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Development and application of elemental fingerprinting to track the dispersal of marine invertebrate larvae

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Abstract

The early life history of many marine benthic invertebrate and fish species involves a planktonic larval stage that allows exchange of individuals among separated adult populations. Here, we demonstrate how natural and anthropogenic trace elements can be used to determine larval origins and assess bay–ocean exchange of invertebrate larvae. Trace elements can be effective site markers for estuaries because run-off and pollutant loading often impart distinct elemental signatures to bay habitats relative to nearshore coastal environments. Crab larvae originating from San Diego Bay (SDB) were distinguished from those originating in neighboring embayments and exposed coastal habitats by comparing multiple trace-element concentrations (''fingerprints'') in individuals. Discriminant function analysis (DFA) was used to characterize stage I zoeae of the striped shore crab, *Pachygrapsus crassipes*, of known origin (reference larvae) via trace-elemental composition (i.e., Cu, Zn, Mn, Sr, Ca). Linear discriminant functions were used to identify the origin and characterize the exchange of stage I *P. crassipes* zoeae between SDB and the nearshore coastal environment during one spring tidal cycle. Elemental fingerprinting revealed that most (87%) of the stage I larvae collected at the bay entrance during the flood tide were larvae of SDB origin that were reentering the bay. Nearly one third of zoeae sampled (32%) at the entrance during ebb tide were coastal larvae leaving the bay and returning to open water. The observed bidirectional exchange contrasts with the unidirectional transport of zoeae out of the bay predicted from stage I vertical migratory behavior. Because *P. crassipes* zoeal survivorship is lower in SDB than in coastal waters, bay–ocean exchange has significant implications for the dynamics of *P. crassipes* populations. Trace-elemental fingerprinting of invertebrate larvae promises to facilitate investigations of many previously intractable questions about larval transport and dynamics.

The early life history for many nearshore and estuarine benthic invertebrate species involves the release of a planktonic larval stage (i.e., meroplankton) which acts as an agent of dispersal and gene flow (Grahame and Branch 1985; Levin and Bridges 1995). The distribution and abundance of marine benthic populations often is determined by events that take place during planktonic stages of development (Gaines et al. 1985; Roughgarden et al. 1988). Initial interest in the influence of planktonic larvae on population dynamics arose from the need to effectively manage declining commercial fish and shellfish invertebrate stocks. A detailed understanding of dispersal trajectories and the magnitude of larval transport is fundamental to understanding the complexities and fate of larvae in the plankton, including the sources and rates of mortality (Rumrill 1990; Morgan 1995) and the origin of recruits (Levin 1990). However, the lack of effective methods to follow meroplankton or to determine their origin once sampled from the plankton has limited the successful tracking of larvae in situ (Levin 1990; Young 1990).

Many temperate marine benthic invertebrate species inhabiting estuarine or exposed coastal environments produce dispersing planktonic larvae. In some species, larval development occurs entirely in coastal waters or within embayments. For others, the life history may involve larval development in both open-water and bay settings. The ability of many of these larvae to exit or return to estuarine habitats has been considered critical for survival (Morgan 1995). This is the case for estuarine species that release planktonic larvae that develop offshore but recruit to adult populations located within estuaries (Epifanio 1988). For species which have adult populations inhabiting both bay and coastal settings, exchange of larvae between these two environments is likely and may have significant consequences for population maintenance (Gaines and Bertness 1992).

The study of larval exchange between bay and coastal habitats has, until recently, involved traditional methods of sampling such as net tows, pump samples, settlement collectors, or larval mimics (e.g., drift tubes; Levin 1983). Such techniques require that scientists infer potential spawning sites and dispersal trajectories from physical oceanographic data (i.e., current directions and speed) coupled with descriptions of larval behavior. For example, studies evaluating the exchange of crab larvae between embayments and coastal habitats have examined larval abundance and vertical distribution patterns in relation to tidal phase (Christy 1978; Epifanio 1988). Further, the determination of bay–ocean flux rates has been restricted to species and larval stages of development that are assumed only to migrate into or out of

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embayments during specified stages of larval development (Lago 1993; Rowe and Epifanio 1994). In the case of organisms with widespread distributions in both bay and exposed coastal habitats, some sort of marker that identifies larval origin is required to characterize bay–ocean exchange. Gaines and Bertness (1992) were able to distinguish newly settled barnacle recruits that had originated as bay-spawned versus coastal-spawned individuals based on size at recruitment. The ability to distinguish larvae originating from bay versus coastal populations allowed them to determine that bay flushing rates influenced recruitment on the outer coast. Here, we demonstrate the use of elemental concentrations to distinguish larval origins and evaluate bay–ocean exchange in decapod larvae.

A variety of methods have been proposed for tagging and tracking marine larvae, including the use of trace elements and isotopes (Levin 1990; Ennevor and Beames 1993; Levin et al. 1993; Kennedy et al. 1997; Anastasia et al. 1998). However, because of difficulties with diffusion and limited larval recovery in the field, such mark–release–recapture methods have not been used extensively. An environmentally induced elemental tag eliminates laboratory manipulations of larvae that could alter natural larval behavior, and avoids problems of diffusion associated with release–recapture methods. Such tags have been developed for fishes (e.g., Edmonds et al. 1989; Campana et al. 1995*a*), but not invertebrates.

