

Egg source, temperature and culture seawater affect elemental signatures in *Kelletia kelletii* larval statoliths

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ABSTRACT: Elemental signatures have been used as a tool to track individual organisms to their natal site in an attempt to understand stock structure and larval dispersal. However, factors that affect elemental signatures are not well understood. We conducted a factorial experiment using whelk *Kelletia kelletii* larvae from Salta Verde Point on Catalina Island, Los Angeles Harbor, and White Point, Palos Verdes peninsula, California, USA, to test the effects of egg source, temperature (10, 14 and 18°C) and culture seawater on the elemental composition of larval statoliths. Intra-capsular contents of newly laid capsules were also analyzed to explore whether maternal contributions might affect larval statolith chemistry. Using inductively coupled plasma mass spectrometry we quantified ratios of 7 elements to calcium in both intra-capsular contents and cultured statoliths and provided the first evidence of significant egg-source effects, independent of subsequently experienced environmental conditions, on statolith elemental signatures for Mg:Ca, Ba:Ca and Pb:Ca. Intra-capsular and statolith element ratios showed no clear relationship that might have indicated possible maternal transfer of elements to larvae. Culture seawater elemental concentration was positively related to statolith Ba:Ca and Pb:Ca, and temperature was negatively related to statolith Sr:Ca, Ba:Ca and Pb:Ca, while no significant effects were found for Mn:Ca or Zn:Ca. Effect-size estimates show that elements responded differently to factors within the variation measured in this study; the major effects for Ba and Pb were temperature and egg source, respectively. The significant effect of egg source on elemental signatures has potentially important implications for tracking free-spawned larvae.

KEY WORDS: Statolith · LA ICP-MS · Dispersal · Egg source · Relative effects · Elemental signature · *Kelletia kelletii*

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INTRODUCTION

Biogenic carbonates (e.g. foraminiferal shell calcite, coral aragonite) have been used for decades as recorders of environmental variation. Elements bound within calcified structures can provide historic information on climate change (Lea et al. 2000), oceanic nutrient geochemistry (Lea et al. 1989) and El Niño Southern Oscillations (Shen & Sanford 1990).

In recent years fishery scientists have used calcified structures of fish (e.g. otoliths) and invertebrates (e.g. statoliths, shells and carapaces) to reconstruct the environmental histories of individual organisms. Theoretically, as individuals move across gradients in temperature, salinity or ambient aquatic chemistry their movements are recorded in the elemental composition of their calcified parts. Thus, elements in fish otoliths and squid statoliths have been used successfully as

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natural signatures of stock structure (e.g. Campana et al. 2000), as records of dispersal and migration pathways (e.g. Ikeda et al. 2003) and for identifying spawning and nursery grounds (e.g. Gillanders & Kingsford 2003).

In particular, the elemental composition of the larval calcified structures of fish (Swearer et al. 1999), crabs (DiBacco & Levin 2000), bivalves (Becker et al. 2007) and gastropods (Zacherl 2005) has potential to elucidate much useful information about larval origin and dispersal trajectories. Many larvae begin forming their calcified structures at or near the site of production and, thus, potentially carry a permanent elemental signature of the site of origin. When larvae enter the water column and move through ocean masses with variable physical and chemical characteristics, these changes can be recorded in the elemental composition of calcified structures. In principle, not only can the site of origin be identified, but subsequent larval movement also can be tracked using statolith elemental signatures.

To identify any particular settler's site of origin the elemental signature of the portion of the calcified structure formed before the individual's release at or near the site of origin must be matched to the unique elemental composition of the site of origin. While the approach is straightforward, the sampling procedure is complicated. All potential source populations must first be identified and spatial variation of the signatures among those potential sources must be documented and mapped. Signatures that vary temporally may require the compilation of a library of elemental signatures over several years to assign a sample to a potential source population with confidence (Gillanders 2002). Finally, the elemental signature in the calcified structure may be analyzed in post-settlement individuals and matched to signatures of potential source populations. Because collection and analysis of samples is costly and time consuming, research would be greatly facilitated if it were possible to use environmental data such as seawater chemical composition and temperature to predict elemental signatures of potential source populations (Warner et al. 2005).

Predicting a site's elemental signature requires an understanding of the factors that influence element uptake, especially in larval structures. Laboratory culture studies have demonstrated that salinity (e.g. Chang et al. 2004), elemental composition of the culture water (e.g. Bath et al. 2000, Elsdon & Gillanders 2003), temperature (e.g. Bath et al. 2000, Zacherl et al. 2003) and diet (Farrell & Campana 1996) can affect the elemental composition of otoliths and statoliths. Single factor laboratory studies are useful for developing general rules about element uptake, but it can be difficult to trace the effects of factors in the field where calcified

structures are probably influenced by multiple interacting factors. Accordingly, several laboratory culture studies of the otoliths and statoliths of larvae (e.g. Zacherl et al. 2003, Bath Martin & Thorrold 2005) and juveniles (e.g. Milton & Chenery 2001, Elsdon & Gillanders 2004) have examined the interactive effects of 2 or more of the above factors. However, only one study attempted to rank the relative importance of the factors studied. Using backwards step-wise regression, Elsdon & Gillanders (2004) eliminated the effect of salinity and determined that temperature and ambient element concentration were the most important factors that affected the barium (Ba) and strontium (Sr) ratios to calcium (Ca) in black bream *Acanthopagrus butcheri* otoliths. If efforts to predict elemental signatures are to be successful, a much more precise understanding of the influence and relative importance of multiple factors must be obtained.

Not only is predicting elemental signatures a complex undertaking, but even interpreting patterns of variation observed in the field is challenging, despite the knowledge gained from laboratory studies. Several field studies have demonstrated spatial variation in the chemistry of larval calcified structures (e.g. DiBacco & Levin 2000, Warner et al. 2005, Zacherl 2005) and that temporal variation is often sufficiently stable for discriminating among individuals produced at different sites (e.g. Zacherl 2005). There has also been some success in field studies with attempts to correlate salinity or temperature with Ba and Sr in calcified structures (e.g. Ikeda et al. 1999). However, correlating the elemental content of seawater with elemental signatures in calcified structures remains elusive despite overwhelming evidence from laboratory studies demonstrating the importance of this factor. The challenge is that the chemistry of calcified structures reflects an integrated signal developed over the entire period of formation when the organism had been exposed to multiple and changing environmental factors. Therefore, a single water sample collected at a particular point in time may not be reflected in the chemistry of the calcified structure. One ambitious field study (Warner et al. 2005) attempted to link seawater and otolith chemistry with the goal of using seawater chemistry as a proxy for larval otolith signatures. Unfortunately, for all but one element (manganese), neither of the 2 physical proxies examined, including seawater elements and a resin-based element accumulator that can sample several trace elements for up to several weeks (Zhang & Davison 1995) showed even qualitative geographic patterns that coincided with those found in larval otoliths.

Failure to find clear links between seawater chemistry and the elemental composition of calcified structures leaves open the possibility that other, yet uniden-

tified, factors may be generating variation among sites. For example, maternal transfer of elements to the progeny's calcified structures via yolk has been suspected (Brophy et al. 2004, Ruttenberg et al. 2005, Chittaro et al. 2006); element enrichment was found in the cores of juvenile otoliths in several species of fish and it was speculated that, among other factors, the enrichment was due to maternal contributions of elements to the eggs. Building on work by Rieman et al. (1994) and Limburg et al. (2001) who initially investigated transgenerational marking, Thorrold et al. (2006) provided conclusive evidence for the maternal transfer of elements via yolk. They injected both a benthic species and a broadcast spawning species of fish with ^{137}Ba and detected elevated levels of ^{137}Ba in the resulting progeny. Maternal contributions could generate site-specific elemental signatures in larval structures due to spatial variation in factors such as maternal diet, bioavailability of elements to mothers and physiological differences among populations. Therefore, as larvae develop they may incorporate elements contained in the yolk into their calcified structures. The resultant chemistry of the calcified structure then might be at least partially independent of environmental conditions experienced by the larva as the calcified structure forms. Thus, elemental signatures of calcified structures might be reflective of both environmental conditions at the site of development and maternal contributions.

