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SPATIOTEMPORAL STABILITY OF TRACE AND MINOR ELEMENTAL SIGNATURES IN EARLY LARVAL SHELL OF THE NORTHERN QUAHOG (HARD CLAM) *MERCENARIA MERCENARIA*

ANDREW M. CATHEY,[1]* NATHAN R. MILLER[2] AND DAVID G. KIMMEL[3]

[1]*East Carolina University Department of Biology, N108 Howell Science Complex, Greenville, NC 27858;* [2]*Department of Geological Sciences The University of Texas at Austin, 1 University Station C1100, Austin, TX 78712-0254;* [3]*Department of Biology/Institute for Coastal Science and Policy East Carolina University, 250 Flanagan Building, Mail Stop 169, Greenville, NC 27858*

ABSTRACT The potential of trace and minor elements within biominerals to track the larval dispersal of bivalves was investigated by examining elemental composition in early larval shell of the northern quahog (hard clam) *Mercenaria mercenaria*. Larvae were cultured in three shellfish hatcheries using the adjacent estuarine waters of the southern Delmarva Peninsula in Virginia. Spatial distinction (~1–50 km) and temporal stability (triweekly) of elemental concentrations was assessed using inductively coupled plasma mass spectrometry. Seventeen minor and trace elements were present at detectable levels in all shell samples: Ca, Mg, Ti, Co, Ni, Zn, Se, Rb, Al, V, Cr, Mn, Cu, Sr, Ba, Pb, and U. Discriminant function analyses using metal-to-Ca ratios as independent variables assigned hard clams to their hatchery of origin correctly, with 100% success. The ratio Cr:Ca proved to be the most effective discriminator, explaining 78.1% of among-group variance. Elemental concentrations within early larval shell also differed temporally. Discriminant function analysis classified individual spawning events with 100% success, with Al:Ca explaining the bulk of among-group variance (81.4%). Despite temporal variability of elements within larval shell, it was possible to resolve elemental signals spatially among hatcheries regardless of spawning date. These results demonstrate for the first time that the chemical composition of hard clam larval shell records spatial elemental signatures with the potential to trace the environment of natal origin as well as subsequent dispersal trajectories of this economically important species.

KEY WORDS: northern quahog, hard clams, *Mercenaria mercenaria*, microchemistry, larval dispersal, population connectivity, aquaculture

INTRODUCTION

Elemental fingerprinting represents a prospective tool for identifying patterns of invertebrate larval dispersal and population connectivity as a result of the potential for all larvae within a particular area to incorporate a geospatially distinct chemical signal (Thorrold et al. 2002, Levin 2006). If habitat-specific signals exist and are stable temporally and spatially on ecologically relevant scales, the potential exists to investigate issues regarding the natal origin and dispersal trajectory of successfully recruited bivalves (Becker et al. 2007). To apply this methodology, it is necessary to confirm that the chemical composition of larval biomineralized structures is stable through time (Gillanders 2002). In addition, the chemical composition of all potential natal origins must be investigated to assess accurately the spatial distinction of trace element incorporation into larval biomineralized structures (Campana et al. 2000). Last, provided the former assumptions are found to be true, chemical analysis of biominerals formed during larval development and retained through subsequent ontogeny is critical to reconstruct larval origin and subsequent dispersal trajectory (DiBacco & Levin 2000). Such biomineralized structures are observed in multiple invertebrates, including gastropods (statocyst and protoconch), cephalopods (statocyst), and bivalves (prodissoconch) (Zacherl et al. 2003, Becker et al. 2007, Zumholz et al. 2007).

Becker et al. (2005) first applied an invertebrate model with a retained larval biomineral (recently recruited mussels *Mytilus* spp.) to investigate shell compositional evolution in relation to geospatial location. Their results, supported by a growing body of literature, suggest that trace element compositions within newly recruited bivalve shell can be relatively stable on both weekly and monthly timescales and can be used to assign individuals to sites of collection at spatial scales on the order of ~12–80 km (Dunphy et al. 2011, Fodrie et al. 2011, Cathey et al. 2012). Using *in situ* larval culturing to investigate patterns of mussel connectivity, Becker et al. (2007) demonstrated that early-juvenile-stage shell trace element composition was retained in the larval shell of the pelagic veliger stage. This research provided the first evidence that trace element analysis can be applied to bivalve larvae to determine the natal origin and dispersal trajectory of individual recruits.

The application of trace element fingerprinting to model patterns of bivalve larval dispersal and population connectivity shows increasing promise. The current study investigates its potential efficacy in tracking the larval dispersal of *Mercenaria mercenaria* (Northern Quahog), hereafter referred to as the hard clam, within the waters associated with the southern Delmarva Peninsula, Virginia. In Virginia, hard clam reproductive periodicity is characterized by a concentration of spawning activity during the spring (March to June), with mature gametes being observed through October (Eversole 2001). External fertilization is followed by pelagic larval development typified by the rapid formation of an initial larval shell, the prodissoconch I (PDI), within ~24–48 h (Carriker 2001). Once the PDI is formed, larvae may be referred to as D-stage larvae. The hydrodynamic properties within and among estuarine systems throughout the range of the hard clam are such that larval residence times within natal estuaries are predicted to span development of their PDI (Brooks et al. 1999, Leuttich et al. 1999, Sheldon & Alber 2002). The PDI of recruited individuals thus carries the potential to

*Corresponding author. E-mail: catheya060@gmail.com
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trace the chemical signature of natal estuaries (Carriker 2001), provided estuaries can be differentiated sufficiently.