In recent years, variations in the trace-element composition of fish otoliths (elemental fingerprints) have been used to discriminate commercial fish stocks (Edmonds et al. 1989, 1991, 1992, 1995; Campana et al. 1994, 1995*a*,*b*; Edmonds and Fletcher 1997), infer migration pathways and environmental conditions experienced by fishes (Gillanders and Kingsford 1996; Secor and Piccoli 1996; Thorrold et al. 1997), and track dispersal trajectories in species with anadromous life-history strategies (Kalish 1990; Secor 1992; Secor et al. 1995; Halden et al. 1995; Thorrold et al. 1998). Trace elements incorporated into the otolith reflect varying physical and chemical characteristics of the ambient seawater (Thorrold et al. 1997). Fish populations growing up in different water masses have been observed to produce otoliths of distinct elemental composition (e.g., Edmonds et al. 1989; Campana et al. 1995*b*). Many marine invertebrate larvae lack calcareous structures equivalent to otoliths. Here, we present the first application of trace-elemental fingerprinting to study the dispersal of marine invertebrate larvae with calcified exoskeletons. Our target taxon was the Decapoda.

The purpose of this study was to develop and test the use of naturally-occurring trace elements and pollutants as viable tags to distinguish origins of planktonic larvae and to use this method to examine bay–ocean exchange processes in the striped shore crab, *Pachygrapsus crassipes* (Grapsidae, Randall 1840). In this paper we: (1) describe an analytical protocol to measure trace-element concentrations in individual crab larvae, (2) characterize the elemental composition of individual *P. crassipes* larvae sampled from embayments and exposed coastal habitats of southern California, (3) test the discriminatory power of observed elemental differences as a tag to determine larval origin, and (4) apply this approach to examine the exchange of stage I zoeae between San Diego Bay (SDB) and nearshore coastal habitats. Based on observations of vertical migration of larvae into the water column during ebb tide and onto the bottom at flood tide, we initially hypothesized that transport of stage I zoeae originating inside SDB should be unidirectional and out of the Bay.

Methods

Target organism, study site, and sampling—Pachygrapsus crassipes was selected for this study because adults and larvae are widely distributed in both bay and exposed-coastal habitats, their spawning cycle is predictable, and larvae are easily sampled and identified (Schlotterbeck 1976). The relatively large size of stage I *P. crassipes* larvae (500-μm carapace length) facilitates elemental analysis of single individuals. *P. crassipes* provides a suitable model for other relatively strong swimming larvae that potentially disperse in the coastal zone. The study was conducted in SDB because trace elements there have been enriched through industrial, commercial, and military activities and by way of rainwater runoff from extensive urban development in the SDB watershed (McCain et al. 1992; Flegal and Sañudo-Wilhelmy 1993; SWRCB 1996). Similar enrichments occur in other heavily urbanized embayments (Kennish 1992).

To examine trace-element concentrations in stage I *P. crassipes* larvae of known origin (reference samples) and to develop fingerprinting algorithms, larvae were collected from seven locations along an axial transect of SDB, five neighboring embayments, and three San Diego coastal sites (Fig. 1). All reference zoeae sampled in this study and used to determine trace-elemental fingerprints were collected over a three-month period (12 June 1997 to 15 September 1997). This ensured that inter-annual variations in elemental fingerprints will not confound results presented. Larval collections were made with a plankton net (250-μm Nitex mesh) towed at approximately 1 m sec^{-1} for 20 min during the nocturnal transition from flood to ebb tide when *P. crassipes* spawns en masse and stage I larvae can be assumed to have originated near the sampling site. Samples were collected within 50 m of the shoreline to ensure that larvae spawned from targeted intertidal areas were sampled. No attempt was made to quantify the concentration of zoeae sampled.

The trace-element concentration of newly-hatched *P. crassipes* zoeae, spawned under laboratory conditions, were compared to concentration levels in field-spawned stage I zoeae. Ovigerous females, collected from inner SDB (i.e., Sweetwater Marsh) and coastal sites (i.e., Dike Rock, Bird Rock, Sunset Cliffs) between 25 June 1997 and 28 August 1997, were transported back to the laboratory in plastic coolers containing local, ambient temperature seawater within 1–2 hours of collection (Fig. 1). Females were transferred to a temperature controlled culture room (20–22°C) and held on a 14 : 10 h, light : dark schedule simulating in situ conditions. Each ovigerous female was placed into an acid-washed, high-density polyethylene bowl (4-L) filled with 5-μm filtered seawater collected at the same sites and times as crabs. All containers were aerated using acid-washed, glass-bonded

