

Temporal, spatial, and interspecific variation in geochemical signatures within fish otoliths, bivalve larval shells, and crustacean larvae

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ABSTRACT: Geochemical signatures of early life stages are increasingly used to study population connectivity. This approach utilizes spatial variability in chemical signatures to predict natal or nursery origins of post-dispersal individuals by comparison with a chemical reference atlas created from individuals of known origin. To examine the relative importance of spatial, temporal, and species variation in elemental signatures, we synthesized the chemical information of otoliths, larval shells, and whole larvae from studies that employed natural geochemical signatures in San Diego County, USA between 1997 and 2009. We compared 8 elements analyzed from 4 bivalve species, 2 larval or juvenile fishes, and Stage 1 crab zoeae. Across all species, different sets of elements best discriminated among open-coast sites or within or among bays and lagoons. In mytilid mussels, which had the most complete record, all 8 elements were more variable over time than space at the site level, highlighting the need to resample the reference atlas during each study. More coarsely, however, bay and lagoon taxa maintained distinct chemical signatures both from each other and from those on the open coast, despite interannual variability. Spatially identifiable signatures for all species were likely imparted by a combination of pollution in bays and export to adjacent coastlines (copper, lead), a heterogeneous distribution of land-sourced elements (manganese, cobalt, uranium), and incorporation that may vary in response to temperature (barium, manganese, strontium) and salinity (7 elements). These results identify important elements for larval tracking of additional species depending on habitat and life history; however, source population signatures appear species-specific.

KEY WORDS: Geochemical signatures · Trace-elemental fingerprinting · Connectivity · Dispersal · *Mytilus californianus* · *Mytilus galloprovincialis* · *Musculista senhousia* · *Ostrea lurida* · *Paralichthys californicus* · *Hypsypops rubicundus* · *Pachygrapsus crassipes*

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INTRODUCTION

Increasingly, researchers are using the chemical composition of larvae and juveniles, or specific calci-

fied structures within them, to answer questions about the largely unknown early life-history movements of marine organisms (Thorrold et al. 2007). An area of origin is inferred by matching concentrations

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of certain elements within larvae (or juveniles) of unknown origin to larval (or juvenile) reference signatures from known possible source populations (Campana et al. 2000). This information helps unravel long-standing ecological questions about the role of early life stages in the structure of marine populations and communities, explores population connectivity patterns, and has practical applications to the spatial management of fisheries, preservation of biodiversity, and control of invasive species (Levin 2006).

In fish, the natal signature is located in the core of the otolith, surrounded by a juvenile signature. In larval molluscs, a signature is found in either the analogous statolith (Zacherl 2005) or larval shell (Becker et al. 2005, 2007). In crustaceans, a similar, spatially variable signature has been found in whole embryos (Carson et al. 2008), whole larvae (DiBacco & Levin 2000), or adult exoskeleton (Jack et al. 2011). Most studies have used these signatures in 'snapshot' fashion, where a short-term, static atlas of chemical variability is used to identify the origins of a recent set of recruits (but see Becker et al. 2005, Cook 2011, Fodrie et al. 2011 for notable exceptions). Often, studies span only 1 or 2 yr, seasons, or cohorts of organisms, with the exception of the 7-yr time series documented by Carson et al. (2010) for mytilid mussels.

As the use of geochemical signatures becomes more widespread, some commonalities among species and locations have begun to emerge (Thorrold et al. 2007). Most notably, the group of elements that exists in readily detectable concentrations and that varies with environment is similar for many species globally. These useful elements include magnesium (Mg), chromium (Cr), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), strontium (Sr), cesium (Cs), barium (Ba), lead (Pb), and uranium (U). Although there is no *a priori* reason to expect teleost fish, bivalves, and crustaceans to incorporate elements into structures the same way, it is helpful to examine similarities to determine if there are elements that are generally discriminating in various habitats.

Geochemical signatures are not only useful for distinguishing among enclosed water bodies, but several studies have found sufficient variation along the open coast at ecologically relevant spatial scales (e.g. Standish et al. 2008). However, the interplay between the spatial and temporal stability of that variation has been difficult to measure due to the relatively short-term nature of the studies. Also, different studies detect different relationships between larval chemistry and physical variables such as seawater temperature and salinity, but it is unclear whether those differences are a result of differing species, habitats, or

both. Due to contamination studies (e.g. Lauenstein et al. 1990) or reconstructions of paleoclimate (e.g. Gillikin et al. 2006), the literature on the incorporation of certain elements into adult organisms is more developed than that for larvae or juveniles (but see 'Discussion: Variability among species').

Between 1997 and 2009, the natural, geochemical signatures for larvae or juveniles from 7 different species were detected along the same ~100 km stretch of the California, USA, coastline, most in separate population-connectivity studies. These species include 4 molluscs, 2 fishes, and 1 crustacean (Table 1). The specific set of sampling sites differed among studies, but many sampling locations were the same or located in close proximity. This overlap in space allows for (1) a comparison of elemental incorporation among species with different physiological and ecological attributes. The range of sites and habitats sampled also allows for (2) an examination of the spatial variability in element incorporation independent of species, and (3) specifically whether there is a relationship between the similarity of geochemical signatures and the distance between sites. The temporal and spatial extent of sampling also permits (4) an evaluation of possible incorporation relationships with temperature and salinity. Finally, sampling of the same species in the same place over several years, as was the case for mytilid mussels, allows for (5) the evaluation of the temporal stability of elemental signatures. In the present study, we discuss these themes, with the objectives of identifying (1) key elements for discrimination within and among various habitats in southern California, (2) the spatial and temporal components of variability in elemental signatures, (3) commonalities and differences among species to test the degree to which trace-element incorporation is taxon-specific, and (4) significant relationships between trace-elemental incorporation and temperature and salinity across species in the same location.

We restrict our discussion here to elemental incorporation trends from the previously published studies on geochemical signatures (Table 1) and do not discuss their population connectivity results, which were previously compared across species in López-Duarte et al. (2012).

METHODS

Area description

All studies were conducted in San Diego County, USA, in the Southern California Bight (Fig. 1). The

Table 1. Species and study details, including the spatial and temporal extent of sampling in San Diego County, USA. N: North County; C: Central County; S: South County; NCL: North County Lagoons; MB: Mission Bay; SDB: San Diego Bay. See Fig. 1 for map of sample locations

Taxon	Common name	Study	Structure	Time period	Spatial coverage						
					Open coast	N	C	S	Embayments	NCL	MB
<i>Hypsypops rubicundus</i>	Garibaldi	Cook (2011)	Embryonic otoliths	Summer 2008, 2009	X	X	X				
<i>Ostrea lurida</i>	Olympia oyster	Carson (2010)	Brooded larval shells	Summer 2006, 2007					X	X	X
<i>Musculista senhousia</i>	Asian mussel	López-Duarte et al. (unpubl.)	Outplanted larval shells	Spring, fall 2008		X			X	X	X
<i>Mytilus californianus</i>	California mussel	Becker et al. (2007), Carson et al. (2010)	Outplanted larval shells	Springs or falls 2003–2009	X	X	X		X	X	X
<i>Mytilus galloprovincialis</i>	Bay mussel	Becker et al. (2007), Carson et al. (2010)	Outplanted larval shells	Springs or falls 2003–2009	X	X	X		X	X	X
<i>Mytilus</i> spp.	Mussel	Becker et al. (2005)	Juvenile shells	Throughout 2001	X	X	X		X	X	
<i>Mytilus</i> spp.	Mussel	Fodrie et al. (2011)	Juvenile shells	Winter 2002		X					X
<i>Pachygrapsus crassipes</i>	Lined shore crab	DiBacco & Levin (2000)	Whole larvae	Spring, summer 1997		X	X		X	X	X
<i>Paralichthys californicus</i>	California halibut	Fodrie & Levin (2008)	Juvenile otoliths	Fall 2003, 2004	X	X	X		X	X	X

San Diego County coastline consists of a narrow northern shelf (~3 km wide), broken by submarine canyons at Carlsbad and La Jolla, and a relatively broad shelf (~10 km wide) south of Point Loma. Farther offshore, circulation is dominated by the southward-flowing California Current, but inshore, flow is dominated by a northward-flowing eddy through the Southern California Bight. Within San Diego County waters (i.e. within 3 nautical miles of shore), the nearshore region is characterized by both episodic and seasonal north–south current reversals (Carson et al. 2010). Mean monthly nearshore sea surface temperatures for the entire study period ranged from 13 to 23°C (www.sccoos.org).