The objective of this study was to investigate the temporal stability of trace element composition resident within generations of D-stage larval quahogs, and the extent to which it may be used as a geospatial tracer of natal estuaries. To assess spatial distinction, D-stage larvae (spawned within a 5-day interval) were obtained from three shellfish hatcheries separated by ~1–50 km. The temporal variability (stability) of trace element composition incorporated into larvae associated with a particular hatchery was assessed by collecting D-Stage larvae from four triweekly spawning events, each from a single hatchery. To date, the validation of geospatially distinct elemental concentrations within larval bivalve shell has been accomplished using *in situ* culturing or out-planting (Becker et al. 2007). An alternative to this technique is to capitalize on the existing infrastructure of commercial shellfish hatcheries. Hatcheries provide a unique and cost-effective opportunity to investigate spatiotemporal chemical variability in rapidly formed invertebrate larval biominerals because newly spawned larvae (i.e., hard clam) incorporate trace elements during growth (biomineralization) within stable environments hosted by natural waters taken directly from adjacent estuaries. This sampling approach, here termed in-planting, is thus beneficial because it avoids logistical difficulties (including large-scale mortality) associated with outplanting larvae into estuaries within temporary enclosures.

Shell major, minor, and trace element chemistry was analyzed by solution mode inductively coupled plasma mass spectrometry (ICP-MS) to determine the temporal variability (stability) of larval shell (PDI) trace element composition from natal hatcheries, and the extent to which larval shell (PDI) trace element composition can be used to discriminate natal origin (hatchery locality). If trace element composition of larval shell (PDI) formed in individual hatcheries does not vary significantly through time and allows for spatial discrimination between hatcheries, PDI elemental signatures offer the potential to track patterns of hard clam larval dispersal and population connectivity. This could potentially allow the identification of subpopulations that may contribute differentially to overall population dynamics by providing a disproportionate supply of larvae. Thus, larval shell (PDI) elemental signature capabilities, if verified, could have significant implications for the management, conservation, and restoration of hard clam populations.

MATERIALS AND METHODS

Sample Collection

During February 2012, three commercial shellfish hatcheries (Cherrystone Aquafarms, Cheriton Virginia; Cherrystone Aquafarms, Willis Wharf, Virginia; and J.C. Walker Brothers, Willis Wharf, Virginia; Fig. 1) provided three replicates of D-stage quahog (hard clam) larvae (>10^6 larvae per replicate) that had been spawned within a 5-day interval. In addition, J.C. Walker Brothers provided three replicates of D-stage larvae from each of three subsequent, triweekly spawning events that spanned February 29 to April 10. Culturing conditions at both Cherrystone Aquafarms locations were 23.3°C and a salinity of 30, and larvae from J.C. Walker Brothers were reared at 25°C and a salinity of 28. Samples were frozen in culture water until processed for elemental analysis.

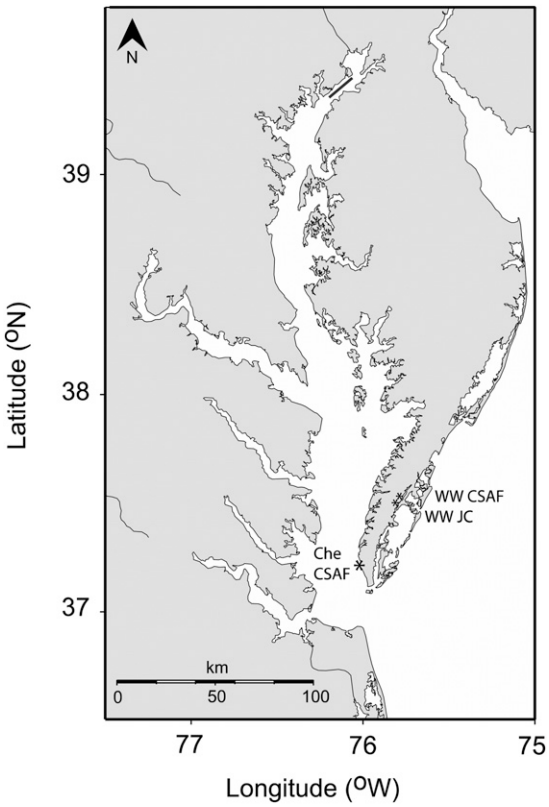

Figure 1. Map of the Chesapeake Bay and Delmarva Peninsula. Asterisks denote hatcheries that supplied D-stage hard clam larvae. Che CSAF, Cherrystone Aquafarms Cheriton; WW CSAF, Cherrystone Aquafarms Willis Wharf; WW JC, JC Walker Brothers. Willis Wharf.

Isolation of larval shell material was conducted in a class 100 laminar flow hood using physical separation methods. Pooled larvae from each hatchery replicate were placed on 20-μm mesh, rinsed with type I ultrapure water (18.2 Ω), and transferred into 1.5-mL acid-washed (7% HNO_3 vol/vol; Fisher Optima Grade) polypropylene vials. Larvae were then suspended in 1-mL type I ultrapure water (18.2 Ω), vortexed for 30 sec, sonicated for 5 min, and finally microfuged for 3 min at 6,000*g* at room temperature. This process results in a stratification of material such that lighter larval somatic tissue and periostracum overlay heavier larval shell. Organic tissue and supernatant were removed, and larval shell was resuspended in 1 mL ultrapure water and treated similarly for an additional 9 cycles. After removal of the final supernatant, resulting larval shell concentrates were dried for 36 h under laminar flow and inspected for residual somatic tissue, which was removed when encountered. When dry, pooled larval shells from each replicate were weighed and transferred to acid-washed polypropylene vials (7% HNO_3 (vol/vol) Optima Grade; Fisherbrand) until analysis.