In controlled laboratory experiments, we cultured larvae in 3 seawater treatments (seawater collected from 3 field locations) to determine whether egg source, independent of subsequently experienced environmental factors (e.g. temperature, ambient elemental concentration), affects the elemental signatures of larval statoliths in the whelk, *Kelletia kelletii*. In addition we tested whether interactions between egg source, temperature and ambient elemental concentration exist. We also tested whether elements deposited in *K. kelletii* eggs are correlated with elements incorporated in larval statoliths. Finally, given the variation measured for the conditions in this study, we determined the relative importance of each of the 3 factors examined.

MATERIALS AND METHODS

Study organism. *Kelletia kelletii* is a predatory buccinid marine gastropod, commonly found on subtidal rocky reefs from Baja California, Mexico, to Monterey Bay, California, USA (Herrlinger 1981). This species was ideal for this study because (1) larvae are deposited in egg capsules on hard substrata, making them easy to manipulate, (2) eggs may have a sufficient

amount of yolk to make maternal transfer of elements a possibility, (3) hatchlings have well developed statoliths and shell before release into the plankton, but the statoliths do not develop until about 2 wk after egg capsules are laid when they are at post-trochophore stages and (4) planktonic duration is between 6 and 8 wk (M. Romero unpubl. data), allowing enough time for substantial larval exchange among populations. Females lay clutches of 20 to 80 egg capsules (Zacherl et al. 2005), each containing between 400 and 1000 eggs (Rosenthal 1970). Larvae develop in egg capsules for about 30 d before they hatch into the water column as veligers that continue to feed on both yolk and phytoplankton (D. C. Lloyd pers. obs.).

Experimental design. Using eggs and seawater collected from 3 sites in southern California, we set up a factorial experiment with all possible combinations of egg source, culture seawater and culture temperature (10, 14 and 18°C) to examine the individual and interactive effects of these factors on the elemental signatures of statoliths of *Kelletia kelletii* larvae. Culture seawater (collected in acid leached 208.2 l plastic drums) and egg capsules (collected in Ziplock bags) were collected at (1) Salta Verde Point (CAT) on the south side of Santa Catalina Island, (2) Los Angeles Harbor (LA) and (3) White Point (PV) off the Palos Verdes peninsula (Fig. 1); Table 1 summarizes all collection dates and locations. The sites were chosen based on the assumption that chemical and physical characteristics in the seawater would vary substantially enough to influence statolith chemistry. Salta Verde Point is about 55 km from the mainland and does not receive direct runoff from the few developed

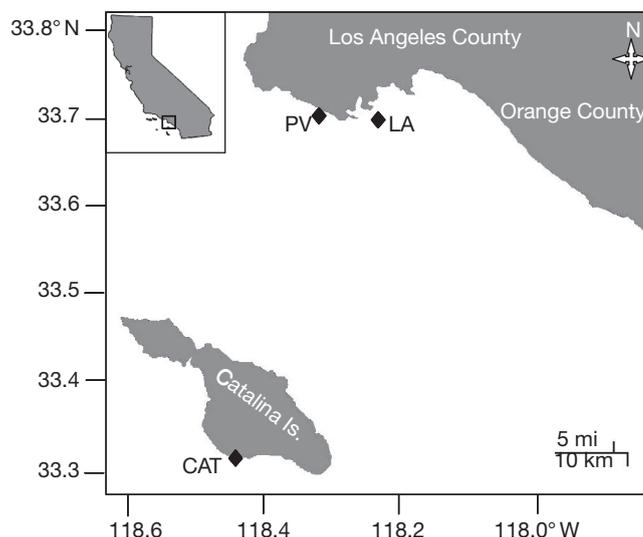


Fig. 1. Collection sites in southern California: CAT = Salta Verde Point, Catalina Island; LA = Los Angeles Harbor; PV = White Point, Palos Verdes peninsula

Table 1. *Kelletia kelletii*. Field site coordinates, mean, highest and lowest site temperatures (T, °C), and culture seawater and egg capsule collection dates (month/day/year). Water temperatures recorded at 12.5 ± 1.5 m, Water = seawater used for cultures collected at 3.0 ± 0.5 m (see Fig. 1 and text for description of site abbreviations)

Site	Location	Mean T	Highest T	Lowest T	Water	Egg capsules
CAT	33°19.188'N, 118°27.160'W	14.2 ± 1.6	17.91	11.33	6/2/05	6/7/05
LA	33°42.631'N, 118°19.060'W	13.7 ± 2.1	19.84	10.38	6/6/05	6/9/05
PV	33°42.671'N, 118°14.660'W	12.9 ± 1.8	19.86	10.85	6/3/05	6/8/05

areas on the island, and, thus, we expected this site to be mostly free of metals associated with municipal runoff. Los Angeles Harbor is a large commercial port with constant boat traffic. In addition, the harbor receives industrial and urban runoff (nonpoint source pollution) from both the Dominguez and Los Angeles River watersheds that combine to include the majority of the Los Angeles basin. Thus, we presumed that the LA site would be characterized by high levels of metal contaminants (e.g. barium, lead and zinc) associated with industrial and municipal runoff. In addition to industrial and urban runoff, White Point is associated with a sewage outfall that discharges approximately 1.2×10^{10} l d⁻¹ (Stull 1995) and is approximately 3 km offshore. Sewage outfall often contains elevated levels of zinc and cadmium (Oakden et al. 1984). Significant upwelling is possible at this site because the shelf extends approximately 3 km offshore to the San Pedro Escarpment where it drops abruptly down to approximately 800 m into the San Pedro basin (Maptech Inc. [www.freeboatingcharts.com] 2005, San Pedro Channel [map], no. 18746, Edn 36). Following upwelling events, elevated levels of barium and/or manganese could be possible at this site.

Seawater was collected at a depth of 3 ± 0.5 m with a PTFE tube attached to an acetal thermoplastic foot valve (Standard Flow D-25 by WATERRA), which was agitated vertically by hand to pump the seawater upward. As seawater was collected it was pre-filtered at the inlet with a 100 µm mesh screen, filtered to 0.45 µm mid-stream and finally filtered to 0.22 µm just before the outlet. Seawater was also sampled with a 3% nitric acid rinsed horizontal Van Dorn bottle (Wildlife Supply) at 3 ± 0.5 m for element analysis. Seawater for the culture experiments was stored in acid leached 208.2 l plastic containers and the cultures were maintained in acid rinsed 250 ml plastic culture flasks (Falcon™ polystyrene with plug-seal cap).

Egg capsules were collected within a few days after they had been laid (indicated by the lack of larval pigmentation and a creamy appearance) to avoid the possibility of newly forming statoliths. Larvae of *Kelletia kelletii* begin forming their statoliths approximately 2 wk (D. C. Zacherl pers. obs.) after egg capsules are laid, at which point larval pigmentation is clearly visible. After egg capsules from each site were collected they were maintained in the laboratory at 14°C in 0.2 µm filtered seawater collected from the pier at the Scripps Institution of Oceanography in San Diego until the culturing experiment was started on June 14, 2005; egg capsules

from CAT, LA and PV were maintained in these conditions for 7, 5, and 6 d, respectively, and none of the larvae had developed any calcified structures when the experiment began. At the beginning of the culturing period encapsulated larvae from all the sites were confirmed to be at pre-trochophore to early trochophore stage by microscopic observation (10× power) and through light diffraction filters that allowed for easy detection of calcium carbonate; calcified structures were not present in any pre-culture larvae. All possible combinations of treatments with egg capsules and culture seawater from each site, and 3 temperatures (10, 14 and 18°C) were represented in the experiment for a total of 27 treatment groups. These temperatures were chosen because they bracket the full range of temperatures experienced by *K. kelletii* in the field throughout its range.

Experiments were performed in temperature controlled growth chambers under ~ 9000 W m⁻² provided by a 15 W fluorescent tube with 12:12 h light:dark cycles. To ensure the range of temperatures in our culturing experiment bracketed those among field sites, TidBit® temperature loggers were placed at each site (June 2005) at a depth of 12.5 ± 1.5 m and temperature recorded every 3 min for the month of peak larval development in the field.