For characterizing enclosed habitats, we draw a distinction between bays, lagoons, and estuaries (Fodrie & Mendoza 2006). The North County coastline is punctuated by several heavily modified lagoons, with constricted circulation due to road and railway causeways across them. Some lagoons, such as Agua Hedionda, have inlets that have been artificially stabilized by armored jetties, while others such as San Dieguito are only open to the ocean seasonally. In North San Diego County, the San Dieguito, San Luis Rey, and Santa Margarita watersheds are drained by seasonal streams. The southern half of the county contains 2 heavily urbanized bays, San Diego and Mission Bay; both bays contain a high density of recreational, commercial, and military vessels.

Because of the semi-arid, Mediterranean climate (~30 cm annual precipitation), the bays are often ‘reverse’ estuaries, with increased temperature and salinity compared to the local ocean. Exchange between these embayments and the ocean is dominated by tidal pumping (Chadwick & Largier 1999). We subdivide these into ‘inner’ and ‘outer’ sectors, with the outer bay characterized by frequent exchange with coastal water compared to the longer residence times of the inner bay.

The largest freshwater sources are from the Tijuana River watershed, whose estuary is located at the US–Mexico border, and the San Diego River, which enters the ocean north of Point Loma. Small freshwater inputs to San Diego Bay include the Sweetwater and Otay watersheds, and for Mission Bay the Peñasquitos watershed. The majority of the coast is underlain by sedimentary rocks, but a heterogeneous distribution of occasional plutonic and metamorphic rock outcrops occurs on the surface (Kennedy & Peterson 1975) and may contribute to chemical differences in freshwater runoff among various watersheds.

Intertidal and shallow subtidal environments of San Diego County are dominated by soft-sediment bottoms, often coarse-grained sand on the open coast and fine-grain mud in the inner bays and lagoons (Fodrie & Mendoza 2006). Intertidal or shallow subtidal bedrock, predominantly sandstone, exists in

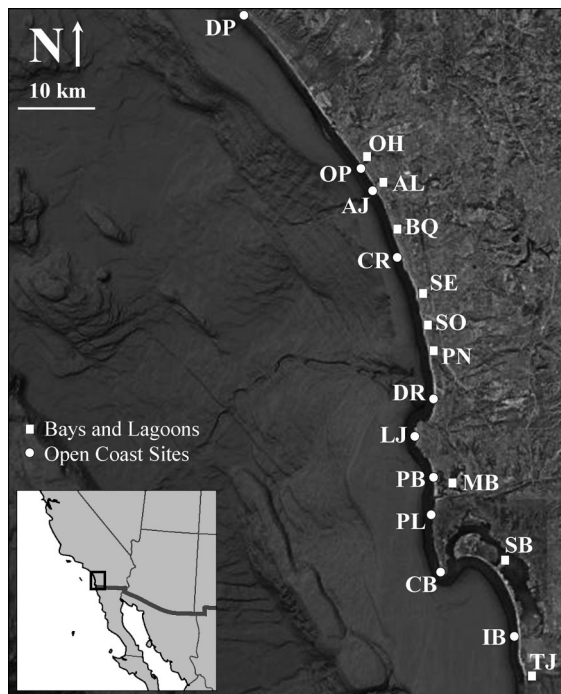


Fig. 1. Sampling sites for all 7 species studied in San Diego County. North County sites are Dana Point (DP), Oceanside Harbor (OH), Oceanside Pier (OP), Agua Hedionda Lagoon (AL), Agua Hedionda Jetty (AJ), Batiquitos Lagoon (BQ), Cardiff Reef (CR), San Elijo Lagoon (SE), San Dieguito Lagoon (SO), and Peñasquitos Lagoon (PN). Central County sites are Dike Rock (DR), La Jolla (LJ), Pacific Beach (PB), Mission Bay (MB), and Point Loma (PL). South County sites are Cabrillo (CB), San Diego Bay (SB), Imperial Beach (IB) and Tijuana River Estuary (TJ). Analogous sites names in the original studies are Carlsbad (AJ), Torrey Pines (DR), Ocean Beach (PL), and Zuniga Point (CB). MB and SB each have multiple sample sites. Not all species were sampled at all sites

patches in the North County near Carlsbad and Cardiff, and in larger stretches in the La Jolla and Point Loma areas (Fodrie & Mendoza 2006). Artificial hard substrates include several wooden and concrete pier structures, seawalls, and rip-rap jetties. The outer portions of the larger embayments are almost completely armored with rock or concrete.

Species descriptions

Hypsypops rubicundus, or *garibaldi*, is a temperate pomacentrid reef fish. Embryos develop in open-coast benthic nests for 2 wk in the summer months, and hatched larvae have 18 to 22 d pelagic larval durations (PLD) before settlement to shallow rocky reefs (Clarke 1970). Chemical information for the present study was obtained from embryonic otoliths,

composed of aragonitic calcium carbonate, removed from naturally produced larvae in benthic nests (Cook 2011).

The California halibut *Paralichthys californicus* is a large flatfish. Adults inhabit deep waters but migrate to shallower coastal waters during the spring or summer to spawn free-floating fertilized eggs. After a 20 to 30-d PLD, settled juveniles use a variety of nursery habitats on shorelines, bays, and lagoons (Fodrie & Levin 2008). Chemical information for the present study was obtained from the blind-side otolith taken from wild-caught juvenile fish.

Female-phase *Ostrea lurida*, the native or Olympia oyster, brood shelled veliger larvae for 7 to 12 d following summer spawning (Strathmann 1988). After release and a subsequent PLD of 7 to 60 d (H. S. Carson pers. obs., Strathmann 1988, reviewed in Baker 1995), larvae settle to intertidal or shallow subtidal habitat in bays and lagoons. Chemical information for the present study was obtained from the aragonitic larval shells (prodissoconchs) removed from inside the shells of naturally occurring, brooding adults (Carson 2010).

In contrast to the preceding 3 species, chemical data for the 3 free-spawning mussel species were obtained via larval shells that had been fertilized in the laboratory and outplanted in larval 'homes' on moorings near adult habitat (Becker et al. 2007). The native California mussel *Mytilus californianus* occurs on intertidal hard substrate located along the open coast, and free-spawns gametes year-round, with peak spawning in the late fall and winter. The PLD for this species is between 9 and 45 d (reviewed by Carson et al. 2010). The introduced bay mussel *Mytilus galloprovincialis* is prevalent on intertidal hard substrate in the outer portions of bays and lagoons, but also coexists in lower abundances with *Mytilus californianus* on the open coast. Adults free-spawn all year but reproduction peaks in the spring, and larvae of this species are thought to have a somewhat longer PLD (14 to 60 d) compared to its congener (reviewed by Carson et al. 2010). In addition to the outplanted larvae, chemical information from newly settled mytilids collected in the field was also considered (Becker et al. 2005, Fodrie et al. 2011). The introduced Asian mussel *Musculista senhousia* creates byssal mats over fine sediments in low-flow areas of inner bays, often associated with seagrass beds (Crooks 1996). They free-spawn year-round, with a PLD between 15 and 21 d (P. C. López-Duarte pers. obs.).