ICP-MS Analysis

Larval bivalve shell concentrates ($n = 18$), weighing 0.7–17.9 mg, were digested for 12 h with 1.25 mL 0.9 M HNO_3 (Fisher trace metal grade) in acid-washed polypropylene microvials, then centrifuged (5 min at11,000*g*) to separate any undissolved residue. The supernatant (1.1 mL) was transferred to acid-cleaned microvials from which aliquots were diluted to

TABLE 1.

ICP-MS operating conditions and instrument parameters.

ICP-MS instrument	Agilent 7500ce ICP-Q-MS
Plasma Conditions	
Nebulizer	PFE microflow with 90 μL/min uptake rate
Nebulizer pump (rps)	0.1
Spray chamber	Scott–type (quartz) with Peltier cooling (2°C)
Sampling depth (mm)	8
RF power (W)	1,600
RF matching (V)	1.7
Carrier gas flow (L/min)	1
Makeup gas flow (L/min)	0.24
Cones	Ni
Reaction cell (ORS) modes and masses measured	
He mode (4.5 mL/min)	23, 24, 27, 39, 43, 44, **45**, 51, 52, 53, **72**, 75
H_2 mode (3.7 mL/min)	28, 40, **45**, 56, **72**, 78
No gas mode	9, 11, 23, 24, 26, 27, 29, 31, 39, 43, 44, 45, 47, 51, 55, 57, 59, 60, 63, 66, 68, 72, 85, 88, 90, 95, 107, 111, 114, 115, 118, 121, 125, 133, 137, 175, 205, 206, 207, 208, 209, 232, 238
Internal standards	**9, 45, 72, 115, 125, 175**
Detection system	Dual-stage (pulse and analog) discrete dynode electron multiplier
Data acquisition	
Scan mode	Peak hopping
Points across peak	3
Integration per mass (sec)	0.100
Replicates	3

Bold values represent isotopes used as internal standards to evaluate instrument drift, specifically: 9 Be, 45 Sc, 72 Ge, 115 In, 125 Te, 175 Lu.

levels appropriate for measurement of trace and minor elements (~2,350× dilution) and major elements (~82,000× dilution). The acid strength and volume used for digestions was capable of dissolving all carbonate in the sample (based on starting weight) by factors of 3–80 (median, 8.9). However, samples typically had prominent tan-colored insoluble residues after centrifugation. To evaluate the noncarbonate insoluble residue content of larval bivalve shell, the insoluble residue proportion of representative dry weight splits (0.7–1.1 mg) was determined for 3 samples of pooled larval bivalve shell isolate. Resulting insoluble residues varied between 26.0 wt% and 43.8 wt% (average, 33.7 ± 9.1 wt%), indicating that insoluble organic shell matrix comprised a substantial proportion of shell concentrate samples.

Cation concentrations (B, Na, Mg, Al, Si, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Tl, Pb, Bi, Th, and U) were determined using an Agilent 7500ce ICP-MS at the University of Texas at Austin (Department of Geological Sciences). The instrument was optimized for sensitivity across the atomic mass unit range, while minimizing oxide production (<1.9%). The analytical method used an octopol reaction system (ORS), operated in helium (collision mode) and hydrogen (reaction mode) for removal of polyatomic interferences. Internal standards, mixed into unknowns via in-run pumping, were used to compensate for instrumental drift, and internal standard sensitivity variations were well within QA tolerances (±50%). Limits of detection, based on the population of blank (2% HNO_3) analyses interspersed throughout the analytical sequence were typically better than 0.173 ppb (median, 0.009 ppb) for analytes measured in optimal modes (with or without the ORS). Analyte recoveries obtained for replicates of two independent quality control standards—NIST 1643e (diluted 10×) and a low-calibration range standard prepared from stocks (VHG Laboratories) independent of those used for preparing calibration standards—were typically within 2% of certified values. Relative precisions (n = 2–3) obtained for these quality control standards were typically within 0.4%–1.6% of replicate averages. Matrix spikes, performed on two randomly selected samples, resulted in analyte recoveries of 97%–98%, indicating that matrices of diluted samples ionized comparable with calibration and quality control standards. Inductively coupled plasma mass spectrometry analytical parameters and quality control recoveries are shown in Tables 1 and 2.

Calcium concentrations calculated based on starting shell weights averaged 56 ± 18% of concentrations expected in stoichiometric $CaCO_3$; these systematically low values are consistent with samples having appreciable insoluble residue contents (in agreement with the range of our insoluble residue determinations described earlier). Accordingly, larval shell cation concentrations were normalized to Ca concentration assuming a stoichiometric $CaCO_3$ composition. This is supported by consistent Ca concentrations within juvenile bivalve shell mineralogy (Cathey et al. 2012). As long as leachate cations derive predominantly from shell carbonate, we note that the metal-to-calcium ratios (Me:Ca) considered subsequently are unaffected by mineral phase composition.

Data Analysis

When assumptions of normality and homoscedasticity were met, analysis of variance (ANOVA) was used to investigate differences in the mean concentration of Me:Ca within pooled larval shells among sampling locations. When these assumptions were not met, nonparametric Kruskal-Wallis tests were used. Similarly, ANOVA and Kruskal-Wallis tests were used to investigate differences in the Me:Ca of larval shell among sampling dates. Discriminant function analysis (DFA) was used

TABLE 2.

Analyte detection limits and recoveries on quality control (QC) standards.