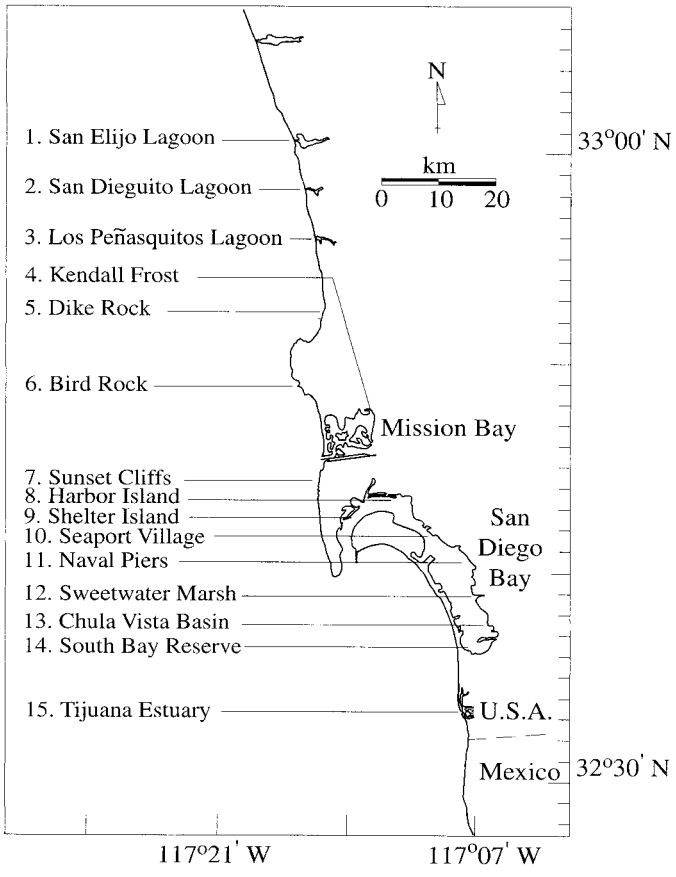

Fig. 1. Map showing sampling locations of stage I *Pachygrapsus crassipes* zoea in southern California embayments and exposed coastal habitat.

air diffusers and standard aquarium air pumps. Ovigerous females were not fed. Zoeae hatching within 24 h of the female collection date were subjected to trace-element analysis.

To study bay–ocean exchange, depth-stratified samples of larvae were collected at one station not previously sampled on the Western side of the channel at the entrance of SDB over an 18-h period (18 August, 1700 h [flood tide, 1700 h to 2227 h] to 19 August 1997, 1100 h [ebb tide 2227 h to 0520 h]). The nighttime tidal cycle was targeted since preliminary field investigations indicated stage I *P. crassipes* zoeae were present in the water column mainly at night (unpubl. data). Samples were collected hourly at three depths; surface (0–2 m), mid-depth (5–7 m) and in the bottom boundary layer (<1 m above the bottom, including the sediment-water interface). A stainless steel pump (0.5 horsepower, 115 V motor), powered by a portable electric generator and fitted with a vortex impeller to minimize damage to larvae, was used to sample consecutive ebbing and flooding tides. Seawater was pumped through a partially submerged 250-μm plankton net to prevent damage to organisms during pump outflow. Plankton samples were concentrated, then stored on ice during transport to the laboratory where they were held at 5°C. To obtain estimates of larval abundance and for elemental analyses, samples were either sorted for *P. crassipes* larvae within 24 h or were concentrated onto acid-washed Teflon filters (2-inch diameter, 250-μm grade mesh), placed in 50-ml centrifuge tubes, stored in liquid nitrogen, and thawed just prior to sorting. *P. crassipes* zoeae were sorted and enumerated with the aid of a dissecting microscope. Zoeae were rinsed with Milli-Q water (deionized water which has been purified further through reverse osmosis and filtered through carbon and Millipore filters) and individually preserved (liquid nitrogen) in acid washed polycarbonate vials until trace-elemental analysis.

Trace-elemental analysis—The trace-element composition of individual organisms or portions of organisms (i.e., otoliths) are usually standardized by weight. Although elemental composition can be determined for single larvae, individual stage I *P. crassipes* zoeae are too small to be weighed accurately. Therefore, 25 to 50 individuals were pooled to estimate weight from a given site and to test for site differences in the estimated mean weight for individual stage I zoea. At least 15 replicate weight measurements were made for larvae at each site. Larvae were individually picked from plankton samples, transferred onto predried and preweighed weighing paper, allowed to dry for 2 hours at 60°C, and allowed to cool in a dessicator for 4 h prior to reweighing to the nearest microgram on a microbalance (Perkin-Elmer, model AD-6).

Elemental analyses of larvae were carried out in solution. Individually preserved larvae were thawed, rinsed with Milli-Q and transferred to 500-μl Teflon digestion vessels (one zoea per vessel). Zoeae were dried for 24 h in an oven at approximately 50°C. Vials were allowed to cool in a desiccator for 3–4 h then larvae were dissolved by adding 5 μl of 15 N nitric acid (HNO_3). Teflon digestion vessels were capped and simultaneously heated (35–40°C) and sonicated for 20–30 min to ensure that larvae were completely digested. Once the larva was in solution, each Teflon vessel was brought to a final volume of 100 μl by adding Milli-Q. Vessels were resealed, shaken, and resonicated to ensure samples were homogenized prior to trace-element analysis. Preliminary tests showed no evidence of evaporative loss from the digestion vessels used, a concern when dealing with such small volumes. Blank vials and internal standards (*see below*) were treated identically, but contained no zoeae.

Characterization of trace-element composition in individual *P. crassipes* larvae was carried out with an inductively coupled plasma-atomic emission spectrometer (ICP-AES; Perkin-Elmer, Optima 3000XL) with an axial viewed plasma for increased sensitivity and detection limits. Four to six emission wavelengths were scanned for each element (Table 1). Element-specific wavelengths without interference from other wavelengths and with the highest sensitivity and detection limits were used to measure trace-element concentrations. For most elements, results from several wavelengths were averaged to obtain trace-element concentrations (Table 1). External standards were used to calibrate the instrument at the beginning of each analytical session. A blank (1% HNO_3) and 4–5 standard solutions spanning expected concentration ranges (0, 25, 50, 100, and 200 μg kg^{-1} for the Al, Cu, Sr, Mn, and Zn matrix; 0, 1, 5, 10, and 20 μg g^{-1} for the Ca and Mg matrix) were run at the start of each

Table 1. Element specific inductively coupled plasma-atomic emission spectrometer (ICP-AES) emission wavelengths employed in this study. Corresponding coefficients of variation (%) and detection limits (3σ of background intensity for the wavelength measured) are given.