Egg capsules from each site were detached at the peduncle and separated into 3 containers with equal representation from all clutches. Each of the 3 containers was then randomly assigned a temperature treatment. Five egg capsules were chosen haphazardly from each of the 3 sets of egg capsules and placed in one of 5 replicate jars for each combination of treatments with culture seawater. Thus, we ensured all treatments with eggs from a site had equal representation of clutches. Fifty percent water changes were performed 3 times a week with seawater corresponding to the assigned treatment. During each water change

seawater from a randomly selected group of 4 or 5 flasks was sampled to test for changes in the concentration of seawater elements over the course of the experiment. The 50 ml seawater samples were immediately acidified with 50 μ l of 12 N HCl and stored at 4°C. Larvae were cultured until egg capsules appeared dark grey in color and the veliger larvae could be observed moving within the capsule, indicating that they were about to hatch; egg capsules were then frozen for subsequent analysis.

Statolith extraction. The statolith isolation and cleaning steps were performed using standard clean lab procedures. All glassware was cleaned with Citranox™ soap, rinsed 5 times with distilled H₂O (resistivity >2 M Ω cm), soaked overnight in 1 N trace metal grade HCl and then rinsed 5 times with ultrapure H₂O (resistivity >18.1 M Ω cm). Egg capsules were handled with acid rinsed Teflon forceps where practicable, but were also handled with polyethylene gloves when necessary. We analyzed 1 egg capsule from each replicate jar resulting in 5 replicate samples per treatment (egg source \times culture seawater \times temperature). Egg capsules were rinsed with ultrapure H₂O (resistivity >18.1 M Ω cm) before the statoliths were extracted; this served to remove any exterior debris and thaw the capsule.

Larvae were removed from the capsule by cutting the peduncle with a sterile scalpel and squeezing the larvae out onto a clean glass slide that had been rinsed with ultrapure H₂O. They were then immediately transferred to an acid-washed 50 ml beaker by squirting with ultrapure H₂O to a total volume of 5 ml. Five milliliters of an equal volume mixture of Ultrapure 30% H₂O₂ buffered with 1 N NaOH (hereafter referred to as the 'cleaning solution') were added to the beaker and heated at 65°C for 12 min. After heating the statoliths dropped out of solution to the bottom of the beaker and were concentrated in the middle of the beaker by vigorous swirling. The remaining steps were performed in a Class 100 (equiv. ISO Class 5) laminar flow hood.

The statoliths were pipetted into a new acid leached 50 ml beaker and the cleaning solution was aspirated down to a volume of 10 ml. The beaker was then filled to 40 ml with ultrapure H₂O and aspirated down again for a total of 5 rinses, after which the statoliths were pipetted onto an acrylic plastic slide. Excess water was pipetted off the statoliths and the slide was stored in a sterile, plastic 35 \times 10 mm Petri dish and allowed to dry overnight in a Class 100 laminar flow hood. Just before analysis statoliths were mounted onto double sided tape (Scotch™) and secured to the slide for laser ablation.

Statolith element analysis. We assayed for a suite of 8 elements: calcium-48 (Ca), magnesium-24 (Mg), strontium-86 (Sr), barium-138 (Ba), lead-208 (Pb),

manganese-55 (Mn), zinc-66 (Zn) and cerium-140 (Ce). All elements except Ce reliably yielded detectable concentrations in statolith samples, thus, Ce was subsequently excluded from analyses. Ten haphazardly chosen statoliths from each slide were introduced via a VG-UV microprobe Nd:YAG laser, frequency-quadrupled to 266 nm with a nominal beam width of 20 μ m, into a Finnigan Element 2 sector field inductively coupled plasma mass spectrometer (ICP-MS) using a micro-flow nebulizer at 20 μ l min⁻¹. Statoliths were completely consumed by the ablation (see Zacherl et al. 2003 for further discussion about statolith ablation). Sensitivity was approximately 1×10^6 counts s⁻¹ for 1 ppb indium (In). Samples from each treatment were analyzed randomly to minimize the effects of instrument drift on the results. The plasma conditions were kept constant during analysis of standards, instrument blanks and laser-ablated samples by constantly aspirating a 1% HNO₃ solution, which was the matrix for both the standards and the blanks (see Zacherl et al. 2003). Matrix-matched, solution-based standards of known element-to-calcium ratio and a mass bias correction applied to the instrument were used to determine the element-to-calcium ratio of the sample (Rosenthal et al. 1999). To check the accuracy and precision of our solution-based measurements we used a consistency standard containing Ca, Mg, Sr, Ba and Mn (Spex Certified primary standard solutions): Mg:Ca (mmol mol⁻¹) = 3.87%, Sr:Ca (mmol mol⁻¹) = 2.64%, Ba:Ca (μ mol mol⁻¹) = 8.97%, Mn:Ca (mol mol⁻¹) = 5.52% (N = 244). We also used National Institute of Standards and Technology (NIST)-612 glass standards as reference materials from which we could estimate the precision of the laser ablation method. The repeatability (relative SD, % rSD) of the method was determined using the results of laser ablated NIST-612 standard reference material except for Sr:Ca, which was determined using an unspecified otolith material: Mg:Ca (mmol mol⁻¹) = 18.8%, Sr:Ca (mmol mol⁻¹) = 9.09%, Ba:Ca (μ mol mol⁻¹) = 14.4%, Pb:Ca (μ mol mol⁻¹) = 14.6%, Mn:Ca (μ mol mol⁻¹) = 14.7%, Zn:Ca (μ mol mol⁻¹) = 38.0% (N = 48 for all elements except Zn where N = 47).

We quantified element concentrations using our matrix-matched standard solution instead of the NIST standard because the NIST glass lacks the aragonite-dominated matrix of statoliths. Our use of solution-based standards is robust as suggested by the quantitative agreement between aragonite-dominated matrix-matched materials analyzed in solution versus laser-based modes (Thorrold et al. 1997). It is important to note, however, that the lack of certified matrix-matched solid reference materials means that our data should be compared with other studies with caution

(Campana 1999). Following Swearer et al. (2003) we calculated detection limits for each element by calculating the standard deviation (SD) of the intensities of the elements in 1% nitric acid (HNO₃) instrument blanks and then multiplying the SD by 3. That value was then added to the blank mean for each element and used as the minimum detectable signal. The intensities of blank-subtracted samples averaged >100 times the detection limit for ⁴⁸Ca, >30 times for Mg, >20 times for Sr, >30 times for Zn, >8 times for Ba, >1.5 times for Pb and >3 times for Mn. To control for differences in statolith sample mass we analyzed for the ratio of each element to ⁴⁸Ca, which is consistent with the convention for trace element analysis in otoliths and statoliths (e.g. Campana 1999).

Intra-capsular contents analysis. Capsules from 10 clutches of eggs frozen before development of any calcified structures (i.e. pre-trochophore) were randomly chosen from each of the 3 sites (CAT, LA and PV). One egg capsule was haphazardly chosen from each clutch (n = 10 capsules per site) for analysis. The intra-capsular contents were removed from each individual egg capsule (as described previously for the larvae), dried in an oven for 16 h at 65°C and weighed. For analysis via ICP-MS the intra-capsular contents of each egg capsule were then digested in ultra-pure HNO₃ and diluted. We did not keep track of which clutch each egg capsule was from; thus, we were unable to analyze the results for each capsule individually, and samples from each site had to be pooled for statistical analysis. To ensure quality data a method of standard additions (Spex Certified primary standard solutions) with internal standards including scandium (Sc), yttrium (Y) and indium (In) was used. Scandium was the internal standard for Mg and Ca, Y was the internal standard for Sr, and In was the internal standard for Ba and Pb. Since the CAT samples were much larger than the LA or PV samples, it was necessary to dilute CAT digests to 100×, while LA and PV digests were diluted to 10×.

Seawater analysis. Both the laboratory and field seawater samples were analyzed via ICP-MS using serial dilution of the samples. For barium we applied a method of isotope dilution using a standard enriched in ¹³⁵Ba (Oak Ridge National Laboratory Ba(CO₃)₂ salt), and determined the ¹³⁵Ba/¹³⁸Ba ratio. For the other elements analyzed we used a method of standard additions (Spex Certified primary standard solutions) with internal standards; Sc was the internal standard for Ca, Mn and Zn, Y was the standard for Sr, and In was the standard for Pb. We have not yet developed a method to analyze for Mg in seawater; thus, no Mg data were collected for the culture seawater samples.