Isotope	Calibration (rho)	LOD (ppb)	ORS mode	QC1 recovery	QC2 recovery	QC3 recovery
^{11}B	1.0000	1.829	No gas	0.94	0.96	0.95
^{23}Na	1.0000	0.288	No gas	1.02	1.02	0.99
^{24}Mg	0.9999	0.058	No gas	1.05	1.04	1.01
^{27}Al	1.0000	0.019	No gas	1.06	1.09	1.01
^{28}Si	0.9996	0.878	H_2	1.05	1.07	—
^{31}P	1.0000	0.942	No gas	0.92	0.93	—
^{39}K	1.0000	0.681	He	1.02	1.00	1.00
^{44}Ca	1.0000	0.827	No gas	1.07	1.06	0.98
^{47}Ti	1.0000	0.011	No gas	1.02	1.05	—
^{51}V	0.9999	0.008	No gas	1.02	1.03	0.99
^{52}Cr	1.0000	0.010	He	1.04	1.04	1.02
^{55}Mn	0.9997	0.006	No gas	1.06	1.08	1.04
^{56}Fe	0.9997	0.028	H_2	1.07	1.08	1.05
^{59}Co	0.9999	0.002	No gas	0.99	1.00	0.98
^{60}Ni	1.0000	0.005	No gas	1.01	1.03	1.00
^{63}Cu	0.9998	0.005	No gas	1.01	1.03	0.98
^{66}Zn	0.9997	0.125	No gas	1.02	1.04	0.94
^{75}As	1.0000	0.015	He	0.99	1.04	0.97
^{78}Se	1.0000	0.008	H_2	1.02	1.04	0.96
^{85}Rb	0.9997	0.002	No gas	1.05	1.06	1.03
^{88}Sr	1.0000	0.007	No gas	1.01	1.01	0.98
^{90}Sr	1.0000	0.004	No gas	0.92	0.93	—
^{95}Mo	0.9997	0.053	No gas	0.95	1.01	0.95
^{107}Ag	0.9997	0.005	No gas	1.05	1.05	—
^{114}Cd	1.0000	0.002	No gas	0.96	0.92	0.92
^{118}Sn	0.9999	0.010	No gas	0.98	1.01	—
^{121}Sb	0.9995	0.015	No gas	0.90	0.91	0.91
^{133}Cs	1.0000	0.002	No gas	1.00	1.01	—
^{137}Ba	1.0000	0.004	No gas	1.00	1.01	0.98
^{205}Tl	0.9995	0.003	No gas	1.05	1.10	1.03
^{208}Pb	0.9999	0.001	No gas	1.05	1.06	1.01
^{209}Bi	1.0000	0.008	No gas	1.02	1.04	0.99
^{232}Th	0.9996	0.014	No gas	0.99	0.99	—
^{238}U	0.9997	0.001	No gas	0.97	0.97	—

QC1, midcalibration range standard (same stock as calibration standards); QC2, midcalibration range standard (stock independent of calibration standards); QC3, NIST 1643e (trace elements in water). Null fields uncertified.

to investigate the ability to classify larval clams from different hatcheries based on their multivariate Me:Ca ratios. Discriminant function analysis is a multivariate statistical test used to produce a predictive model composed of discriminant functions derived from linear combinations of the independent variables (Me:Ca of larval shell) that provide the best discrimination among our dependent variables (hatchery of origin and date of collection). All analyses were performed using SPSS version 20 (Manly 2005).

RESULTS

Spatiotemporal Variability of Elemental Signals

Of 34 trace and minor elements investigated, 17 were present at detectable levels in larval shell samples (Figs. 2 and 3). For D-stage larvae sampled from February 2012, ANOVA revealed significant differences in Mg:Ca, Ti:Ca, Co:Ca, Ni:Ca, Zn:Ca, Se:Ca, and Rb:Ca among hatcheries ($P < 0.01$), and Kruskal-Wallis tests revealed significant differences in the means of Al:Ca, V:Ca, Cr:Ca, Mn:Ca, Cu:Ca, Sr:Ca, Ba:Ca, Pb:Ca, and U:Ca among hatcheries ($P < 0.01$; Fig.re 2). Discriminant function analysis with Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, Pb:Ca, Al:Ca, Mn:Ca, and Cr:Ca serving as independent variables assigned larval clams to their hatchery of origin with 100% success (Fig. 4A, Table 3). The primary driver of our elemental signal, Cr:Ca was responsible for 78.1% of the observed variance among natal locations.

Significant temporal differences were detected in the Me:Ca of pooled larval shell for samples collected triweekly from J.C. Walker Brothers between February 12 and April 10. Analysis of variance revealed differences in Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, and Pb:Ca ($P < 0.05$). Similarly, Kruskal-Wallis tests revealed significant temporal differences in Al:Ca, Mn:Ca, and Cr:Ca ($P < 0.05$; Fig. 3). Discriminant function analysis using Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, Pb:Ca, Al:Ca, Mn:Ca, and Cr:Ca as independent variables assigned pooled individuals correctly to their date of collection with 100% success (Fig. 4B, Table 4). The ratio of aluminum to calcium accounted for 81.4% of the observed variance among collection dates.

