during three (SIO) or two (HI) randomly selected weeks were compared to settlers from all other weeks.

	SIO	HI
Elements	Mg, Sr	Sr, Cd, Ba, Pb
MANOVA Score	0.087	0.434
df	2	2
df-residual	108	43
F-value	4.563	9.335
p-value	0.013	<0.001
p-value (Random)	0.393	0.494

Figure 1. Location of collection sites along the southern California coastline, including one on the open coast at the Scripps Institution of Oceanography Pier (SIO) and one within a protected embayment, San Diego Bay, at Harbor Island (HI).

Figure 2. Images captured from the New Wave UP 213-nm laser ablation unit before (a., c.) and after (b., d.) sampling the larval and post-settlement shell of *Mytilus californianus* (a.-b.) and *M. galloprovincialis* (c.-d.). In 'before' images, larval (L) and post-settlement (Settler) shell are distinguished, and the dorsal apex is noted when visible (DA). In 'after' images, 2 laser tracks are visible and labeled as either "early" or "late" larval shell. Only data from the "early" larval shell and DA ablations are included in the results of this study (e., dark bars). "Early" and "late" larval shell are relative, qualitative definitions based on the primary growth axis and torsion of growing shell material observed for mytilid larvae spawned and raised in the lab (f., dark arrows).

Figure 3. Temporal patterns of multielemental signatures in post-settlement mussel shell collected from Scripps Pier (SIO) and Harbor Island (HI). Mg:Ca (A), Mn:Ca (B), Cu:Ca (C), Sr:Ca (D), Cd:Ca (E), Ba:Ca (F), Pb:Ca (G) and U:Ca (H). Gray vertical bars indicate an apparent shift in environmental conditions following week 8. Cd, Ba, Pb and U were included in a DFA to compare multielemental signatures in post-settlement shell between sites for the entire sampling period; Ba, Pb and U were included in a DFA for only the first eight weeks; and Mg, Sr, Cd and U were included in a DFA for only the last five weeks.

Figure 4. Settlement of mytilid mussels (settlers gram-byssus-thread^{-1}) during the winter and spring of 2002 at Scripps Pier (SIO) and Harbor Island (HI). Weeks classified by "high" settlement events (> 3 SD above mean settlement) are denoted by *H* (SIO) or **H** (HI).

Figure 5. Elemental concentrations (X:Ca) in early larval shell of mytilid mussels collected during "low" and "high" (> 3 SD above mean settlement) settlement phases at Scripps Pier (SIO) and Harbor Island (HI). For each element used in MANOVAs testing, element-by-element comparisons between recruitment phases were generated via t-tests, with significant results denoted by * ($p < 0.05$) and ** ($p < 0.001$).

Figure 1.

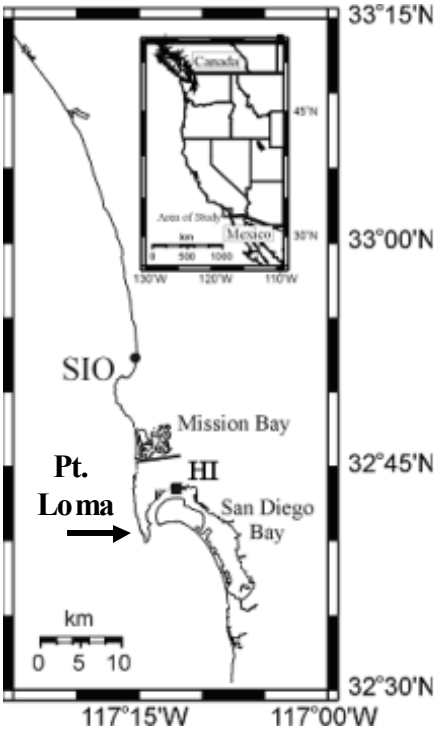

33°15'N
33°00'N
32°45'N
32°30'N
117°15'W
117°00'W
Canada
45°N
30°N
Area of Study
Mexico
km
0 500 1000
130°W
120°W
110°W
SIO
Mission Bay
Pt.
Loma
HI
San Diego
Bay
km
0 5 10

Figure 2.