The final species discussed here is the lined shore crab *Pachygrapsus crassipes*. Adults inhabit both

rocky intertidal and marsh habitats in open-coast and bay habitats, and brood fertilized embryos beneath the abdomen. The planktonic larvae molt through 5 zoeal stages over a period of 3 mo before recruiting to adult habitat as megalopae (Schlotterbeck 1976). Chemical information for the present study was obtained from entire, dissolved, Stage 1 zoeae collected during highly predictable *in situ* spawning events (DiBacco & Levin 2000).

Chemical analysis and standardization among studies

All studies measured the concentration of trace elements in ratio to the amount of calcium (Ca) using inductively coupled plasma mass spectrometry (ICP-MS), except for the lined shore crabs, which lacked calcium-carbonate shells or otoliths. Whole shore-crab larvae were digested and analyzed using ICP-atomic emission spectroscopy (ICP-AES) standardized per larva. The shell and otolith studies used laser ablation to directly sample natal regions of the solid shell or otolith. For the present study, methodological information from each original study has been summarized; species-specific sampling and data processing specifics are described in detail in the relevant published studies (Table S1 in the supplement at www.int-res.com/articles/suppl/m473p133_supp.pdf).

Because analyses were made using differing techniques, or using similar techniques in different laboratories, the absolute concentrations or ratios to Ca among species across studies are not directly comparable (Campana et al. 1997). Variation in preparation, instruments, operators, and background levels could confound interspecific differences of interest, despite the use of standards. Therefore, all comparisons between studies were made using non-parametric rank order instead of element concentration. Sites were ranked for each element concentration from highest to lowest within each species and sampling event, so that consistent 'high' or 'low' levels at particular sites could be identified independent of absolute levels. The rank scales were standardized by number of sites considered so that analyses with larger number of sites had the same range of ranks as those with smaller numbers of sites. Only when questions could be answered using individual species, such as the temporal stability of signatures in the mytilid-mussel time series, or relationships with temperature or salinity, were actual ratios to Ca utilized.

We incorporated data from 8 of the elements used in the fish, bivalve, and crab studies, although not all these elements were used to discriminate among populations of each species or on each date. Three elements were common to the 7 species: Mg, Mn, and Sr, while studies of all species except *Pachygrapsus crassipes* examined Ba, Pb, and U. Cu was measured in the bivalves, the shore crab, and the halibut, and Co was studied in only the 3 mussels and the halibut.

Temperature information was gathered using HOBO temperature data loggers (Onset Corporation) fixed to the substrate near source populations for *Ostrea lurida* and *Hypsypops rubicundus* or on outplant moorings for the 3 mussel species. Sub-surface (i.e. 5 m water depth) seawater temperature data were obtained from the Scripps Institution of Oceanography pier (32° 52.0' N, 117° 15.5' W) manual shore station (<http://shorestation.ucsd.edu>). Similarly, salinity information was gathered from Scripps Pier, in which salinity of daily water samples was measured using a Model 8410 inductive salinometer (Guildline Instruments). Salinity and sub-surface temperature data were used for comparison only to organisms sampled in the vicinity of Scripps Pier during the same dates for which data were available.

Statistical methods

Kruskal-Wallis tests were used to differentiate adjusted ranks for each element, with post hoc pairwise comparisons among sites, years, or species. Bray-Curtis similarity was used in SIMPER analyses to compare ranks as a function of site, year, or species in multivariate space (using all 8 elements' ranks) in the program PRIMER. Bray-Curtis similarity in the chemistry of sites was compared to geographic distance using linear regression. Relative variability of elements in space or time in the *Mytilus* spp. time series were compared using the coefficients of variation (CV; standard deviation as a percentage of the mean) on raw element-to-Ca ratios. Multidimensional scaling (MDS) plots were employed to visualize the variation in mytilid chemistry at sites over consecutive fall sampling events. Linear regressions and corresponding pairwise correlations were used to test for relationships between element ratios and temperature or salinity. The relative importance of an element in discrimination among sites was evaluated using the 'F-to-remove' values in linear discriminant function analyses.

Table 2. Comparison of elevated elements across species and regions. Means for elements shown in **bold** were significantly elevated in univariate *t*-tests ($p < 0.05$) where sample size allowed. Remaining listed elements were $>150\%$ of the mean of the comparison group and thus highly likely to be useful in the multi-variate discrimination among groups

Taxon	Sampling events	Whole bay vs. whole coast	Outer bay vs. inner bay	Inner bay vs. outer bay	Whole coast vs. whole bay	North coast vs. south coast	South coast vs. north coast	Lagoon or estuary vs. whole coast
<i>Hypsypops rubicundus</i>	Summer 2008					Sr	Pb	
	Summer 2009				Mn, Ba	Mn, Ba		
<i>Ostrea lurida</i>	Summer 2006	Cu, Pb	Cu	Mg, Ba				
	Summer 2007	Cu, Pb	Pb	Mn				
<i>Musculista senhousia</i>	Spring 2008	Mn, Co, Cu	Mg, Cu, Ba	Mn, Co	Pb, U			Mn, Co, Cu, Ba
	Fall 2008	Mn, Cu, Pb	Cu, Ba, Pb	Mg, Mn, Co, U	Ba			Mn, Co
<i>Mytilus californianus</i>	Spring 2003						Cu, U	Pb
	Fall 2004	Mn, Cu	Cu, Ba, Pb	Co, Sr	Co, U	Mn		Mn, Ba
	Spring 2005			Mn, Co, Sr	U	Mn, Co, U		
	Fall 2006	Mn, Co, Cu, Ba, Pb		Mn, Co		Mg, Mn, Ba		Mn, U
	Fall 2007	Mg, Mn, Cu, U	Mg, Cu, Ba, U	Mn		U		Mg, Mn, Co, Ba
<i>Mytilus galloprovincialis</i>	Fall 2008	Mn, Cu		U	Ba	Mg, Mn, Pb, U		Cu
	Fall 2009	Mg, Mn, Pb					Cu, U	Cu, U
	Spring 2003	Mn, Cu, Pb						
	Spring 2005		Mg	Co, Cu	Mn, Co	U	Cu, Pb	Mg, Mn, Co, Cu, Ba
	Spring 2006	Mn, Cu, Sr, Ba, Pb	Mg, Ba	Mg, Mn, Co, Cu, Pb	U		Mg, Co, Pb, U	Mg, Ba
<i>Mytilus spp.</i>	Spring 2007	Mn	Cu		Ba	Mn		Cu, Ba
	Spring 2007	Mn, Cu	Cu, U	Mg, Mn, Pb	Mg, Sr, Pb		Pb	Cu
	Fall 2007	Pb		Mg, Mn, Ba, U		Mn, Ba	U	Mg
	Fall 2008	Mg, Mn, Co, Cu, Ba	Mg, Co	Cu				
	Fall 2009	Mn, Pb		Ba	Sr		Pb	
<i>Pachygrapsus crassipes</i>	Winter 2002	Mn, Cu, Ba, Pb			Sr			
	Spring 1997	Mn		Mn, Cu, Sr	Mg			Mn, Cu
<i>Paralichthys californicus</i>	Summer 1997	Mn, Cu	Mn, Cu	Mg, Sr	Sr			Mn, Ba, Pb, U
	Fall 2003	Mn, Cu, Ba, Pb, U		Cu, Sr, Ba, U	Mn, Ba, Pb, U	Mg	Cu, Pb	Mn, Ba, Pb, U
Fall 2004	Cu	Mg					Mn, Cu, Pb, U	Ba