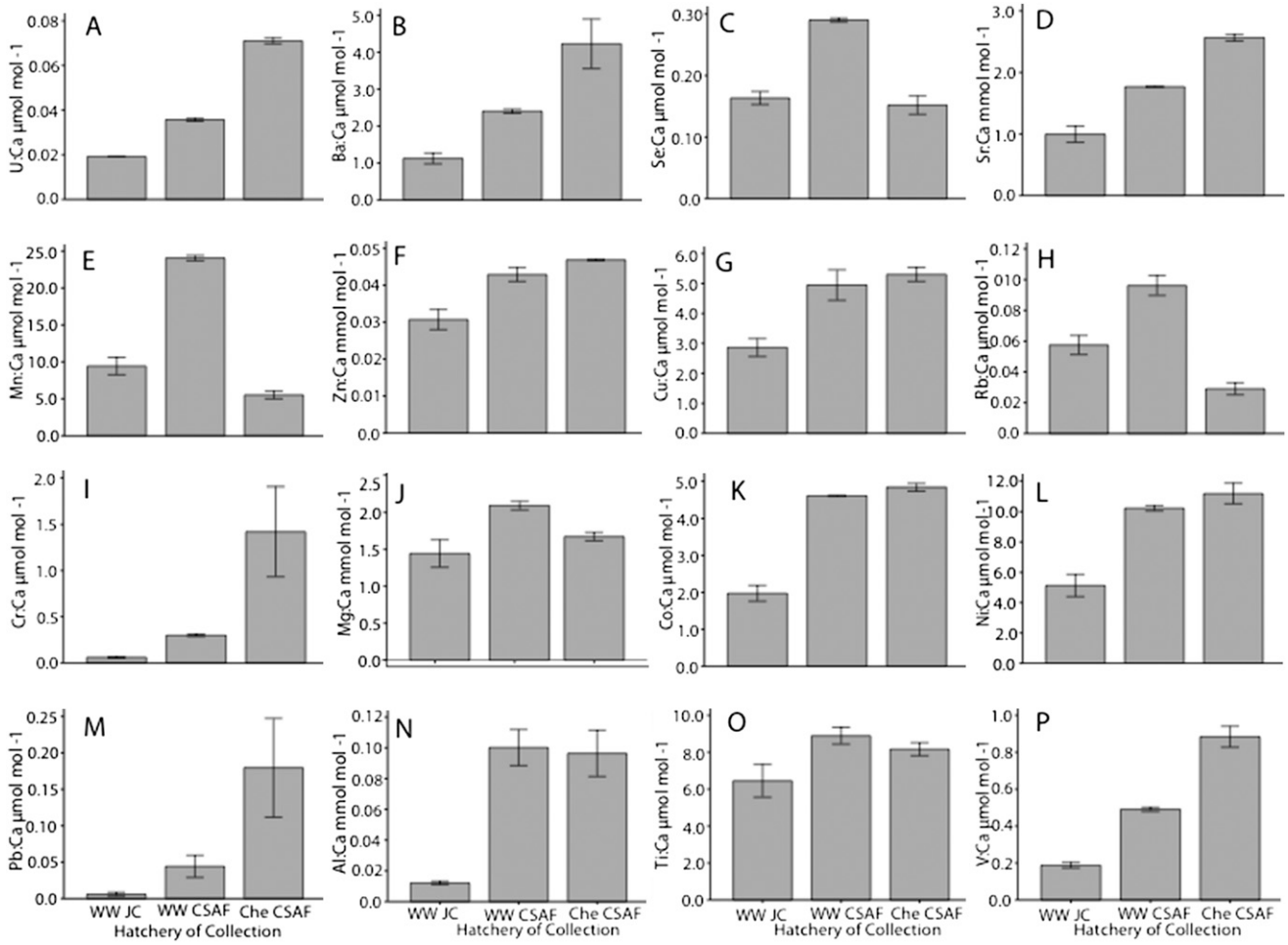

Figure 2. Mean (± 1 SE) of Me:Ca (metal-to-calcium ratio) within pooled D-stage hard clam larvae collected from JC Walker Brothers. in Willis Warf (WW JC), Cherrystone Aquafarms in Willis Warf (WW CSAF), and Cherrystone Aquafarms in Cheriton (Che CSAF). (A) U:Ca. (B) Ba:Ca. (C) Se:Ca. (D) Sr:Ca. (E) Mn:Ca. (F) Zn:Ca. (G) Cu:Ca. (H) Rb:Ca. (I) Cr:Ca. (J) Mg:Ca. (K) Co:Ca. (L) Ni:Ca. (M) Pb:Ca. (N) Al:Ca. (O) Ti:Ca. (P) V:Ca.

DISCUSSION

Spatial Distinction of Elemental Compositions

These results demonstrate for the first time the existence of geospatially distinct elemental compositions within distinct populations of hard clam larval shell (Fig. 4A). Concentrations of Mg:Ca, Sr:Ca, Zn:Ca, Pb:Ca, U:Ca, Ba:Ca, and Mn:Ca within pooled larval shell samples were consistent with values reported for pooled encapsulated gastropod veligers (Zacherl 2005). Spatial variability of Me:Ca within larval shell samples among the three hatcheries allowed a DFA using Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, Pb:Ca, Al:Ca, Mn:Ca, and Cr:Ca to classify larvae to their natal hatchery with 100% accuracy (Fig. 4A, Table 3). A subsequent DFA using only the most effective discriminators from our original model (Cr:Ca > Co:Ca > Ba:Ca) also assigned pooled larvae correctly to their hatchery of origin with 100% success. The ability to discriminate among natal location using a reduced suite of elements will serve, potentially, to reduce analytical operating costs as well as increase the speed of future analyses (Dunphy et al. 2011).