Element	ICP-AES emission wavelength	Coefficients of variation (%)	Detection limits (μg kg^{-1})
Aluminum	396.192	3.68	2.251
Copper	213.598	2.54	1.017
	324.754	2.48	1.226
	327.396	2.87	2.092
Zinc	202.548	1.37	0.364
	206.200	1.36	0.385
	213.856	1.55	0.596
Magnesium	279.079	2.57	15.269
	279.553	3.07	11.782
	280.270	2.79	9.962
	285.213	2.56	9.408
Manganese	257.610	1.51	0.709
	260.569	1.71	3.170
	294.920	1.76	0.772
Strontium	407.771	1.85	0.333
	421.552	1.88	0.311
	460.733	3.27	0.910
Calcium	315.887	2.69	8.419
	317.933	3.46	10.334
	422.673	2.70	11.631

analytical session. The blank intensity was subtracted from standard and sample intensities to obtain net intensities and concentrations. Blanks spiked with defined concentrations of standards were used to monitor instrument drift every 5–10 samples (15–30 min of instrument operation time). Trace elements which were included in preliminary analyses, but were not consistently detectable in individual stage I *P. crassipes* zoea, included Ag, Ba, Cd, Cr, Hg, Ni, Pb, Se, Sn, and Ti. Subsequent analyses focused on Al, Cu, Zn, Mn, Sr, Mg, and Ca.

Statistical analyses—One factor analyses of variance (ANOVA) and a posteriori Student *t* tests were used to determine significant differences in trace-element composition of zoeae sampled from the inner and outer regions of SDB, neighboring embayments and coastal sites. Student *t* alpha levels were Bonferroni adjusted to reduce the likelihood of Type I errors. A linear discriminant function analysis (DFA) was performed on larval trace-elemental concentration data and ratios calculated from key elemental concentrations (e.g., Cu/Mg) to develop a classification model which would discriminate those larvae originating in SDB from those from neighboring embayments, coastal sites or both. The classification algorithms were validated using a jackknife routine to test the model's accuracy and robustness (Manly 1986). Zoeae of unknown origin, sampled at the entrance of SDB, were categorized by linear discriminant functions as having originated from either SDB populations or from populations outside SDB, which included neighboring embayments or exposed coastal habitats. Trace elemental data were log transformed to standardize data for homogeneity of variance.

Results

Instrument sensitivity and precision—Seven of 17 elements analyzed in individual *P. crassipes* zoeae were found at concentrations above detection limits of the ICP-AES (Table 1). Detection limits for element-specific emission wavelengths were defined as three times the standard deviation (in μg kg^{-1}) of the background intensity. Coefficients of variation (%; i.e., % relative standard deviations) for replicate assays were used to test and monitor ICP-AES precision for element-specific emission wavelengths. The coefficients of variation were found to be less than 4% for all emission wavelengths used in this study (Table 1).

Trace-element concentrations of individual larvae—The effect of individual zoeal weight on elemental concentration is preferably tested by regression analysis (Campana et al. 1995*b*). Since individual stage I zoeae can not be accurately weighed, this analytical approach was not possible in this study. The potential effect of larval size on measured trace-element composition at each sampling location was evaluated by comparing larval weights. There was no statistical difference observed in the estimated weight of individual stage I zoeae collected from SDB (0.011 ± 0.004 mg, [mean $\pm$ SD], $N = 15$), neighboring embayments (0.015 ± 0.005 mg, $N = 25$), or coastal sites (0.012 ± 0.009 mg, $N = 23$; one-factor ANOVA, $F_{2,60} = 1.332$, $P = 0.272$), suggesting that observed differences in the elemental composition of individual larvae analyzed in this study were not attributable to between-site variations in individual zoeal weight.

Stage I *P. crassipes* zoeae sampled from inner and outer SDB, neighboring embayments, and coastal sites exhibited differences in trace-elemental composition (Table 2). On average, Cu, Zn, Al, and Mn concentrations were higher in zoeae sampled from SDB than neighboring embayments or coastal sites (Table 2). Copper concentrations were higher in larvae sampled from inner than outer SDB. Magnesium concentrations in individual zoeae were higher outside than inside SDB, while strontium and calcium concentrations varied little among sites (Table 2).

The elemental concentration of laboratory hatched, stage I *P. crassipes* zoeae spawned from ovigerous females collected from inner SDB and exposed coastal sites were comparable to field-sampled larvae from the same locations (Table 2). Copper and aluminum follow the same gradients observed in field-sampled larvae, while both Sr and Ca revealed larger differences between sites in laboratory-hatched larvae than field-sampled larvae (Table 2).

Differentiating larvae by their elemental fingerprints—DFA resulted in one significant algorithm that effectively distinguished between larvae originating from inside and outside SDB (reference samples) based on the multiple elemental composition of individual zoeae (Fig. 2A). The first of two canonical variables captured most of the observed difference (98%) among groups, effectively discriminating between larvae originating from SDB versus sites outside of

Table 2. Mean (and SD) elemental concentrations (μg kg^{-1}) of stage I *Pachygrapsus crassipes* reference zoea sampled from inner and outer regions of San Diego Bay (SDB), neighboring embayments and coastal sites (*see Fig. 1*). One factor analysis of variance (ANOVAs) was used to determine significant differences among sites. Concentrations for laboratory hatched stage I *P. crassipes* zoeae are also presented. All mean estimates are based on $N = 96$ individuals for reference zoea and $N = 6$ to 12 individuals for laboratory-hatched zoea. *A posteriori* multiple comparison results for reference zoeae (^{a,b} and ^{c}) and laboratory hatched zoeae (^{x} and ^{y}) are presented as superscripted letters next to mean estimates. Mean estimates for reference larvae which share a letter are not statistically different at the Bonferroni corrected significance level ($P = 0.017$) for *a posteriori* multiple comparisons. Sampling sites pooled within each region are indicated by numbers in parentheses that correspond to numbered sites in Fig. 1.