Statistics. Effects and possible interactions of egg source, culture seawater and temperature on element-

to-calcium ratios in statoliths were analyzed via a nested 3-way ANOVA for each element; replicate statoliths on a slide (from a single egg capsule) were nested and randomized within each treatment. We then compared differences among sites in statolith elemental ratios to calcium using a Tukey's multiple-comparisons procedure. We also tested for differences among sites in element-to-calcium ratios in the intra-capsular contents via a 1-way ANOVA followed by Tukey's multiple comparisons. Finally, we tested for differences in element-to-calcium ratios in the culture seawater among sample dates and among sites using a 2-way ANOVA followed by a Tukey's multiple comparisons procedure. Data that did not meet ANOVA assumptions were log- or log(x + 1)-transformed, and included all elements in the statolith data, Pb and Zn in the seawater data and the Ba in the intra-capsular data. Regression analyses tested for relationships between culture seawater and statolith elemental ratios, temperature and statolith elemental ratios, and intra-capsular and statolith elemental ratios. Statistical analyses were conducted using SAS statistical software (version 9.1).

To determine which of the factors was most influential on statolith chemistry within the constraints of the variation measured in our experiment, we estimated the relative effect size of each factor for each element analyzed using partial η^2 , which is estimated by:

$$\eta^2 = \frac{\text{SS effect}}{(\text{SS effect} + \text{SS error})}$$

This is a measure of the percentage of variance explained by a particular factor (Grissom & Kim 2005). To avoid complications from the mixed model, we simplified the analysis by calculating the mean statolith elemental concentration per culture flask and performed a 3-factor ANOVA; in all cases the results corresponded to the nested 3-way models. This simplification also allowed us to calculate 95% confidence intervals (CIs) for the measures of effect size. We calculated exact CIs based on the non-central *F* distribution (Fidler & Thompson 2001). However, since this method assumes balanced data and our data were unbalanced, we also calculated 95% CIs using a bootstrap resampling procedure in R (version 2.4.0; Roff 2006). Bootstrap samples were generated in proportion to the number of jars within a particular combination of treatments (e.g. eggs from CAT, water from LA, cultured at 18°C) and 1000 bootstrap samples were used to generate percentile-based CIs for η^2 . Since the data were slightly unbalanced and the bootstrap estimates were similar to the exact intervals, we only present the bootstrap intervals.

RESULTS

Culturing conditions

Element-to-calcium ratios in the culture seawater were significantly ($p < 0.05$) different among sites for Ba:Ca ($F_{2,64} = 11.86$, $p < 0.0001$), Pb:Ca ($F_{2,64} = 7.61$, $p = 0.0011$) and Mn:Ca ($F_{2,64} = 25.06$, $p < 0.0001$), but not for Sr:Ca ($p = 0.8380$) or Zn:Ca ($p = 0.1896$) (Fig. 2). Because we archived one water sample from each site for use in the cultures, we tested for differences in elemental ratios over storage time. Culture seawater samples showed no significant change over time in the elemental ratios of Ba:Ca ($p = 0.3923$), Pb:Ca ($p = 0.1925$) or Sr:Ca ($p = 0.0781$), but did show a significant change over time for Mn:Ca ($F_{18,64} = 4.16$, $p < 0.0001$) and Zn:Ca ($F_{18,64} = 2.01$, $p = 0.0219$). Temperatures in the growth chambers were stable at the set temperatures (10.45 ± 1.05 , 14.44 ± 0.79 and $18.87 \pm 0.86^\circ\text{C}$) and

bracketed the full range of temperatures measured in the field (Table 1).

Factorial experiment

Egg-source effects. Egg source significantly ($p < 0.05$) affected the elemental signatures of Mg:Ca, Ba:Ca and Pb:Ca in statoliths of laboratory cultured *Kelletia kelletii* larvae (Fig. 3, Table 2). Average values of Mg:Ca and Ba:Ca in statoliths over all treatments plotted against egg source indicate that Mg:Ca and Ba:Ca were higher in statoliths from CAT regardless of the origin of the culture seawater or the temperature treatment (Fig. 3). Similarly Pb:Ca was lowest in statoliths from LA (Fig. 3), which is contrary to our original assumptions. Elemental ratios not significantly affected by egg source included Sr:Ca, Zn:Ca and Mn:Ca, despite the fact that Mn:Ca was significantly different in seawater among sites.

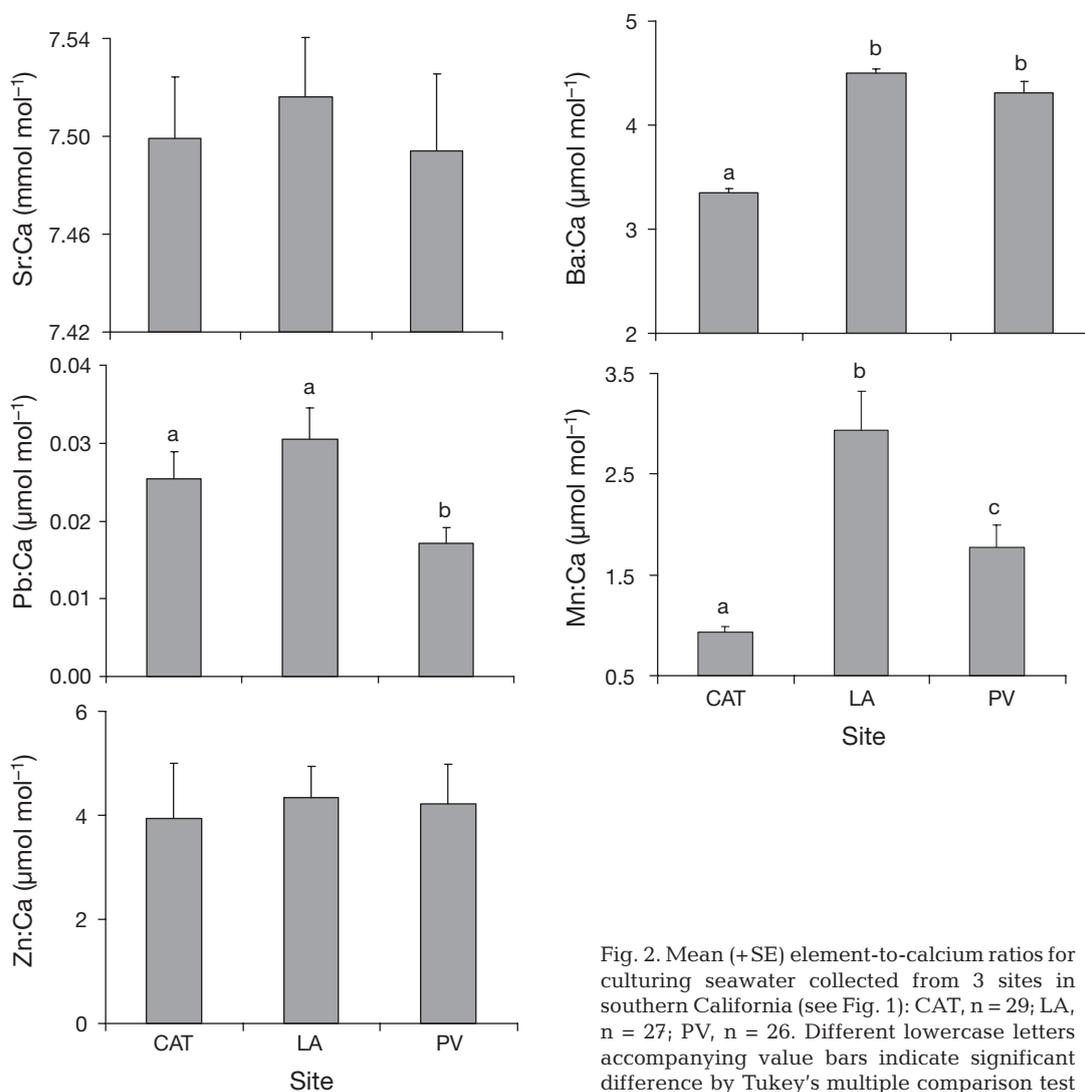


Fig. 2. Mean (+SE) element-to-calcium ratios for culturing seawater collected from 3 sites in southern California (see Fig. 1): CAT, $n = 29$; LA, $n = 27$; PV, $n = 26$. Different lowercase letters accompanying value bars indicate significant difference by Tukey's multiple comparison test