The results of this investigation are supported by a growing body of literature suggesting that elemental fingerprints within invertebrate larval biomineralized structures can be used to assign individuals to their site of collection at regional spatial scales (~40 km) (DiBacco & Levin 2000, Zacherl 2005). In addition, work by Cathey et al. (2012) demonstrates that shell isolates of newly recruited hard clam contain distinct elemental signatures that can be used to assign individuals to their site of collection seasonally with very high levels of accuracy at even smaller spatial scales (~12 km). More important, our current results provide the first evidence that elemental fingerprints within invertebrate larval shell can be used to identify natal origin at local scales (~1 km). However, the extrapolation of these data to natural systems must be taken with great care because the possibility exists that the hatcheries themselves may be influencing the incorporation of some elements. In addition, the strength of signal observed at such a small spatial scale could be the result of analyzing pooled larvae in solution, because this method serves to increase the limits of detection of elemental species compared with microbeam assays, such as laser ablation ICP MS (Campana 1999). The refinement of microbeam techniques such as laser ablation ICP-MS to analyze the chemical composition of individual hard clam larval shell will be requisite for the application of elemental fingerprinting as a methodology

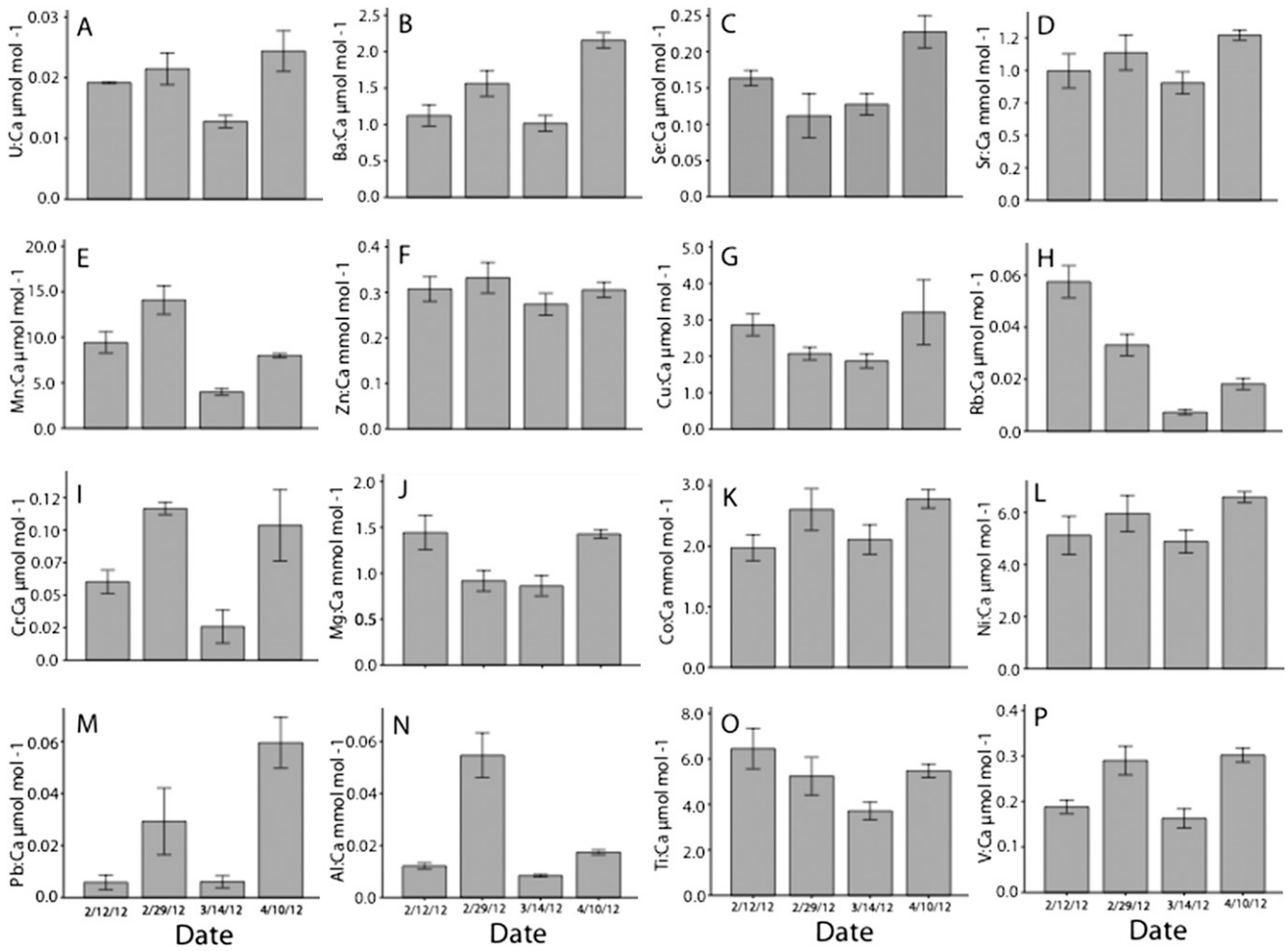

Figure 3. Mean (±1 SE) of Me:Ca (metal–to-calcium ratio) within pooled D-stage hard clam larvae collected from JC Walker Brothers. in Willis Wharf from 4 triweekly spawning events spanning Feb 12 to April 10. (A) U:Ca. (B) Ba:Ca. (C) Se:Ca. (D) Sr:Ca. (E) Mn:Ca. (F) Zn:Ca. (G) Cu:Ca. (H) Rb:Ca. (I) Cr:Ca. (J) Mg:Ca. (K) Co:Ca. (L) Ni:Ca. (M) Pb:Ca. (N) Al:Ca. (O) Ti:Ca. (P) V:Ca.

to model the larval dispersal of hard clams. If the small-scale elemental signals observed in pooled samples are identified within naturally occurring hard clam larvae, this will have significant logistical implications for the chemical characterization of all possible natal origins (Campana et al. 2000).