	Reference zoeae						Laboratory hatched zoeae		
	Inner SDB (11–14)	Outer SDB (8–10)	Neighboring embayments (1–4, 15)	Coastal sites (5–7)	ANOVA $F_{3,92}$	ANOVA P	Inner SDB (12, 14)	Coastal sites (5–7)	t-test
Aluminum	34.1^{a} (23.6)	12.3^{a,b} (7.5)	12.3^{b,c} (10.6)	8.9^{c} (5.7)	17.34	$P < 0.001$	24.9^{x} (10.2)	8.8^{y} (3.5)	$P < 0.001$
Copper	23.5^{a} (29.1)	6.0^{b} (8.8)	1.7^{b} (2.2)	1.0^{b} (0.8)	12.51	$P < 0.001$	27.5^{x} (28.1)	1.8^{y} (0.6)	$P = 0.029$
Zinc	25.8^{a} (17.7)	18.1^{a} (13.0)	7.3^{b} (6.6)	3.4^{b} (2.6)	21.53	$P < 0.001$	—	—	—
Strontium	132.1^{a,b} (82.2)	75.9^{a} (77.3)	99.1^{a,b} (57.2)	14.5^{b} (103.1)	3.12	$P = 0.030$	93.4^{x} (23.7)	181.6^{y} (54.1)	$P = 0.046$
Manganese	10.4^{a} (8.2)	8.5^{a} (7.3)	1.2^{b} (0.9)	1.0^{b} (1.0)	22.33	$P = 0.001$	—	—	—
Magnesium	1700.9^{a} (1203.0)	2057.6^{a,b} (1087.1)	3632.0^{a,b} (3345)	5699.3^{b} (7198.6)	3.96	$P = 0.011$	1094.4 (428.5)	—	—
Calcium	11011.5^{a} (5453.6)	13016.2^{a} (5076.1)	8706.5^{a} (5625.7)	10690.2^{a} (5342.8)	2.21	$P = 0.092$	8701.1^{x} (4665.7)	12504.9^{x} (2250.2)	$P = 0.072$

SDB (Fig. 2A). The second canonical variable did not add significant discriminatory power, but was employed to plot reference and unknown larvae in two-dimensional space (Fig. 2A). The DFA included only five of the seven detectable trace elements (Table 3): Mn, Cu, Zn, Sr, and Ca. The Al and Mg contributions were negligible and were not included in the linear discriminant functions. DFAs did not accurately discriminate between larvae originating from inner versus outer regions of SDB or between larvae originating from neighboring embayments versus coastal sites.

A validation of the classification algorithms indicated that 93% of SDB larvae ($N = 39$ larvae) and 96% of non-SDB larvae ($N = 57$ larvae) were correctly identified (Table 4A). This classification algorithm is biased toward allocation of individual larvae to their site of origin, since the same observations were used to create the classification algorithm. To test this bias we carried out a jackknife classification of sampled larvae, allocating each individual to its closest sampling site without using that individual to calculate the classification algorithm (Manly 1986). The jackknife classification algorithm correctly identified 90% of SDB larvae and 95% of non-SDB larvae (Table 4B). The results of both validations confirm the ability of the classification algorithm to predict whether larvae sampled in our study area originated from SDB or outside of SDB.

In addition to multiple trace-element concentrations for individual larvae, the discriminatory power of various elemental ratios was also tested. Most ratios tested (e.g., Al : Mg, Cu : Mg, Zn : Mg, Sr : Mg, Mn : Mg, Sr : Ca, Mg : Ca) had no effect on ''classification matrix'' or ''jackknifed classification matrix'' results (Table 4). The Mn : Ca ratio improved the ''classification matrix'' results, but decreased the ''jackknifed classification matrix'' results. Since the jackknifed classification algorithms represent a more rigorous test of the predictability of classification algorithms, the Mn : Ca ratio was not used.

Bay–ocean exchange of stage I zoeae—Trace-element fingerprinting and the discriminant functions developed from reference samples were used to identify the origins of larvae sampled at the entrance of SDB and to describe the potential for exchange of stage I *P. crassipes* zoeae between SDB and the nearshore coastal environment. Once discriminant functions have been generated from a data set of reference samples, these algorithms can be used to classify any new data point (zoeae of unknown origin, unknown samples) into one of the groups. Any point in the discriminant function space will be classified regardless of where it lies, even if it is located well outside well-defined clusters (Fig. 2A). To determine the reliability of our classification algorithms, we have plotted DF1 and DF2 values calculated for larvae sampled from the mouth of SDB (Fig. 2B) in the same discriminant function space defined by the reference samples (Fig. 2A). The points of unknown larvae (Fig 2B) fall neatly within the two clusters of SDB and non-SDB larvae plotted in Fig. 2A. This result suggests that we have captured the natural variability in our sampling regions, including SDB, neighboring embayments, and coastal sites, and that DF1 is effectively discriminating SDB and non-SDB zoeae on the basis of trace-element composition.