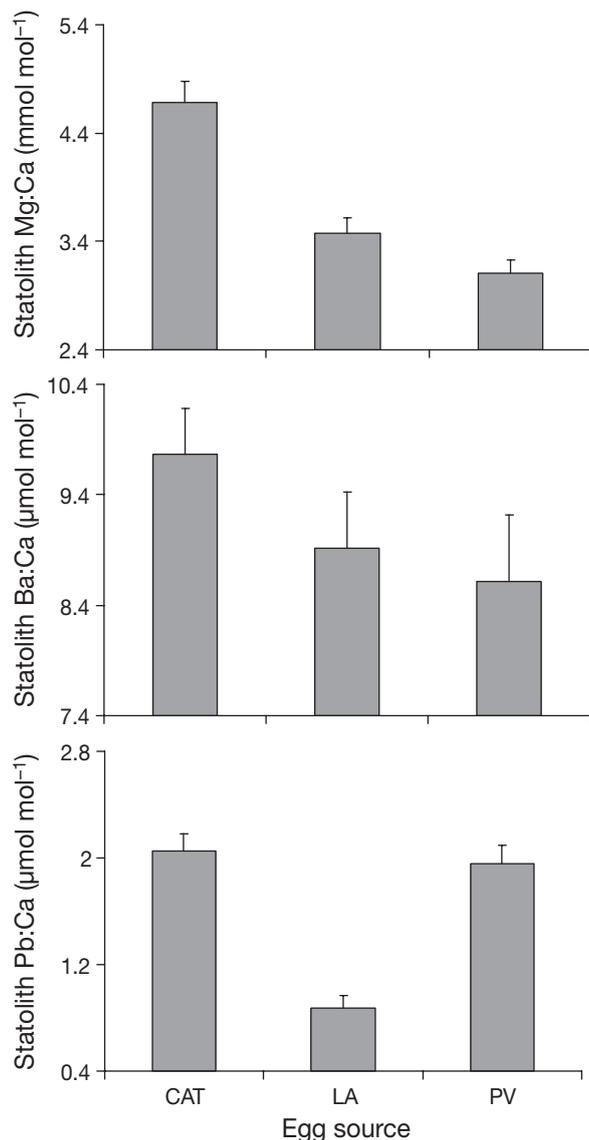


Fig. 3. *Kelletia kelleitii*. Mean (+SE) element-to-calcium ratios in statoliths of laboratory reared larvae plotted against egg source. Means are over all temperature and culture seawater treatments for larvae from each site

Seawater effects. Culture seawater significantly ($p < 0.05$) affected larval statolith element signatures for Ba:Ca and Pb:Ca, but did not significantly affect elemental signatures for Mg:Ca, Sr:Ca, Mn:Ca or Zn:Ca (Table 2). We found significant relationships between element-to-calcium ratios in larval statoliths and element-to-calcium ratios in the culture seawater Ba:Ca ($F_{2,1161} = 8.53$, $p = 0.007$) and Pb:Ca ($F_{2,1161} = 11.11$, $p = 0.003$). Elemental ratios in statoliths averaged over all temperature and egg-source treatments plotted against culture seawater elemental ratios showed positive trends (Fig. 4a,b). Although seawater Mn:Ca was

Table 2. *Kelletia kelleitii*. Nested 3-way ANOVA of statolith elemental ratios from larvae cultured in treatments of 3 factors: E = egg source, T = temperature, W = culture seawater. Nested within each treatment are 10 statoliths ablated per slide, $n = 5$ for each treatment. Significant p-values (< 0.05) in **bold**

Element/Factor	df	SS	MS	F	p
Mg:Ca					
E	2	35.67	17.84	21.93	<0.0001
T	2	1.77	0.88	1.09	0.3412
W	2	0.34	0.17	0.21	0.8110
E × T	4	7.59	1.90	2.33	0.0607
E × W	4	3.39	0.85	1.04	0.3893
T × W	4	1.53	0.38	0.47	0.7568
E × T × W	8	1.75	0.22	0.27	0.9747
Statolith (E × T × W)	102	82.98	0.81	20.66	<0.0001
Error	1161	45.72	0.04		
Sr:Ca					
E	2	0.09	0.04	0.78	0.4630
T	2	6.36	3.12	55.46	<0.0001
W	2	0.01	0.01	0.10	0.9044
E × T	4	0.10	0.02	0.43	0.7865
E × W	4	0.17	0.04	0.75	0.5618
T × W	4	0.05	0.01	0.20	0.9378
E × T × W	8	0.34	0.04	0.73	0.6629
Statolith (E × T × W)	102	5.85	0.06	6.69	<0.0001
Error	1161	9.95	0.01		
Ba:Ca					
E	2	5.02	2.51	4.10	0.0194
T	2	34.30	17.15	27.98	<0.0001
W	2	14.25	7.12	11.62	<0.0001
E × T	4	3.22	0.81	1.31	0.2697
E × W	4	0.42	0.11	0.17	0.9517
T × W	4	1.91	0.48	0.78	0.5416
E × T × W	8	4.62	0.58	0.94	0.4856
Statolith (E × T × W)	102	62.54	0.61	17.13	<0.0001
Error	1161	41.56	0.036		
Pb:Ca					
E	2	54.10	27.05	36.75	<0.0001
T	2	7.12	3.56	5.16	0.0073
W	2	7.09	3.55	4.82	0.0100
E × T	4	2.36	0.59	0.80	0.5278
E × W	4	1.13	0.28	0.38	0.8203
T × W	4	1.70	0.43	0.58	0.6794
E × T × W	8	2.52	0.31	0.43	0.9023
Statolith (E × T × W)	102	75.08	0.74	30.48	<0.0001
Error	1161	28.03	0.02		
Mn:Ca					
E	2	1.10	0.55	0.43	0.6497
T	2	2.06	1.03	0.81	0.4464
W	2	1.49	0.75	0.59	0.5567
E × T	4	1.02	0.25	0.20	0.9373
E × W	4	2.55	0.64	0.50	0.7336
T × W	4	6.83	1.71	1.35	0.2575
E × T × W	8	7.25	0.91	0.72	0.6773
Statolith (E × T × W)	102	129.15	1.27	12.52	<0.0001
Error	1161	117.40	0.10		
Zn:Ca					
E	2	16.05	8.03	1.92	0.1524
T	2	16.53	8.26	1.97	0.1443
W	2	1.35	0.68	0.16	0.8511
E × T	4	8.08	2.02	0.48	0.7487
E × W	4	3.71	0.93	0.22	0.9259
T × W	4	3.52	0.88	0.21	0.9323
E × T × W	8	27.22	3.40	0.81	0.5932
Statolith (E × T × W)	102	427.23	4.19	38.12	<0.0001
Error	1161	127.55	0.11		

different among sites, statolith Mn:Ca was not, and there is no relationship between statolith and seawater Mn:Ca ($F_{2,1161} = 0.01$, $p = 0.914$; Fig. 4c).

Temperature effects. Temperature significantly ($p < 0.05$) affected the element-to-calcium ratios in larval statoliths for Sr:Ca, Ba:Ca and Pb:Ca, but did not affect statolith elemental ratios of Mg:Ca, Mn:Ca or

Zn:Ca (Table 2). We found significant relationships for Sr:Ca ($F_{2,1161} = 119.34$, $p < 0.0001$), Ba:Ca ($F_{2,1161} = 27.23$, $p < 0.0001$) and Pb:Ca ($F_{2,1161} = 4.50$, $p = 0.044$). Element-to-calcium ratios in larval statoliths averaged over all egg-source and seawater treatments plotted against temperature showed strong negative trends (Fig. 5).