RESULTS

Variability in space

Bays and lagoons versus open coast

Organisms in San Diego County bay and lagoon habitats have distinct chemical signatures compared to those same taxa along the open coast. Six species were sampled in San Diego and Mission Bays for a total of 24 cases (a 'case' is a particular species and sampling event), and they commonly but not uniformly demonstrated elevated levels of Mn, Cu, and Pb compared to the open coast. The 6 species were the California halibut, lined shore crab, *Olympia* oyster, Asian mussel, and 2 mytilid mussels. Relatively high Mn was detected in 17 out of 24 cases in the bays versus 2 cases along the open coast. Elevated Cu was detected in 16 of 24 cases; in no cases was Cu higher on the open coast. Pb was higher in 11 cases in bays versus 3 cases along the open coast (Table 2). The same species in lagoon habitats mirrored the pattern seen in the 2 large bays, including increased Mn (8 of 20 cases elevated in lagoons to 2 cases on the open coast) and Cu (7 to 0), but not Pb (2 to 3). Additionally, Ba was elevated in lagoon samples 8 times, compared to 3 instances where it was elevated along the open coast (Table 2).

Within and among bays and lagoons

In the studies using geochemical signatures from San Diego and Mission Bays, samples of the same 6 species were collected from both the inner and outer halves of the bay. Inner-bay organisms had higher levels of both Mn (10 of 20 cases) and Co (7 of 20 cases) relative to the outer bays (Table 2). No elements were consistently elevated in the outer-bay individuals, which often more closely resembled those from the open coast, possibly due to bay-ocean water exchange.

We compared element incorporation by the 6 bay-inhabiting species across all San Diego embayments, regardless of habitat type (e.g. large bay, lagoon, or estuary). We found significant between-

embayment variation in average rank across species for Mn, Cu, Pb, and U. San Diego Bay had high ranks for Mn, Cu, and Pb, while Mission Bay showed increased U. San Dieguito Lagoon also showed high ranks for these 4 elements, while Agua Hedionda and Batiquitos Lagoons had low ranks (Fig. 2).

Open coast

Considering open-coast sites only, 5 species were examined in 22 sampling events, including the California halibut, garibaldi, lined shore crab, and 2

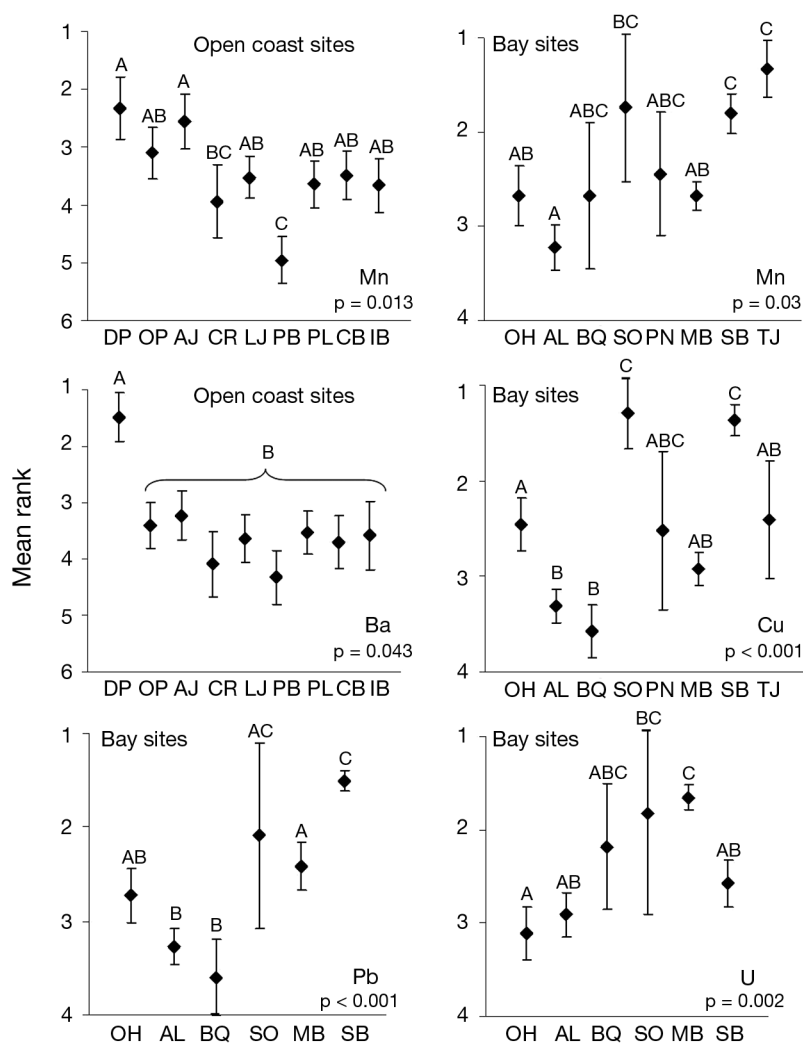


Fig. 2. Results of Kruskal-Wallis tests on the element-concentration ranks for all species (mean \pm 1 SEM) at open-coast (Mn, Ba) and bay and lagoon sites (Mn, Cu, Pb, U). The results of post hoc pairwise comparisons are listed above each point as group membership designations (A, B, or C). Sites are listed from north to south, left to right, and name abbreviations are given in the caption for Fig. 1

mytilid mussels. The northern part of the county, between Dana Point and Cardiff Reef, had elevated Mn in 7 of 22 cases, compared to 1 case of higher Mn in the south (Table 2). The southern county, between Torrey Pines–La Jolla and Imperial Beach, had higher Cu (5 cases) and Pb (7 cases) compared to northern sites. Comparing these sites individually, instead of by region, showed significant differences in average rank for Mn and Ba (Fig. 2) but not other elements. Sites in the extreme north had elevated ranks for both Mn and Ba, whereas Pacific Beach in the central county imparted weak Mn signals within animals regardless of taxon.

Similarity versus distance

To address whether sites in closer proximity had similar chemical signatures, we used the Bray-Curtis similarity index, considering all elements simultaneously. In general, geographic distance between sites was not a good predictor of elemental ranks. Only the mytilid mussels showed a significant negative distance–similarity relationship. In both cases, R^2 values were <0.07 ($p < 0.021$), indicating geographic distance accounted for a small but significant amount of the variation in chemical differences among sites.

Variability in time

Space versus time in *Mytilus* spp. time series

We examined the variability of element ratios over space and time using both species of *Mytilus*, which had 7 sampling events over 7 yr, rather than the other species with only 1 or 2 yr of data. The CVs for each element over time at the same sites (time) were greater than the CVs among sites within each sampling year (space) (Fig. 3). Averaged across elements, no sites had significantly more variability than others over the time series. Some elements, however, were significantly more variable in both time and space than others. Mg and Sr had the lowest CVs; Cu, Ba, Pb, and U had intermediate coefficients; and Mn and Co had relatively high variability (Fig. 3).