The majority (>81%) of stage I *P. crassipes* zoeae sampled at the entrance of SDB during nocturnal ebbing tides

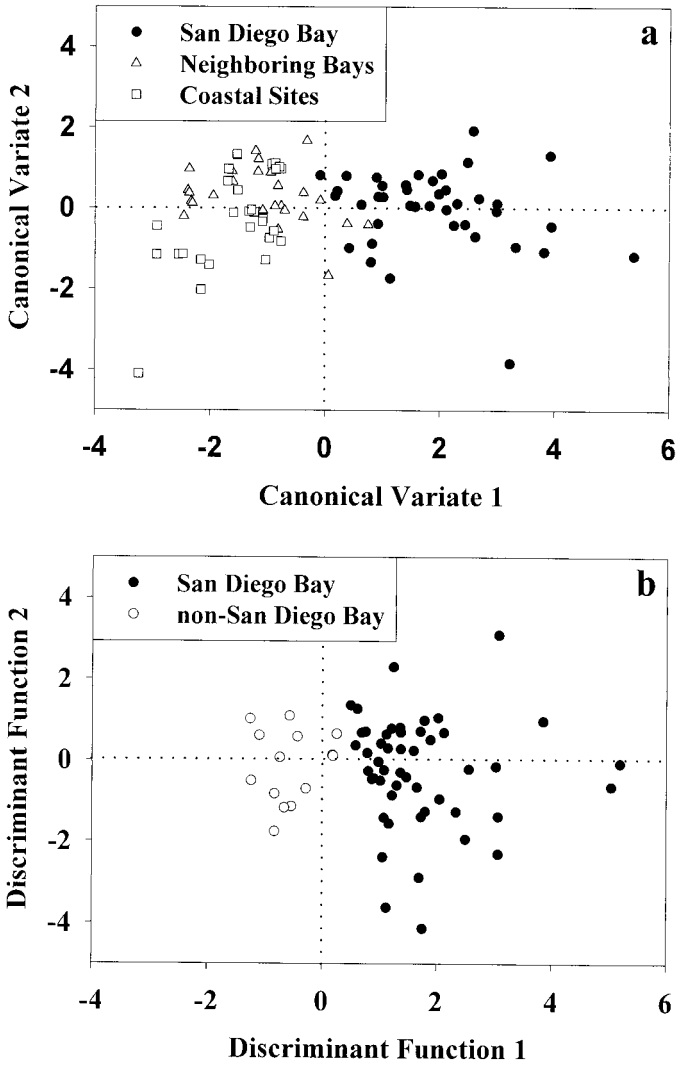

Fig. 2. Trace elemental fingerprints characteristic of stage I *Pachygrapsus crassipes* zoea sampled from San Diego Bay, neighboring embayments and coastal sites. (A) First and second canonical variable scores correspond to individual zoea of known origin. The first canonical variable (Canonical Variate 1) accounts for 98% of the dispersion between groups. (B) Plot of two discriminant functions for zoea of unknown origin sampled at the entrance of San Diego Bay over a tidal cycle. Note that the points cluster within San Diego Bay and non-San Diego Bay groupings defined by discriminant function analysis shown in Fig. 2A.

were observed in surface (0–2 m) and middle layers (5–7 m; Fig. 3). Fewer than 19% of sampled larvae were in the bottom boundary layer, including the sediment–water interface, during ebbing tides (Fig. 3). Conversely, most stage I zoeae sampled during flood tide were concentrated in the bottom boundary layer (52%). Similar *P. crassipes* stage I zoeae vertical distribution patterns were observed on three other occasions in SDB (DiBacco 1999). The vertical distribution of stage I *P. crassipes* zoeae suggests a tidally or diurnally timed vertical migratory behavior that should facilitate the export of SDB spawned larvae to nearshore coastal waters. However, the effect of tidal versus diel light cycles on zoeal vertical migratory behavior could not be assessed individually, since both cues were in phase during spring tide sampling periods targeted in this study. This vertical migratory behavior, referred to as selective tidal stream transport, has been observed for other grapsid crabs (Christy and Stancyk 1982). This behavior also should prevent larvae originating outside the bay from entering SDB. Despite this vertical migratory behavior, approximately 32% of stage I zoeae sampled at the Bay entrance during the ebb tide apparently originated outside SDB, while 87% of larvae collected at the entrance during the flood tide originated from within SDB (Fig. 3). The observed bidirectional exchange counters the prediction of unidirectional transport out of the bay expected from vertical migration.

Table 3. Standardized canonical coefficients of canonical variate 1 that accounted for greater than 95% of the total dispersion between groups.

Trace element	CV 1
Cu	0.4588
Zn	0.5714
Sr	−0.7704
Mn	0.6585
Ca	0.8922

Discussion

Effectiveness of element based larval fingerprinting—The development of tags that effectively discriminate invertebrate larvae originating from adjacent bay and coastal populations has been elusive (*but see* Gaines and Bertness 1992). Here we have shown that naturally induced elemental tags can be an effective and practical method for determining the spawning origin of brachyuran zoeae sampled in situ. Trace-elemental fingerprints developed from a linear discriminant function analysis of elemental gradients were sufficiently distinct (classification success rate between 93% and 96%) to distinguish individual, stage I *P. crassipes* zoeae originating from inside or outside of SDB. These fingerprints effectively served as environmentally induced tags

Table 4. Validation results of the classification algorithm. (A) Classification matrix indicating the predicted origin for larvae comprising the model data set and (B) Cross-validation using a 'jackknifed' classification matrix (*see Methods*) to test the robustness of the classification algorithm.