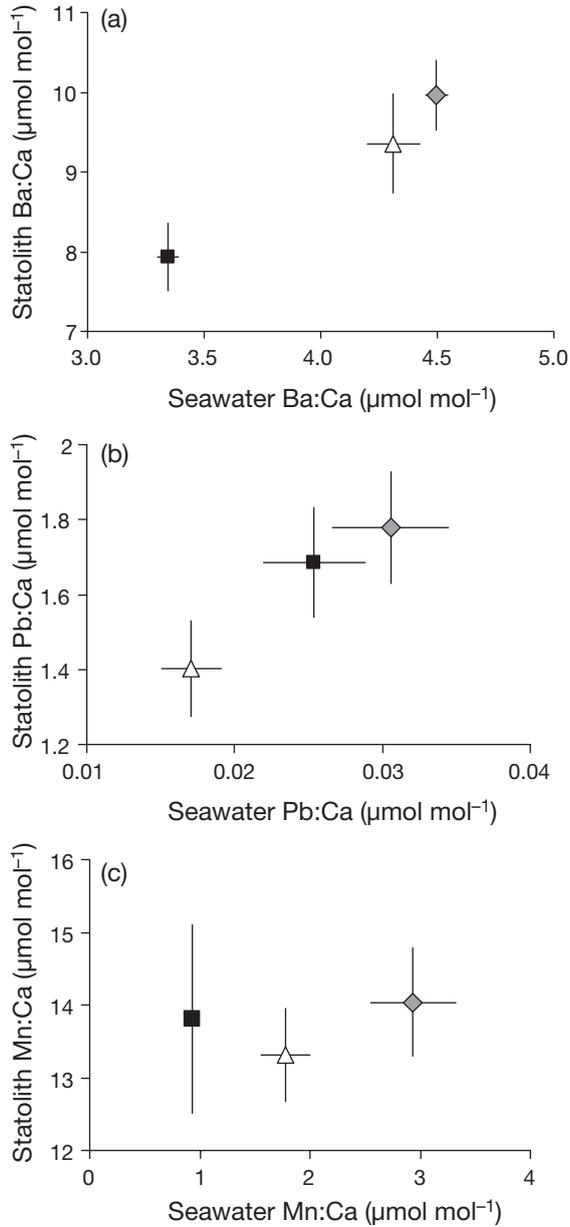


Fig. 4. *Kelleteria kelleteria*. Mean (\pm SE) element-to-calcium ratios of larval statoliths plotted against element-to-calcium ratios in culture seawater for (a) Ba:Ca ($F_{1,25} = 8.53$, $p = 0.0073$), (b) Pb:Ca ($F_{1,25} = 11.11$, $p = 0.0027$) and (c) Mn:Ca ($F_{1,25} = 0.01$, $p = 0.9139$); $n = 27$. Points are means over all temperature and egg-source treatments for statoliths cultured in water from each site vs. means of culture seawater from each site. Symbols indicate culture seawater source: ■ = CAT, ◆ = LA, Δ = PV

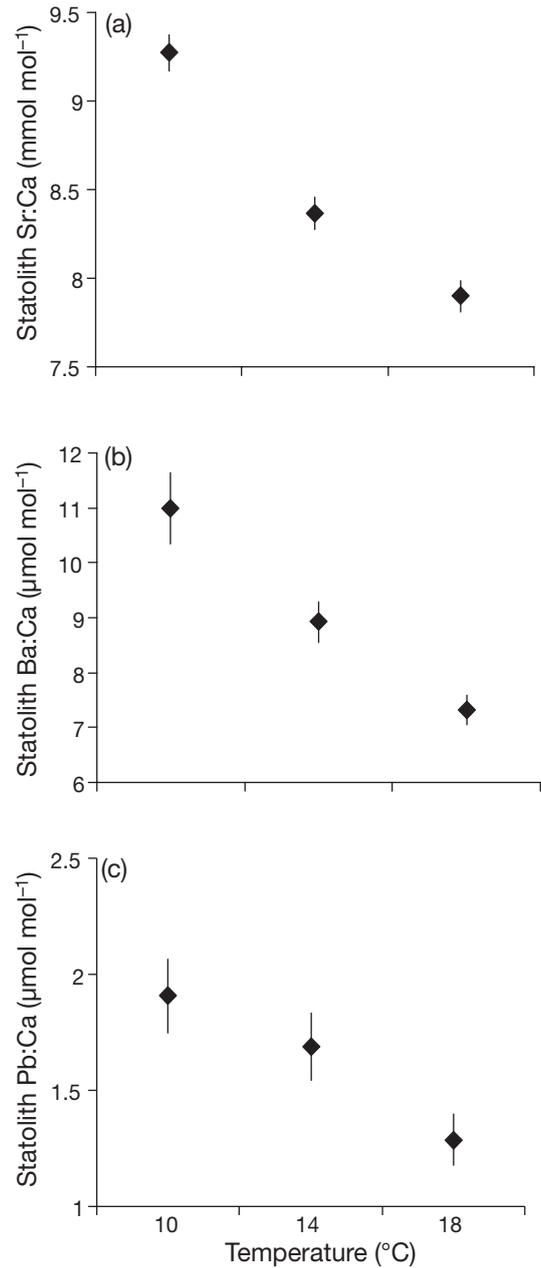


Fig. 5. *Kelleteria kelleteria*. Mean (\pm SE) element-to-calcium in larval statoliths plotted against culture temperature for (a) Sr:Ca ($F_{1,25} = 119.34$, $p < 0.0001$), (b) Ba:Ca ($F_{1,25} = 27.23$, $p < 0.0001$) and (c) Pb:Ca ($F_{1,25} = 4.50$, $p = 0.0440$); $n = 27$

Intracapsular contents

Analysis of the entire intra-capsular contents of newly laid egg capsules sampled at each site revealed that Sr:Ca ($F_{2,27} = 10.42$, $p = 0.0004$), Ba:Ca ($F_{2,27} = 34.02$, $p < 0.0001$), Pb:Ca ($F_{2,27} = 18.97$, $p < 0.0001$), Mn:Ca ($F_{2,27} = 12.06$, $p = 0.0002$) and Zn:Ca ($F_{2,27} = 4.30$, $p = 0.0238$) were significantly ($p < 0.05$) different among sites, while Mg:Ca ($p = 0.6412$) was not (Fig. 6). Relationships between element-to-calcium ratios in larval statoliths and elemental ratios in intra-capsular contents were not significant for Mg:Ca ($p = 0.376$), Ba:Ca ($p = 0.425$) or Pb:Ca ($p = 0.377$) based on ANOVA (Fig. 7).

Relative importance of factors

Both Ba:Ca and Pb:Ca in statoliths were affected by all 3 factors, including egg source, temperature and culture water (Table 3). Within the constraints of the

variation measured in this study for each factor, we found that for Pb:Ca, the most influential factor was egg source (i.e. where the eggs were laid), followed by temperature and culture seawater, although the latter 2 factors may have the same effect sizes (Table 3). In contrast, effect sizes for Ba:Ca show that temperature was the most influential factor, followed by culture seawater and egg source (Table 3). The effect size estimates for other elements confirm the significance of factors with an effect. Statolith Mg:Ca was most influenced by egg source and Sr:Ca was most influenced by temperature (Tables 2 & 3).

DISCUSSION

Egg-source effects

This is the first study to show that egg source may be a significant factor that contributes to among-site differences in natal elemental signatures of larval