Using MDS, we examined whether *Mytilus* spp. individuals sampled at different sites from the same sampling year were more similar in terms of elemental composition, or whether individuals from the same site group together across 4 consecutive fall sampling events (2006 to 2009). In contrast to the temporal instability noted above, differences among

individuals from whole bays and lagoons were generally consistent across years, suggesting that for larvae or juveniles from these habitats, variation in time does not overwhelm that in space. There was no overlap between *M. galloprovincialis* shell chemistry over time in the 4 embayments tested, and little overlap in *M. californianus* shells (Fig. 4). The one exception for *M. californianus* was 2007, where 3 embayments (except San Diego Bay, which remained distinct) clustered close together and muddled the otherwise clear spatial differentiation. On the open coast, however, the variability among consecutive fall sampling events clouded the spatial structure of sites. Although some sampling events for some sites remained close together in multivariate space (e.g. Point Loma for *M. californianus*), there was considerable overlap with other sites. Again, particular years (e.g. *M. californianus* in 2007, or *M. galloprovincialis* in 2008) clustered closer together than the other years (Fig. 4).

The elements useful in discriminating among sites in the *Mytilus* spp. time series were similar to those used to discriminate among sample years or seasons (spring or fall). They did differ significantly between habitat and species, however. In *M. californianus*, U, Co, and Ba had the highest mean contributions to dissimilarity among the open-coast sites in a SIMPER analysis, no matter whether the data were grouped by site, year, or season. Among bays, the prominent elements contributing to dissimilarity were Mn, Cu, and Pb, independent of the grouping (Fig. S1 in

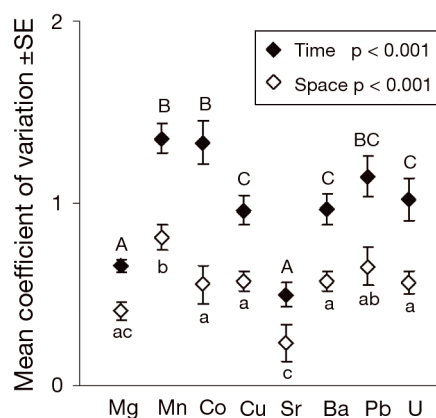


Fig. 3. *Mytilus californianus* and *M. galloprovincialis*. Comparison of the variability of 8 elements in the larval shell averaged across all years at each site (time) and across all sites within each year (space). The results of a 1-way ANOVA with post hoc pairwise comparisons are depicted at each point as group membership designations (time: upper-case letters; space: lower-case letters). Information for every element was not available for all sites in all years

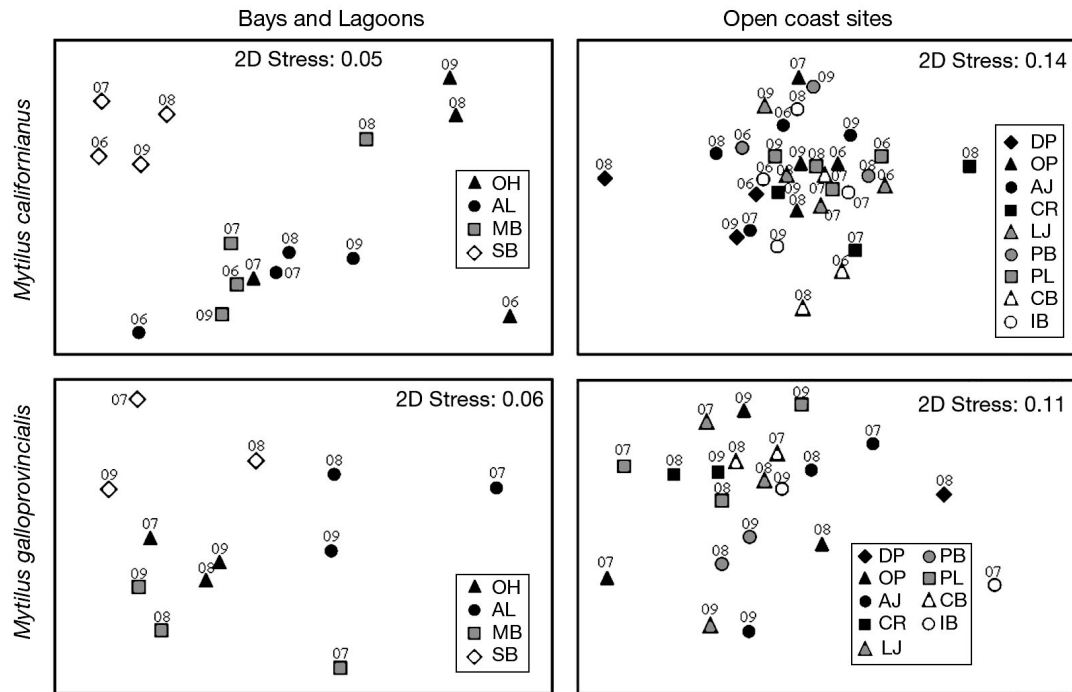


Fig. 4. *Mytilus californianus* and *M. galloprovincialis*. Multidimensional scaling plots of variability in 8 elements over consecutive fall sampling events. Sites are depicted as different symbols with northern sites in black, central sites in gray, and southern sites in white. Site name abbreviations are listed in the caption for Fig. 1. Sample year (2006–2009) is listed next to each point. Two-dimensional (2D) stress is the percentage variability not visibly represented by 2 axes

the supplement at www.int-res.com/articles/suppl/m473p133_supp.pdf). For *M. galloprovincialis*, Pb, U, and Mn contributed to dissimilarity of sites, years, or seasons along the open coast, whereas Mn, Pb, and Ba were the most discriminatory among the bays and lagoons (Fig. S1 in the supplement).

Temporal variability at multiple scales

There is broad agreement among these studies regarding patterns of temporal variability in trace-elemental signatures. Over weekly scales, analyses of *Hypsypops rubicundus* (Cook 2011) and juvenile *Mytilus* spp. (Becker et al. 2005, Fodrie et al. 2011) indicated that individual elemental signatures could appear stable across sites over several weeks, and then quickly shift to new, steady concentrations for several more weeks. Across the 3-mo spawning season of garibaldi, elemental signatures of sites early in the spawning season were indistinguishable from different sites later in the spawning season, suggesting sampling and analysis should occur on time scales able to capture shifts in elemental signatures (Cook 2011). Significant temporal variability in element-to-Ca ratios was also a consistent result ob-

served across seasons (Fodrie & Herzka 2008, López-Duarte et al. unpubl.) and years (Fodrie & Levin 2008, Carson 2010).

Variability among species

Utility of discriminating elements

There was species-specific variation in utility of certain elements to discriminate among sites in linear discrimination function analyses. At bay sites, ranks of importance in discrimination for the 3 bay-dwelling bivalve species were similar for all elements except Mg and Ba (Fig. 5). The importance of elements for the juvenile halibut at bay sites was markedly different than the larval bivalves; halibut otoliths exhibited greater relevance of Co, Sr, and Ba in discrimination, and lower importance for Pb and U. In all 4 bay species, Mn and Cu were moderately to highly important for discriminating among sites (Fig. 5). Along the open coast, 2 bivalve species had similar ranks for all elements except Mn and Ba. The 2 fish species did not have similar element importance, with wide difference in relevance of U, and also Co and Cu, which were important to the halibut