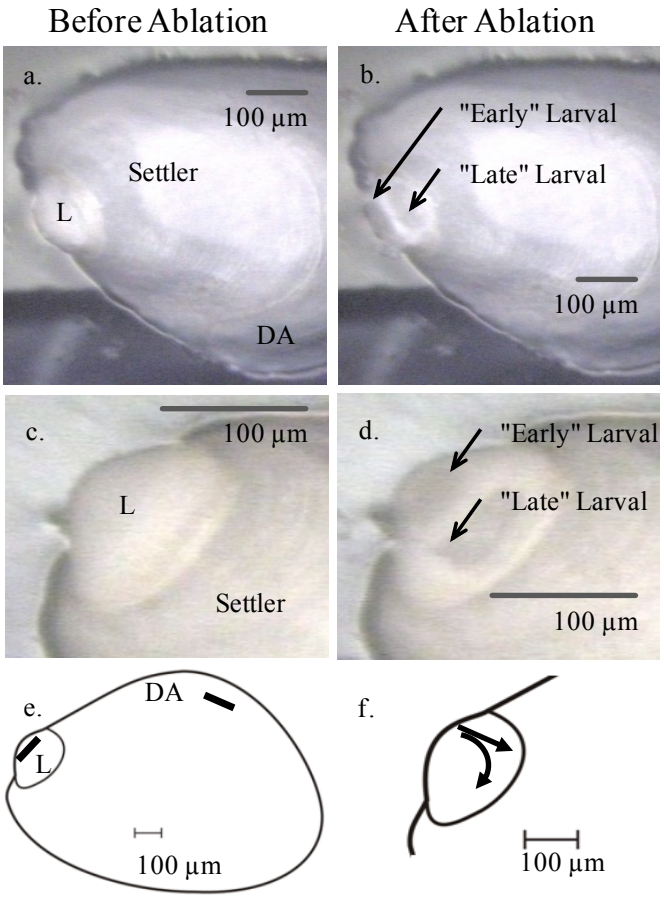

Before Ablation
After Ablation
a.
100 µm
Settler
L
DA
b.
"Early" Larval
"Late" Larval
100 µm
c.
100 µm
L
Settler
d.
"Early" Larval
"Late" Larval
100 µm
e.
DA
L
100 µm
f.
100 µm

Figure 3.