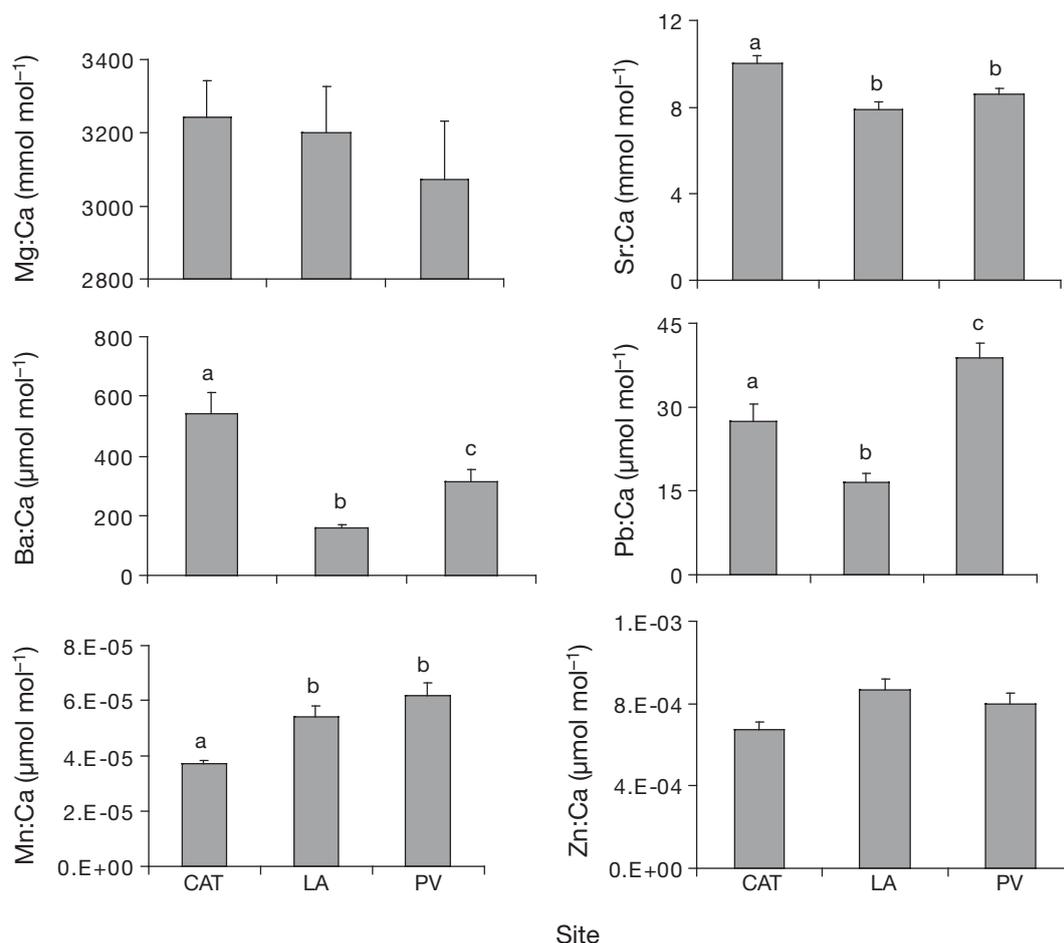


Fig. 6. Mean (+SE) intra-capsular element-to-calcium ratios across site. Different lowercase letters accompanying value bars indicate significant difference by Tukey's multiple comparison procedure

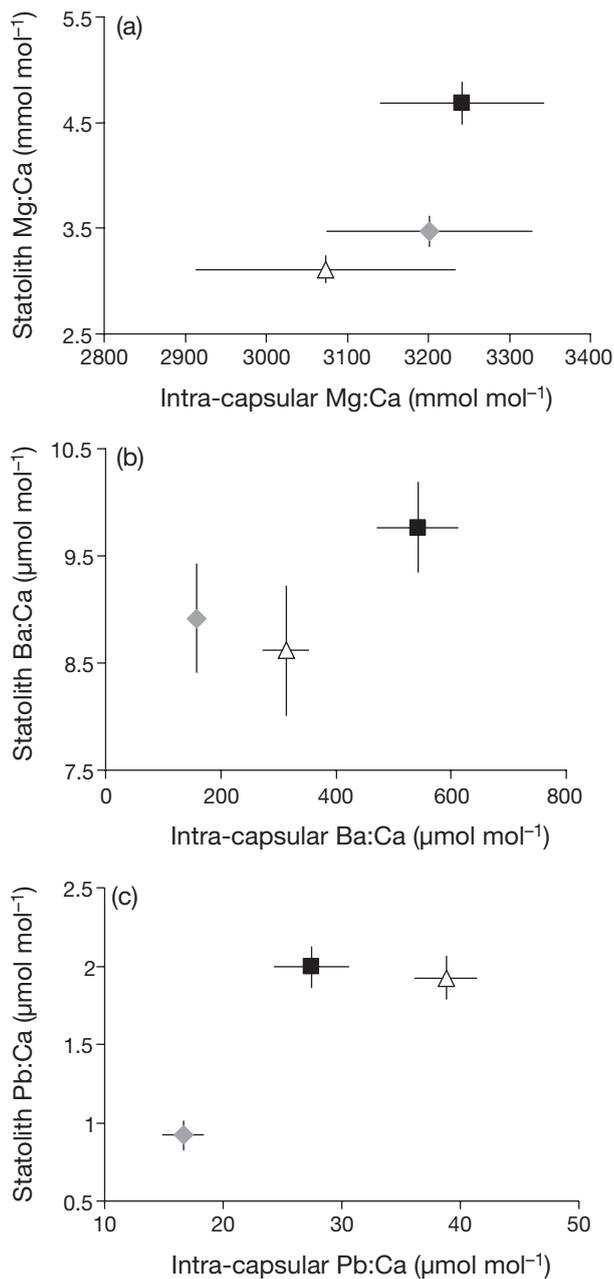


Fig. 7. *Kelleitia kelleitii*. Mean (\pm SE) element-to-calcium ratios of larval statoliths plotted against intra-capsular element-to-calcium ratios for (a) Mg:Ca ($F_{1,1} = 2.23$, $p = 0.3757$), (b) Ba:Ca ($F_{1,1} = 1.61$, $p = 0.4246$) and (c) Pb:Ca ($F_{1,1} = 2.21$, $p = 0.3773$); $n = 3$. Points are means over all temperature and culture seawater treatments for statoliths from each egg-source treatment vs. means of intra-capsular contents. Symbols indicate egg capsule source: ■ = CAT, ◆ = LA, Δ = PV

statoliths. These egg-source effects must be due to factors unique to an area or population of individuals. For example, prey items consumed by *Kelleitia kelleitii* differ among sites (D. C. Zacherl pers. obs.), and element uptake or bioaccumulation in these prey items may dif-

Table 3. Partial η^2 measure of relative importance of main effects on statolith elemental signatures. CI = 95 % confidence intervals calculated using bootstrap procedures, E = egg source, T = temperature, W = culture seawater, * $p < 0.05$

Element/Factor	Partial η^2	Lower CI	Upper CI
Mg:Ca			
E	31.80*	21.00	45.70
W	0.26	0.05	7.06
T	1.40	0.0004	3.43
Sr:Ca			
E	1.57	0.19	12.12
W	0.16	0.04	7.22
T	52.10*	38.70	69.73
Ba:Ca			
E	6.91*	2.10	21.50
W	17.35*	8.38	33.40
T	33.80*	26.40	49.50
Pb:Ca			
E	41.50*	28.40	57.80
W	8.40*	2.33	20.63
T	8.72*	2.21	21.70
Mn:Ca			
E	0.90	0.10	8.82
W	0.71	0.08	9.38
T	1.77	0.003	7.58
Zn:Ca			
E	4.04	0.30	17.68
W	0.32	0.07	6.72
T	3.97	0.002	8.62

fer depending on the prey species (e.g. Deheyn & Latz 2006). In addition, the bioavailability of elements to the mother from ambient seawater may vary among sites (e.g. Geffen et al. 2003) due to variation in the input (e.g. runoff, atmospheric deposition) and removal of elements in seawater (e.g. sorption onto substrate and suspended particles). Therefore, the maternal contribution of elements to the yolk and egg cells may be influenced by elements in the diet or water.

Compelling evidence exists to suggest that elements deposited in yolk can be incorporated into fish otoliths. Volk et al. (2000) crossed captive salmonid fishes reared in freshwater to wild anadromous salmonids and reared the progeny in a common environment. They found that the progeny's otolith Sr:Ca signature reflected the origin of the female; otoliths of larvae from freshwater females had very low levels of Sr:Ca compared with otoliths of larvae from seawater females. Volk et al. (2000) were also able to clearly distinguish between the progeny of salmon in a channel where freshwater captive adults spawned with wild anadromous adults, demonstrating that the maternal origins of salmon can be distinguished based on Sr:Ca otolith signatures. Perhaps most convincingly, Thorrold et al. (2006) demonstrated trans-generational transfer of ^{137}Ba by injecting the isotope into the abdominal cavity of gravid female

Amphiprion melanopus (benthic-spawning coral reef fish) and *Centropristis striata* (planktonic-spawning temperate reef fish) using a range of dosages (0.45 to 23 $\mu\text{g g}^{-1}$). Eggs of both species were reared to settlement in a controlled environment before otolith extraction. Analysis of embryonic otoliths revealed that larvae of both species had incorporated maternal ^{137}Ba . Transects across the otolith showed ^{137}Ba only in the embryonic core, which corresponded to the period of yolk absorption; thus, ^{137}Ba was presumably transferred via the yolk to the embryonic otoliths. Importantly, despite the fact that planktonic-spawned eggs have relatively less yolk than benthic-spawned eggs, ^{137}Ba was easily detected in embryonic otoliths of planktonic-spawned larvae.