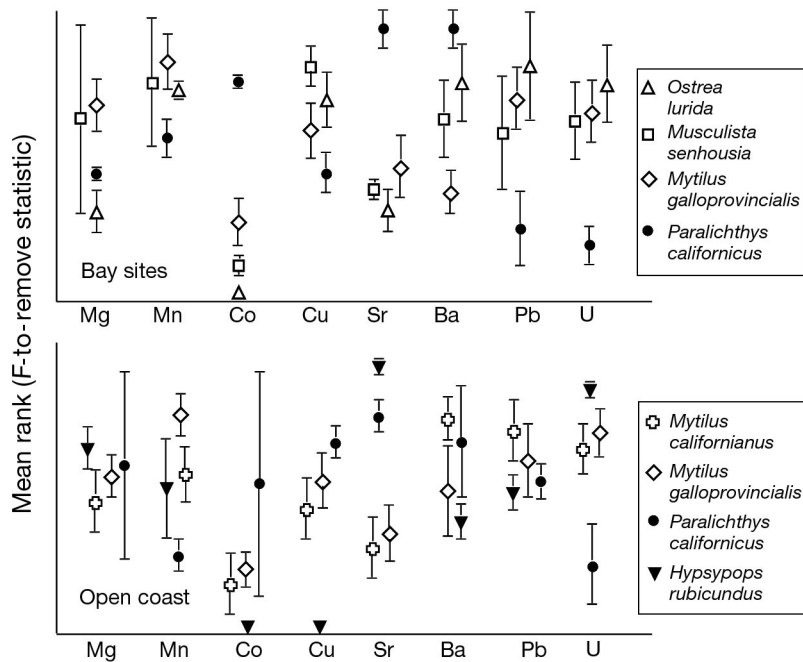


Fig. 5. Relative importance of 8 elements used to discriminate among sites in bays and along the open coast. The y-axis values are the *F*-to-remove statistics from linear discriminant function analyses converted to ranks and averaged across sampling years. Open symbols are bivalve larval shells, closed symbols are fish otoliths. *Mytilus galloprovincialis* and *Paralichthys californicus* samples were split among bay and open-coast locations

but not reliably detected in the garibaldi study (G. S. Cook unpubl. data). As with the bays, Sr was used to discriminate fish otoliths more than bivalve shells. For all 4 open-coast species, Mg, Ba, and Pb were used to discriminate among sites with moderate to high importance (Fig. 5).

Relationship to temperature

Correlations between chemical incorporation and physical parameters of the seawater are often considered in population connectivity studies using geochemical signatures, including the 4 bivalves and 1 of the fishes considered here (Table 3). Carson (2010) detected a positive relationship between Ba and temperature during brooding ($R^2 = 0.385$, $p < 0.001$) in bay-dwelling Olympia oysters. Two other elements, not used to discriminate regions, also had positive relationships to temperature for oyster larvae in summer 2007: Mn ($R^2 = 0.331$, $p < 0.001$) and Sr ($R^2 = 0.241$, $p = 0.005$). The 3 elements showed similar but non-significant relationships to temperature during summer 2006, perhaps due to a 50% lower sample size.

The oyster occurred in many of the same bays and lagoons as the Asian mussel, which was sampled in

2008 using outplanted embryos (López-Duarte et al. unpubl.). *Musculista senhousia* showed similar increases in metal incorporation with temperature for Ba ($R^2 = 0.287$, $p = 0.033$), Mn ($R^2 = 0.335$, $p = 0.018$), and Sr ($R^2 = 0.219$, $p = 0.067$) when both spring and fall events were pooled. Information from another bay-dwelling bivalve, *Mytilus galloprovincialis*, did not support these trends when only bay and lagoon sites were considered over its entire 2003–2009 time series. However, when sampling events were analyzed separately, and open-coast sites were included, Mn had a positive relationship with temperature in 3 of the 7 yr ($R^2 = 0.424$ to 0.755 , $p = 0.003$ to 0.030). Sr was only significantly positively correlated in 2003 ($R^2 = 0.156$, $p = 0.029$), and Ba incorporation was never significantly related to temperature in *Mytilus galloprovincialis* ($R^2 = 0.007$ to 0.259 , $p = 0.188$ to 0.803). For *Mytilus californianus*, outplanted at open-coast and bay sites from 2003 to 2009, Mn was positively related to temperature in 3 of 7 yr ($R^2 = 0.295$ to 0.779 , $p = 0.003$ to 0.045) but only one of the events (fall 2007) overlapped with the significant *Mytilus galloprovincialis* Mn–temperature relationships.

Temperature data were available for the protracted (~3 mo) spawning season of *Hypsypops rubicundus* in 2008 and 2009. The significance of pairwise correlations varied between years, but was generally opposite of the trends shown in bivalve larval shells. In 2008, Mn ($R^2 = 0.053$), Sr ($R^2 = 0.017$), and Ba ($R^2 = 0.063$) were negatively correlated with subsurface temperature (all $p < 0.01$), while U ($R^2 = 0.360$) and Pb ($R^2 = 0.026$) were positively correlated (both $p < 0.0001$). In 2009, while trends in relationships were similar, there were no significant relationships with subsurface temperature.

Relationship to salinity

Subsurface (i.e. 5 m water depth) salinity data were available for sampling sites adjacent to the Scripps Institution of Oceanography for the 3 mussel species and 1 species of fish (*Hypsypops rubicundus*), which allowed for testing of elemental relationships with salinity over time but not space. For all mussel spe-

Table 3. Relationships between trace-element incorporation in larval shells and otoliths and temperature (top, measured using data loggers and samples from each site) and salinity (bottom, measurements and samples from the vicinity of Scripps Pier only). '+' denotes a significant positive correlation, '-' a significant negative correlation, and 'ns' no significant relationship. Blank entries mean an element was not measured for that species. There were no near-La Jolla sites for *Ostrea lurida* to compare with salinity measurements at Scripps Pier. All entries include data pooled across all sample years; however, significant interannual variability existed (see 'Results' and footnotes to this table)

Element	<i>Mytilus californianus</i>	<i>Mytilus galloprovincialis</i>	<i>Musculista senhousia</i>	<i>Hypsypops rubicundus</i>	<i>Ostrea lurida</i>
Incorporation relationships with temperature by species					
Mg	ns	ns	ns	ns	ns
Mn	ns ^a	ns ^a	+	-	+
Co	ns	ns	ns		
Cu	ns	ns	ns		ns
Sr	ns	ns ^b	+	ns ^b	+
Ba	ns	ns	+	-	+
Pb	ns	ns	ns	ns ^b	ns
U	ns	ns	ns	+	ns
Incorporation relationships with salinity by species					
Mg	-	ns	+	ns	
Mn	-	+	+	+	
Co	ns	ns	ns		
Cu	-	+	ns		
Sr	-	-	+	ns	
Ba	-	+	ns	+	
Pb	-	-	ns	ns	
U	-	-	+	-	
^a 3 of 7 years showed a significant positive relationship					
^b 1 sample year showed a significant relationship					

cies, Co was never significantly related to salinity (Table 3). For *Mytilus californianus*, all other elements were negatively related to subsurface salinity when elemental data were pooled across all sample years (all $p < 0.0001$), ranging from $R^2 = 0.09$ (Ba) to 0.57 (Pb).

For the other 2 species of mussel (*Mytilus galloprovincialis* and *Musculista senhousia*), relationships with salinity were not as consistent. *Mytilus galloprovincialis* microchemistry was significantly positively related ($p < 0.05$) to salinity for Mn ($R^2 = 0.24$), Cu ($R^2 = 0.07$), and Ba ($R^2 = 0.20$), and significantly negatively related to Sr ($R^2 = 0.44$), Pb ($R^2 = 0.07$), and U ($R^2 = 0.13$) (all $p < 0.004$). *Musculista senhousia* had significant positive correlations (all $p < 0.008$) between salinity and Mg ($R^2 = 0.41$), Mn ($R^2 = 0.36$), Sr ($R^2 = 0.52$), and U ($R^2 = 0.59$).