The incorporation of trace and minor elements into invertebrate biomineralized structures is influenced by a complex interplay between geochemical and biological processes (Schöne 2008). The incorporation of some elements appears to be influenced predictably by the physical and chemical composition of the water in which they form (Gillikin et al. 2006, Zacherl et al. 2009). In the current investigation, hatchery waters, in which hard clam larvae were cultured, had relatively small differences in temperature and salinity. Specifically, conditions at both Cherrystone Aquafarms hatcheries were 23.3°C and 30‰ salinity, with larvae from J.C. Walker Brothers cultured at 25°C and 28‰ salinity. Empirical evidence supports an inverse temperature effect regarding the incorporation of Ba, Pb, and Sr into gastropod and cephalopod statoliths (Zumholz et al. 2007, Lloyd et al. 2008). Significant differences in shell Mg:Ca, Se:Ca, Zn:Ca, U:Ca, V:Ca, Ni:Ca, Mn:Ca, Pb:Ca, Sr:Ca, Ba:Ca, and Cu:Ca among all three hatcheries despite identical culturing conditions at the two Cherrystone hatcheries suggests the small temperature differences in culture water were likely negligible. Additional experiments demonstrate that salinity does not influence the incorporation of Mn, Mg, Ba, and Sr into fish otoliths and cephalopod statoliths appreciably (Martin & Thorrold 2005, Zumholz et al. 2007). A reduced contribution of salinity concerning the incorporation of trace and minor elements is supported by significant differences in Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, Pb:Ca, Al:Ca, Mn:Ca, and Cr:Ca among collection date from clams spawned under identical salinity conditions by J.C. Walker Brothers.

Estuaries are dynamic systems that experience differential concentrations of trace elements resulting from varying geomorphology, atmospheric deposition, pollution, and inputs from local watersheds (Swearer et al. 2003, Thorrold et al. 2007). Laboratory experiments have demonstrated a positive linear relationship between the concentration of Mg:Ca, Pb:Ca, and Ba:Ca within molluscan biominerals and the concentration of these elements within culture water (Lorens & Bender 1980, Lloyd et al. 2008). Unfortunately, chemical analysis of culture water was not conducted in the current investigation, preventing a direct comparison with elemental concentrations of hard clam larval shell. Regardless, these results provide compelling evidence that elemental signatures in larval shell can discriminate the natal

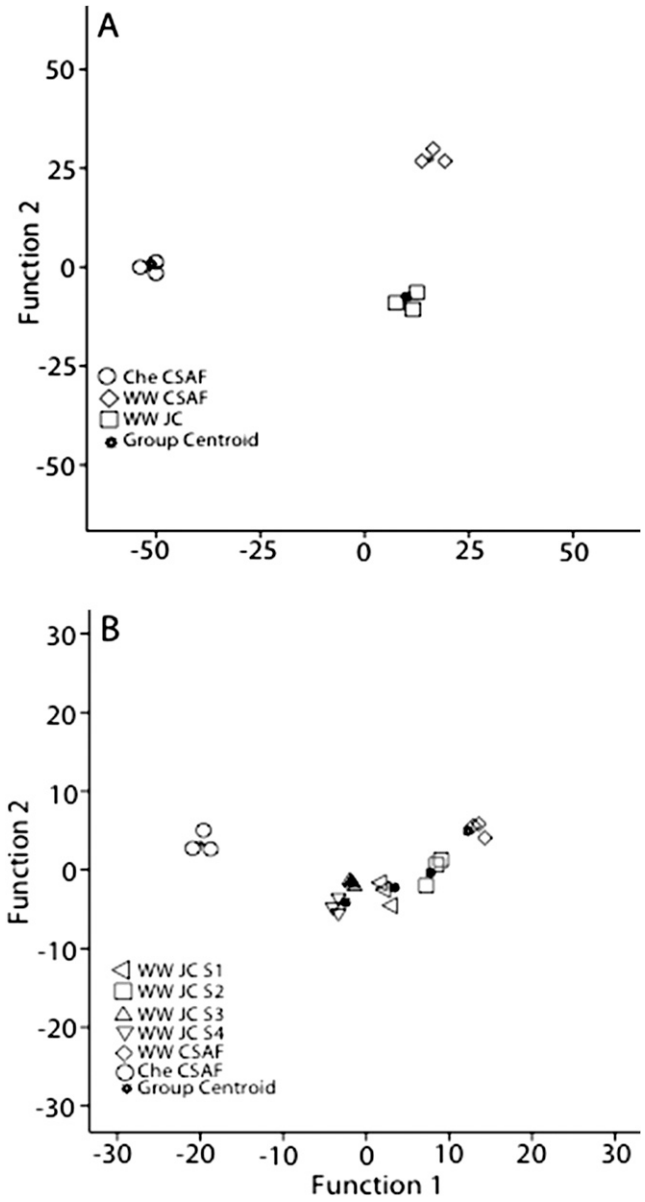

Figure 4. (A) Scatterplot of discriminant function analysis (DFA) scores of element (Mg, V, Se, Rb, Ba, Pb, Al, Mn, and Cr)-to-Ca ratios in hard clam larvae collected from JC Walker Brothers. in Willis Wharf (WW JC), Cherrystone Aquafarms in Willis Warf (WW CSAF), and Cherrystone Aquafarms in Cheriton (Che CSAF). Grouped as WW JC, WW CSAF, and WW CSAF. (B) Scatterplot of DFA scores of element (Mg, V, Se, Rb, Ba, Pb, Al, Mn, and Cr)–to-Ca ratios in hard clam larvae collected from JC Walker Brothers. in Willis Wharf (WW JC; with S1–S4 denoting each of 4 triweekly spawning events), Cherrystone Aquafarms in Willis Wharf (WW CSAF), and Cherrystone Aquafarms in Cheriton (Che CSAF). Grouped as WW JC S1–4, WW CSAF, and WW CSAF.