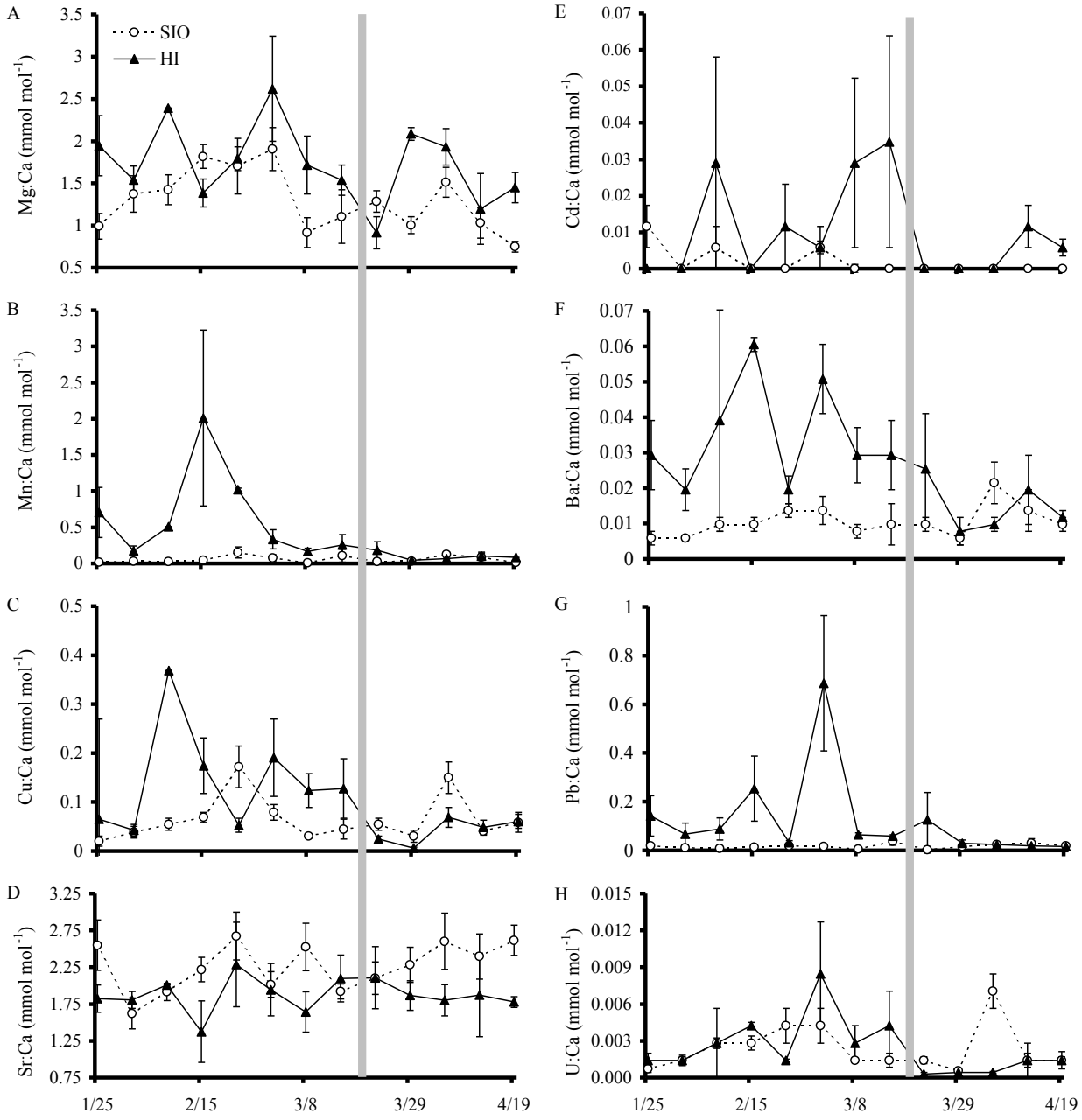
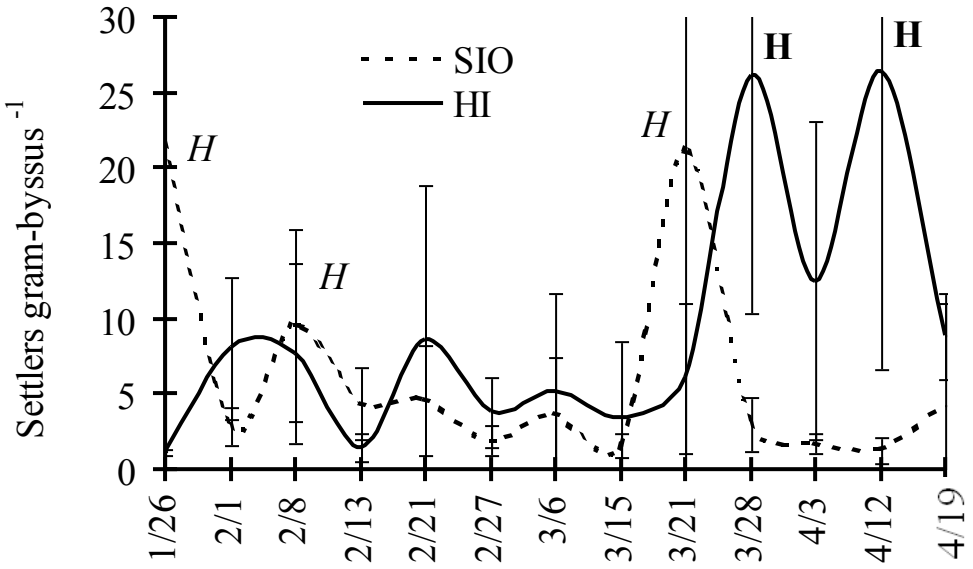
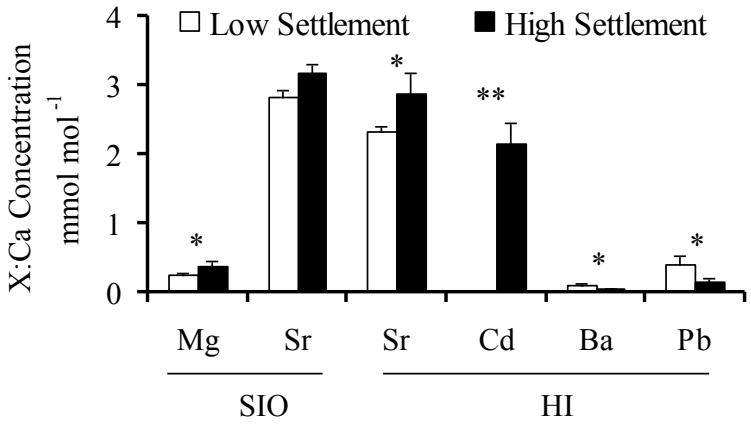

A
SIO
HI
Mg:Ca (mmol mol-1)
B
Mn:Ca (mmol mol-1)
C
Cu:Ca (mmol mol-1)
D
Sr:Ca (mmol mol-1)
E
Cd:Ca (mmol mol-1)
F
Ba:Ca (mmol mol-1)
G
Pb:Ca (mmol mol-1)
H
U:Ca (mmol mol-1)
1/25
2/15
3/8
3/29
4/19

Figure 4.


Settlers gram-byssus -1
30
25
20
15
10
5
0
SIO
HI
H
H
H
H
H
1/26
2/1
2/8
2/13
2/21
2/27
3/6
3/15
3/21
3/28
4/3
4/12
4/19

1 Figure 5.

2 
Low Settlement
High Settlement
X:Ca Concentration
mmol mol-1
4
3
2
1
0
*
*
**
*
*
Mg
Sr
Sr
Cd
Ba
Pb
SIO
HI