As with subsurface seawater temperature, subsurface (i.e. 5 m) salinity data were available across the 3-mo spawning season of *Hypsypops rubicundus* in 2008 and 2009, and relationships between salinity and elemental chemistry varied among years. In 2008, 3 elements were significantly negatively corre-

lated (all $p < 0.003$) with salinity: Mg ($R^2 = 0.01$), Sr ($R^2 = 0.02$), and U ($R^2 = 0.24$). Two elements were significantly positively correlated (both $p < 0.0001$) with salinity: Mn ($R^2 = 0.06$) and Ba ($R^2 = 0.10$). However, in 2009, only Mg was significantly positively correlated with salinity ($R^2 = 0.02$, $p = 0.04$).

When trends in correlations among elemental chemistry and salinity were compared among species, generalities were few (Table 3). At most 3 of the 4 species had similar correlations between microchemistry and salinity. Relative concentrations of Mn in *Mytilus galloprovincialis*, *Musculista senhousia*, and *Hypsypops rubicundus* were positively correlated with salinity; Mn in *Mytilus californianus* was negatively correlated with subsurface salinity. In a similar fashion, 2 of the 3 mussel species (*Mytilus californianus* and *Mytilus galloprovincialis*) and *H. rubicundus* were negatively correlated with U, while relative concentrations of U in *Musculista senhousia* were positively correlated with salinity.

DISCUSSION

Variability in space

Different sets of elements were useful as natural geochemical signatures in 7 species to differentiate between bay and open-coast environments, among various bays and lagoons, within larger bays, or among sites on the open coast. Between bays and open coasts, elevated Cu, Pb, Mn, and in some cases Ba were often diagnostic for bay origin or residency (Table 2). In San Diego County, these elevated levels within bays could be partially attributed to anthropogenic sources, particularly Pb and Cu (Flegal & Sañudo-Wilhelmy 1993, Schiff et al. 2007), although the inputs of Pb may be more diffuse than Cu (Alexander & Young 1976). Elevated Mn and Ba levels in bays and lagoons may be related to increased temperature and salinity (see below, 'Variability among species').

Within the sometimes hypersaline bays of San Diego County, elevated Mn and Co were often indicators of inner-bay origins (Table 2) and may be

related to the prevalence of muddy, anoxic environments and associated redox cycles. Both of these elements were likely sourced from weathering processes on land, and have been shown to be highly correlated with salinity in the Northeast Pacific (Knauer et al. 1982). Volpe & Esser (2002) found a uniform increase in salinity and Mn concentration in surface water as they moved inward from the mouth of San Diego Bay. Several metals are in higher concentrations in the sediments of inner San Diego Bay (Deheyn & Latz 2006), although that study finds that bioavailability of those metals may be similar throughout the bay.

When comparing entire bays and lagoons to each other, Mn, Pb, Cu, and U were often useful in discrimination. Anthropogenic pollution in large, urbanized bays with thousands of military and civilian vessels such as San Diego Bay (Schiff et al. 2007) may be the source for increased Cu and Pb there compared to less-urbanized lagoons. Other elemental trends, such as consistently higher U in Mission Bay shells and otoliths (Fig. 2), could not be explained by obvious sources. U is a conservative element and might be expected to vary with salinity (Borole et al. 1982); however, a positive salinity–U incorporation relationship was detected only for *Musculista senhousia*. U can also vary among watersheds due to the weathering of different source rock minerals (Rengarajan et al. 2006). However, it reaches the ocean primarily from river and groundwater inputs (Dunk et al. 2002), neither of which are plentiful in Mission Bay.

Along the open coast, Mn, Ba, Cu, and Pb were most useful in distinguishing among sites or regions. Elevated Cu and Pb in the south region may be due to exchange with Mission Bay or San Diego Bay (Volpe & Esser 2002, Chadwick et al. 2004). Bioavailability of sediment-sourced metals at the mouths of bays may increase due to turbulent exchange (Deheyn & Latz 2006). Mn and Ba levels in the south region were not apparently influenced by the large bays, however, and instead were elevated more often in the individuals from northern sites (Fig. 2).

Other mechanisms exist to explain the observed spatial variation in element incorporation, aside from the aforementioned anthropogenic inputs and relationships to temperature and salinity, which are discussed in ‘Variability among species’. These include variation in upwelling intensity along the coast (Fodrie et al. 2011, Send & Nam 2012), variable shelf width (Fig. 1), and differences in the land-use and bedrock geology of county watersheds (Kennedy & Peterson 1975), although precipitation and run-off are generally low. Spatial variation in biological pro-

cesses such as phytoplankton blooms (Vander Putten et al. 2000) or growth rate and maternal effects can affect trace-elemental incorporation (Lloyd et al. 2008), although for most elements, physical factors such as temperature and salinity may overwhelm them (Beer et al. 2011). Genetic differences have been shown to influence otolith chemistry (Clarke et al. 2011) although the larval exchange detected (summarized in López-Duarte et al. 2012) for these species is likely to negate significant genetic differentiation at this spatial scale (barring strong selection within cohorts).

In general, we did not detect consistent differences in the variability of element-to-Ca ratios at particular sites in the *Mytilus* spp. data. We also did not detect strong relationships between similarity of multi-element signatures and proximity of sites in any species. The lack of a strong similarity–distance relationship may be due to the complexity of the San Diego Coast circulation, which is sensitive to eddies, wind reversals, upwelling events, El Niño Southern Oscillation (ENSO), and other climate influences (Rasmussen et al. 2009). The complex shoreline, which is punctuated by a number of submarine canyons, bays, and lagoons of varying size, and kelp forests, also influences inshore circulation patterns and land–river–rock coastal interactions. A small but significant distance–element similarity relationship was detected in the mytilid time series, which suggests that similar trends might have emerged in the other 5 species, had more data been available. Also, expressing the connection between sites using realized oceanographic distances, as did Watson et al. (2011), instead of using simple geographic distances, may improve the ability to explain the spatial variability of larval chemistry.

Variability in time

For each of the 8 elements detected in *Mytilus* spp. larval shells, the mean CV within a site across sample years (time) was greater than that among sites in the same year (space) (Fig. 3). This result emphasizes the need for geochemical signature studies to resample possible source populations during each season or year. One important caveat, however, is that most analysis artifacts are incorporated into the variation among years rather than within them. In general, samples from one event are analyzed together, whereas other events may be analyzed years later. Standards run with samples can help compare among studies, and all *Mytilus* spp. larval shells were

run under similar protocols. However, it is impossible to completely control and quantify possible instrumental or environmental artifacts that may influence the variability detected among sample years (Campana et al. 1997).

Despite the high temporal variability, certain sites and regions remained distinct from all others in multivariate space over a period of years, especially in bay habitats (Fig. 4). These relatively stable signatures are most useful for species limited to bays, without potentially confounding source populations on the open coast. The fact that bay signatures varied considerably while still remaining distinct suggests that sites are defined by multivariate signatures that rise and fall together. On the open coast, however, year-to-year variation generally scrambled the spatial structure of these signatures.

Comparing among elements, Mg and Sr ratios to Ca had relatively low CVs (<1) in both space and time (Fig. 3). These are both conservative elements in seawater (along with U), and most abundant in shell, so their relative stability is not surprising. In time, 4 elements (Cu, Ba, Pb, and U) had coefficients of approximately 1, where the standard deviation equals the mean. In space, only Mn had significantly higher variability in *Mytilus* spp. larval shells than the other non-conservative elements. Since Mn is often released from sediments, the depth of water or resuspension forces may increase variability. This variability may contribute to the importance of Mn in discrimination within and among all habitat categories examined here. Mg and Sr, by contrast, were less variable and less valuable in discrimination, except in some fish otoliths (Fig. 5).