origin of distinct larval hard clam populations. Indeed, elemental fingerprints within biominerals can be used as natural tags without a full comprehension of all factors influencing elemental incorporation (Gillanders & Kingsford 1996). Future work will be essential to elucidate other factors that may influence the availability and incorporation of trace and minor elements into hard clam larval shell.

TABLE 3.

Classification success using larval shell microchemistry to determine natal hatchery from JC Walker Brothers. in Willis Wharf (WW JC), Cherrystone Aquafarms in Willis Wharf (WW CSAF), and Cherrystone Aquafarms in Cheriton (Che CSAF).

	Hatchery	WW JC	WW CSAF	Che CSAF	Total
Count	WW JC	3	0	0	3
	WW CSAF	0	3	0	3
	Che CSAF	0	0	3	3
Percent classified	WW JC	100.0	0.0	0.0	100.0
	WW CSAF	0.0	100.0	0.0	100.0
	Che CSAF	0.0	0.0	100.0	100.0

Grouped as WW JC, WW CSAF, and WW CSAF. Rows denote actual grouping using the discriminant function analysis model.

Temporal Stability of Elemental Fingerprints

The current study investigates the temporal stability of elemental signatures within pelagically formed bivalve larval shell (PDI). The protracted spawning period of our hard clam model coupled with the rapid formation of this larval biomineral (~24–48 h) within highly dynamic estuarine systems underscores the importance of validating any small-scale temporal variability of chemical signals (Peterson & Fegley 1986, Carriker 2001, Swearer et al. 2003). Evidence to date suggests that elemental compositions within recruited bivalve shell can be reproducible on weekly, monthly, and seasonal timescales (Becker et al. 2005, Dunphy et al. 2011, Cathey et al. 2012). In the current investigation, Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, Pb:Ca, Al:Ca, Mn:Ca, and Cr:Ca differed among spawning dates (Fig. 2). Discriminant function analysis using Mg:Ca, V:Ca, Se:Ca, Rb:Ca, Ba:Ca, Pb:Ca, Al:Ca, Mn:Ca, and Cr:Ca as independent variables assigned pooled individuals correctly to their date of collection with 100% success (Fig. 4B, Table 3). Despite these results suggesting temporal variability in the chemical composition of hard clam larval shell, it was possible to discriminate between J.C. Walker Brothers and both Cherrystone Aquafarms facilities regardless of collection date (Fig. 4B, Table 3). In addition, DFA scores plotted for independent spawning dates cluster more closely to one another than either of the Cherrystone hatcheries (Fig. 4B). Furthermore, Fodrie et al. (2011) demonstrated the ability to resolve elemental signals consistently and spatially within recruited bivalve shell despite weekly variability in Mn:Ca, Cd:Ca, Ba:Ca, Pb:Ca, U:Ca, Cu:Ca, and Sr:Ca. If the overall spatial resolution in the chemical composition of larval shell among hatcheries is maintained through time, then any interlocation temporal variability could potentially be used to identify more precisely the date of birth for an individual recruit.

These results support the possibility that elemental signatures in early larval shell of the hard clam can be used as geospatial tracers of natal origin, on small regional scales (hatchery spacings of ~1–50 km; Fig. 3A, Table 1). Despite temporal variability in elemental signatures, it was possible to discriminate between J.C. Walker Brothers and both Cherrystone Aquafarms facilities regardless of collection date (Fig. 3B, Table 1). Thus, for a given location, elemental compositions obtained within growing larval shell of individuals from different spawnings, although variable, are consistent enough to discriminate natal origin (hatchery site).

TABLE 4.

Classification success using larval shell elemental signatures to determine spawning date for hard clam larvae collected from JC Walker Brothers. in Willis Wharf from each of 4 triweekly spawning events spanning February 12to April 10.

	Date spawned for larvae from JC Walker Brothers in Willis Wharf	2/12/12	2/29/12	3/14/12	4/10/12	Total
Count	2/12/12	3	0	0	0	3
	2/29/12	0	3	0	0	3
	3/14/12	0	0	3	0	3
	4/10/12	0	0	0	3	3
Percent classified	2/12/12	100.0	0.0	0.0	0.0	100.0
	2/29/12	0.0	100.0	0.0	0.0	100.0
	3/14/12	0.0	0.0	100.0	0.0	100.0
	4/10/12	0.0	0.0	0.0	100.0	100.0

Grouped by date spawned. Rows denote actual grouping using the discriminant function analysis model.

Future work should focus on the refinement of assays to analyze the shell of individual larvae because these assays will be required to identify the natal origin of recruited individuals. Additional studies are necessary to investigate the interplay between exogenous and endogenous factors that influence the availability and incorporation of trace and minor elements from the environment into early larval shells of the hard clam. These data could elucidate the nature and geospatial extent of dispersal on the population dynamics of this species through the identification of subpopulations that may supply disproportionate numbers of larvae. From a management perspective, these source populations would benefit the most from conservation and restoration efforts.

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