	Predicted identity		
True identity	SDB	Non SDB	% correct
(A) Classification matrix			
SDB	37	3	93
Non SDB	2	54	96
Total	39	57	95
(B) Jackknifed classification matrix			
SDB	36	4	90
Non SDB	3	53	95
Total	39	57	92

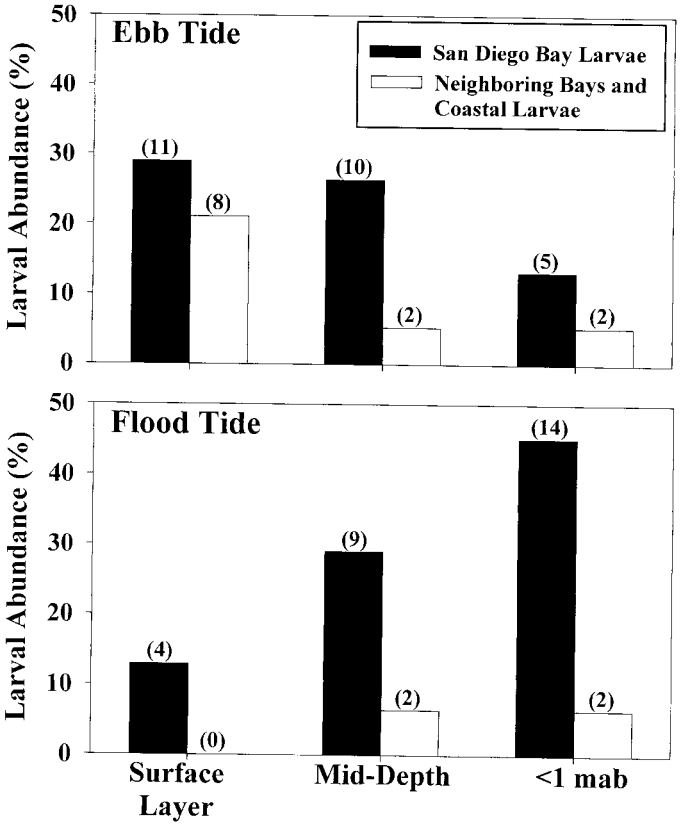

Fig. 3. Depth-stratified distributions (in % abundance of total pooled from all depths) of stage I *Pachygrapsus crassipes* larvae as a function of predicted origin (determined by discriminant function analysis). The number of larvae analyzed is listed in parentheses. (Surface layer = top 2 m, mid depth = 5–7 m, <1 mab = less than 1 m above the bottom)

to monitor the exchange of newly hatched zoeae between SDB and nearshore coastal waters.

Changes in the amount of freshwater runoff among years and between sites may influence elemental or isotopic composition of organisms (Gillanders and Kingsford 1996; Kennedy et al. 1997). Campana et al. (1995*b*) found small differences in the elemental composition of fish otoliths sampled from the same location between 2 years. No variation in zoeal elemental fingerprints were expected within the three-month sampling period of this study, since freshwater input from rain runoff and river sources into SDB is negligible during summer months. Further, there was no reported dredging operation in SDB during our sampling period, which might have liberated large amounts of trace elements sequestered in sediments and pore waters.

The trace-element composition of newly hatched stage I *P. crassipes* zoeae likely is imparted during gametogenesis and brooding, but can subsequently be altered through uptake and retention of elements following hatching and exposure to ambient environmental conditions (DiBacco 1999). The trace-element composition of newly hatched zoeae from laboratory-maintained ovigerous crabs collected from SDB and coastal sites reflected concentration levels observed in zoeae sampled in situ from the same sites (Table 2), suggesting that elemental fingerprints were persistent in the newly hatched stage I zoeae considered in this study. The elemental signature is likely to change following larval molting, therefore we limited the application of trace-elemental fingerprints to the identification of stage I zoeal origins.

Source of elemental signatures—Overall, trace-element concentrations (Cu, Al, Zn, Mn) were highest in zoeae collected from inner SDB and generally decreased in larvae sampled from outer SDB, neighboring embayments, and exposed coastal sites, respectively (Table 2). These gradients were expected for SDB, which has been subject to heavy industrial, commercial, and military development and ranks as one of the most contaminated urbanized coastal areas in the nation (O'Connor 1990). The larval body burden estimates mirror elemental concentrations for SDB seawater, sediments, and adult invertebrate and fish species which have been reported to be higher in SDB than in neighboring embayments or coastal sites (Young et al. 1979; McCain et al. 1992; Flegal and Sañudo-Wilhelmy 1993; Fairey et al. 1998).

The accumulation of trace metals in marine organisms can occur through: (1) the ingestion of food and suspended particulate material, (2) uptake of metals directly from bottom sediments or interstitial waters, or (3) direct uptake (i.e., absorption) from the surrounding seawater. In the case of newly hatched *P. crassipes* zoeae, ovigerous females probably impart the elemental signal to their larvae (DiBacco 1999). The majority of adult crabs inhabit high intertidal zones of salt marshes or crevices and tidepools of exposed rocky intertidal habitats (Hiatt 1948). Their dominant food source includes benthic algal mats (e.g., diatoms) that they scoop or scrape off sediment surfaces or off the top and sides of rocks and boulders (Hiatt 1948). Ingestion of benthic algae, sediments, and associated pore waters, all repositories for heavy metals (Kennish 1998), can enhance the signal of trace elements in SDB adults and their larvae relative to individuals from more pristine bays and coastal habitats.