The evidence for short-term (weeks) stability in chemical signatures from *Mytilus* spp. (Becker et al. 2005, Fodrie et al. 2011) and *Hypsypops rubicundus* (Cook 2011) could be instrumental to the success of geochemical signature studies when it is difficult to determine an accurate PLD or age of recent recruits. However, the finding that signatures can rapidly shift to a new, stable state means that studies that span such a shift without detecting it may improperly assign recruit origins. Estuaries have been shown to have variability in the amounts of Ba and Mn over weeks, days, and even tidal cycles (Elsdon & Gillanders 2006).

Variability among species

The 3 bivalves that inhabit bays and lagoons incorporated the 8 elements in similar ways, allowing dis-

crimination among sites, whereas in the halibut, Pb and U were less useful, and Co, Sr, and Ba more useful, in discriminating the locations of otoliths (Fig. 5). There are many interspecific differences that could account for this discrepancy (see Swearer et al. 2003), including formation processes for otoliths compared to larval shells. Chief among the differences, perhaps, is habitat. Larval bivalves are either brooded within adults attached to intertidal rock (*Ostrea lurida*) or free-spawned into the water (*Mytilus* spp). Juvenile halibut, on the other hand, are likely to be located near or in contact with bottom sediments throughout development, where metals are more bioavailable (Deheyn & Latz 2006). Habitat-use patterns may have contributed to observed differences in how the elements were used in discrimination on the open coast, such as increased use of Sr in the fish otoliths (Fig. 5). Pre-dispersal garibaldi embryos develop on red algal benthic nests on hard-bottom substrate, unlike halibut juveniles. The mytilid larvae were located in the water column on subsurface moorings.

Studies often yield different relationships between temperature or salinity and the incorporation of trace elements into biogenic calcium carbonate, alternately finding a positive, negative, or no relationship (cf. Martin & Wuenschel 2006). Here we present evidence from multiple bivalve species of a positive relationship between the incorporation of Mn, Ba, and Sr and temperature, although the relationship was not detected in every year of sampling. For the temperate damselfish, Ba was negatively correlated with temperature.

A positive Mn–temperature relationship found here in all 4 bivalves is consistent with an increase in partition coefficient with temperature observed in the larval softshell clam *Mya arenaria* (Strasser et al. 2008) and the juvenile spot *Leiostomus xanthurus* (Martin & Thorrold 2005), although the Mn:Ca ratio did not increase with increasing temperature in spot otoliths. No relationship was detected in gray snapper *Lutjanus griseus* (Martin & Wuenschel 2006), black rockfish *Sebastes melanops* (Miller 2009), black bream *Acanthopagrus butcheri* (Elsdon & Gillanders 2002), or Kellet's whelk *Kelletia kelletii* (Lloyd et al. 2008).

Higher Ba:Ca ratios were found in larval shells at increased temperatures for the oyster and Asian mussel, but this was not observed in the mytilid data, and the Ba:Ca ratio was lower in the garibaldi. Other studies with larval or adult molluscs have shown a negative relationship between Ba and temperature (Zumholz et al. 2007, Lloyd et al. 2008, Strasser et al.

2008). Increased Ba:Ca ratios with temperature have been found in some fish otoliths (Elsdon & Gillanders 2002, Miller 2009), but others have detected a negative (DiMaria et al. 2010) or no relationship (Martin & Thorrold 2005, Martin & Wuenschel 2006).

There is evidence for a positive relationship between Sr:Ca ratio and temperature in *Olympia* oyster, bay mussel, and Asian mussel larval shells, but only in a few years. Dodd (1965) found a positive relationship between Sr and temperature in adult mytilid mussel shell, although the differing crystal structure between larval and adult shell makes comparisons difficult. Negative relationships have been detected in other molluscs, such as the common squid *Todarodes pacificus* (Ikeda et al. 1998) and Kellet's whelk *Sepia officinalis* (Lloyd et al. 2008). No relationship was found in the common cuttlefish *Sepia officinalis* (Zumholz et al. 2007), jumbo flying squid *Dosidicus gigas* (Ikeda et al. 2002), or softshell clam (Strasser et al. 2008). Fish otolith studies also provide mixed results on Sr, with both positive (e.g. Elsdon & Gillanders 2002) and negative (e.g. DiMaria et al. 2010) Sr-temperature relationships detected. The garibaldi otoliths showed a negative relationship one year, and none the other.

Salinity can be even more influential on trace-element incorporation than temperature, as can temperature-salinity interactive effects (Elsdon & Gillanders 2002). Salinity has been shown to affect incorporation of nearly all the elements considered here in one species or another, in studies including both molluscs (e.g. Beer et al. 2011) and fish (e.g. Martin & Wuenschel 2006). Unfortunately, we did not have access to fine-scale, spatially explicit salinity data with which to examine the relationships between salinity and shell or otolith chemistry across our study area. From our more limited exploration of correlations between elemental chemistry and subsurface salinity at sampling sites near La Jolla, results support the idea that salinity effects on incorporation are also species-specific. The trends observed across 4 species (3 mussels and 1 fish) indicate that generalities are few; at best 3 of the 4 species had similar relationships between a given element and salinity (Table 3). The lack of consistent trends in interspecific relationships between microchemistry and salinity may be a result of the species-specific physiological processes responsible for the incorporation of trace elements into calcareous structures. To gain a mechanistic understanding of how this process actually occurs will require further laboratory-based studies (Martin et al. 2004).

CONCLUSIONS

Different sets of elements discriminate among biogenic structures collected at open-coast sites, bay or lagoon sites, or a combination of both. Some elemental discrimination patterns are, however, often shared across fish and invertebrate species. Elements such as Mn, useful in discrimination among and within all San Diego County habitats tested, can be highly variable in both space and time. In contrast, elements such as Mg and Sr are less variable and generally less useful in natural tagging studies, although they still often add to discriminatory power. In mytilid mussels, all 8 elements were more variable over time than they were in space (Fig. 3), highlighting the need to continue to resample the chemistry of source populations during each new tagging study. This is an important finding for future studies, considering the great expense and effort needed to measure larval dispersal via the use of geochemical signatures. However, in general, the bays and lagoons had sufficiently distinct chemical characteristics so that they each occupied separate multivariate space despite interannual variability (Fig. 4).

The ability to differentiate source populations for 7 species of larvae or juveniles in San Diego County is most likely due to a combination of anthropogenic pollution (Cu, Pb) in large bays and their influence on nearby coastlines, elevated levels of elements from terrestrial runoff (Mn, Co, U) and their retention in certain areas, and elements that may be incorporated differently in response to temperature (Ba, Mn, Sr, U) and salinity (all except Co) shifts throughout the county. Although some consistent relationships between temperature and trace-element incorporation among bivalves emerged, they were not identified in every year. Access to fine-scale salinity data to match with shell or otolith chemistry would add to our understanding of the mechanisms controlling trace-element variation in these structures. Direct sampling of seawater chemistry during geochemical signature studies, and further controlled laboratory experiments, could also aid our understanding of incorporation mechanisms.

Trends uncovered here did not always agree with past studies. This suggests that there are species-specific mechanisms by which fishes and invertebrates incorporate trace elements into their otoliths, statoliths, exoskeletons, and shells. Findings here will aid in targeting important elements for tracking the movement of larvae of additional species depending on their habitat and life history. However, it is unlikely that the source population chemistry for

one species can be used to track the larvae of another species. In future studies, geochemical signatures should be combined with other methods such as hydrodynamic modeling and population genetics to gain a more complete understanding of marine population connectivity.

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