Body burden estimates of Mg and Sr also differed in larvae sampled from inner SDB and coastal habitats (Table 2). Seawater temperature, salinity, food resources, and individual growth rates may influence Sr and Mg concentrations in crustacean exoskeletons (De Deckker et al. 1988). Strong correlations have been reported between the concentration of Sr and Mg in inorganically precipitated calcite and temperature (reviewed in Rosenthal et al. 1997). Variations in Mg and Sr content of biogenic calcite may be driven by temperature in adult animals such as corals (Beck et al. 1992), foraminifera (Rosenthal and Boyle 1993), clams (Hart and Blusztajn 1998), and ostracods (De Deckker et al. 1988). A similar relationship may be expected for crabs since the exoskeletons of ostracod and decapod crustaceans are fundamentally similar in composition; both groups have an endocuticle that is impregnated with calcium carbonate (Brusca and Brusca 1990).

Utility and application of elemental larval tags—The earliest reported attempt to tag marine invertebrate larvae was made over 50 years ago (Loosanoff and Davis 1947), yet very few studies have applied invertebrate larval tracking

techniques in field studies (Levin 1990). This is because advection, mixing, and mortality experienced by most larvae under ambient conditions make the recovery of labeled larvae impractical. Naturally induced elemental tags employed in this study are effectively applied to all larvae within the experimental system without any effort or expense to the investigator. As a result, every larva sampled from a system represents a recovery. Unlike artificially applied tags, naturally induced tags do not pose additional environmental hazards or alter larval mortality rates, development, or behavior as a result of laboratory handling. Trace-elemental signals observed in stage I *P. crassipes* zoeae were persistent in this study. However, in order to track post-stage I larvae and postlarval (megalopae) stages of development, it will be necessary to evaluate the uptake and retention of elements used to distinguish origins, as has been done in studies examining the utility of artificially induced tags (Levin et al. 1993; Anastasia et al. 1998).

There are several aspects of trace-elemental fingerprinting of marine invertebrate larvae that merit additional research if the method is to be expanded. In addition to decapods, other marine invertebrates that should be considered promising candidates for naturally induced elemental tagging studies are those with calcified body parts, such as bivalves and gastropods. The temporal stability of elemental signals needs to be addressed in study organisms. This is essential in crustacean larvae, which replace their exoskeleton following ecdysis, if post-stage I zoeae and postlarval megalopae are being considered. Organisms that maintain calcified body parts throughout their development need to be monitored for ontogenetic changes in the uptake and retention of trace elements. The source of trace-elemental signals, whether passively accumulated from seawater or actively assimilated from ingested food, sediments, and water, should be examined under controlled laboratory studies. Finally, in addition to trace-element abundance data, elemental ratios (e.g., Sr/Ca) as well as isotopic ratios can be considered as potential invertebrate tags. These are often highly sensitive indicators of environmental differences among habitats, such as salinity or temperature gradients.

Consequences of bay–ocean exchange of larvae—Application of trace-elemental fingerprinting to stage I *P. crassipes* zoeae suggest that a significant proportion of larvae experience bidirectional exchange between SDB and nearshore coastal habitats. This is contrary to inference drawn from the vertical migratory patterns of zoeae relative to tidal phase. The bidirectional exchange of larvae between bay and coastal habitats also may be explained by bay-specific flow dynamics (Dyer 1997). The early ebb tide from SDB consists of bay and coastal water. The bay water is concentrated in the surface layer and in the middle of the channel while recently entered coastal water is concentrated at the channel sides (Chadwick et al. 1996). Since larvae in this study were collected on the western side of the channel, this may help explain the high proportion of coastal larvae sampled during the ebbing phase of the tide (Fig. 3). Seawater reentering the bay during flood tide consists of well-mixed bay and ocean water (Chadwick et al. 1996). This water contained mainly crab larvae which originated from SDB (Fig. 3) and may reflect the concentration of stage I zoeae recently exported on the previous ebbing tide.

The above scenario of bay–ocean exchange should influence the genetic structure of both bay and coastal populations. If SDB populations are self-seeding, reduced availability of recruits by pollution could result in reduced population size. A laboratory-based study (DiBacco, in press) demonstrated that *P. crassipes* zoeae cultured in SDB seawater or brooded in SDB experienced lower survivorship, resulting in fewer megalopae than zoeae reared in coastal seawater or brooded on the outer coast. If larvae originating from SDB are less likely to survive and ultimately settle than those from other bays, populations of other sites may help maintain SDB populations.

A viable technique for assessing the degree of self-seeding of estuarine populations will aid future management of coastal habitats, especially for fragmented systems such as mudflats and salt marshes of southern California (Fairweather 1991). SDB, like many other urbanized bays, has lost much of its natural habitat to industrial, commercial, and military development. It is important to assess the extent to which bay populations interact with coastal populations via larval exchange. This is now possible through larval tagging. Observations have considerable bay–ocean exchange has implications for the maintenance of local marine invertebrate populations, as well as for maintenance of genetic diversity. Discrimination of larval invertebrate origins based on elemental fingerprints offers the potential for improved understanding of many aspects of larval dynamics in estuarine and nearshore systems, including larval behavior, physiological responses, gene flow, survivorship, and ultimately population dynamics.

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