Our results show clear egg-source effects in that Mg:Ca and Ba:Ca were higher in statoliths from CAT and Pb:Ca was lowest in statoliths from LA, regardless of the origin of the culture seawater or the temperature treatment (Fig. 3). We found no clear relationships between intra-capsular and statolith chemistry for Mg:Ca, Ba:Ca and Pb:Ca (Fig. 7); the lack of statistical significance was perhaps due to lack of statistical power from the small sample sizes used ($n = 3$). While the egg-source effect might have been partly influenced by incorporation of elements from yolk into larval statoliths (Fig. 7), some other factor obscured the relationships or the egg-source effect was due to some other aspect specific to each site. Clearly, the mechanism driving the egg-source effect warrants further investigation.

Results for Pb:Ca in samples from LA were particularly interesting. Statoliths from LA had the lowest values of Pb:Ca (Fig. 3) regardless which treatment the larvae had been cultured in, and the intra-capsular contents (Fig. 6) from LA had the lowest values of Pb:Ca. However, Pb:Ca was highest in the culture seawater from LA (Fig. 2). We speculate that perhaps a detoxification mechanism could be contributing to the low Pb baseline in adult and/or larval *Kelletia kelletii* from Los Angeles Harbor. Adaptation to heavy metals in populations of a variety of aquatic taxa (including bivalves and gastropods) exposed to polluted environments has been documented (see review by Klerks & Weis 1987). Detoxification methods known to occur in some gastropods include metal-binding proteins (e.g. Han et al. 2003) and sequestration in metal-containing granules within digestive gland cells (e.g. Mason & Nott 1981). While the populations at our study sites may be too close in proximity to produce local adaptation, perhaps phenotypic plasticity can occur within embayments such as Los Angeles Harbor. Studies examining whether *K. kelletii* exhibits any methods of heavy metal detoxification would improve our understanding of these results.

Natural element tagging has been of limited utility for larvae of broadcast spawners (but see Becker et al. 2007), because the larvae develop their calcified structures after release into the plankton and researchers have previously assumed that variation in statolith/otolith chemistry is primarily due to environmental conditions (Ruttenberg et al. 2005, Chittaro et al. 2006). Consequently, the assumption has been that larvae of planktonic spawners form calcified structures whose elemental signatures reflect environmental conditions encountered in the plankton rather than signatures representative of their natal site. However, this first empirical demonstration that egg source affects elemental signatures of larval calcified structures implies that it may even be possible to track larvae of planktonic spawners if they incorporate elements reflective of their egg source during yolk consumption. The findings that the egg-source effect on statolith Pb:Ca overwhelmed the effects due to the environment (Fig. 3) and that egg source was statistically the most significant factor influencing Pb uptake into statoliths (Table 3) provide provocative evidence that larvae of planktonic spawners might be 'tagged' by their natal site despite never spending any time there.

Culture seawater effects

We found significant positive relationships between culture seawater and statolith chemistry for Ba:Ca and Pb:Ca (Fig. 4), confirming past work that showed Ba:Ca (e.g. Zacherl et al. 2003) and Pb:Ca (Geffen et al. 1998, Milton & Chenery 2001) increase in calcified structures with increasing seawater concentration. These results contrast those found by Warner et al. (2005), who found no relationship between ambient levels of these elements and the trace element composition of natal otoliths of fish. This contrast might be due to differences in physiology between the species studied. A brief comparison of uptake of Mg:Ca, Sr:Ca and Ba:Ca in coral skeletons, bivalve shells, squid statoliths and fish otoliths showed that discrimination of elements tended to be least for the simplest animals, with more discrimination occurring in more complex animals (Campana 1999). Another explanation is that Warner et al. (2005) conducted a field study, which was subject to environmental variation with multiple factors probably affecting otolith chemistry, whereas we conducted a controlled laboratory study and were able to isolate seawater effects. Finally, the relationship we found between statolith and seawater chemistry may be an artifact of using a single sample of water from each site for the cultures, while Warner et al. (2005) used the average of several seawater samples taken at each site over time to correlate to otolith chemistry.

Warner et al. (2005) attempted to link seawater and a resin-based element accumulator as proxies for otolith elemental signatures in an open-coast fish. However, they found only Mn concentrations to be similar between otolith chemistry and the 2 physical proxies, with qualitative regional patterns being similar over 2 yr. Multiple field studies have found Mn in calcified structures useful for discriminating among individuals from different sites (e.g. DiBacco & Levin 2000, Geffen et al. 2003), and correlative studies on coral aragonite have found that Mn:Ca ratios are inversely related to upwelling intensity (e.g. Delaney et al. 1993). However, no laboratory studies have conclusively demonstrated relationships between Mn in calcified structures and Mn in seawater. We found that while Mn:Ca was significantly different in the culture seawater among 3 sites, no significant difference in statolith Mn:Ca signatures existed among the sites (Table 2), and no significant relationship existed between statolith and seawater Mn:Ca (Fig. 4c).

In fact, it has proven difficult to experimentally pinpoint the factors responsible for Mn signatures in calcified structures. Bath Martin & Thorrold (2005) found no effect of temperature or salinity on Mn:Ca ratios in otoliths. The authors detected changes in Mn:Ca over time in the culture seawater, which they attributed to bacterial oxidation of Mn that may have been responsible for part of the variation in otolith Mn:Ca. Two other studies also did not find an effect of temperature and/or salinity (Elsdon & Gillanders 2002) or seawater (Elsdon & Gillanders 2003) on Mn:Ca ratios in fish otoliths. Our results are similar to the results in those studies. As with Bath Martin & Thorrold (2005), Mn:Ca in our culture seawater samples was significantly different among experiment days, with Mn:Ca decreasing over time. Microbial oxidation rapidly oxidizes dissolved Mn in marine systems (e.g. Rosson & Nealson 1982), and we observed some bacterial growth in the culture flasks despite regular 50% water changes. As in Bath Martin & Thorrold (2005), bacterial oxidation may have occurred during our experiment causing dissolved Mn to decrease over time, meaning our results concerning Mn must be interpreted with caution.

The crystal structure of calcium carbonate affects incorporation of elements in bivalve shells (Onuma et al. 1979). The most common forms of biogenic carbonates are orthorhombic aragonite and rhombohedral calcite (White et al. 1977). Elements with an ionic radius smaller than Ca^{2+} , including Mn^{2+} and Zn^{2+} , are more likely to fit within a calcite lattice, while elements with an ionic radius greater than Ca^{2+} are more likely to substitute for Ca^{2+} in an aragonite lattice (Speer 1983). Brophy et al. (2004) reasoned that the enrichment of Mn in the otolith core could be a result of the otolith being composed of calcite in the core and then

shifting to aragonite outside the core. Although the crystal structure of *Kelletia kelletii* statoliths has not been determined, relative concentrations of Mg^{2+} , Sr^{2+} , Ba^{2+} and Pb^{2+} suggest that aragonite formation would be favored over calcite (Carlström 1963). Statoliths composed mostly of aragonite would be less likely to incorporate Mn, and this may at least partly explain why statolith Mn:Ca did not differ among sites even though seawater Mn:Ca did.

Temperature effects

We found strong, significant, inverse relationships between temperature and statolith Sr:Ca, Ba:Ca and Pb:Ca (Fig. 5). Our results support past research that shows Sr:Ca and Ba:Ca vary inversely with temperature in gastropod statoliths (Zacherl et al. 2003). In addition, to our knowledge, we have provided the first empirical demonstration of a negative relationship between statolith Pb:Ca and temperature. Since Pb^{2+} and Sr^{2+} have similar ionic radii, Pb^{2+} might behave similarly to Sr^{2+} during incorporation in CaCO_3 . Our results are consistent with circumstantial evidence showing that average core Pb: ^{43}Ca signatures in European hake *Merluccius merluccius* otoliths were 1 order of magnitude higher at sites in the northeast Atlantic Ocean with a mean annual temperature of 8.9°C than in otoliths of fish from the western Mediterranean Sea with a mean annual temperature of 13.7°C (Morales-Nin et al. 2005). More studies are needed to understand the relationship between temperature and Pb:Ca in calcified structures.

Relative importance of factors

Only one other study has attempted to estimate the relative effects of factors on elemental uptake in calcified structures. Elsdon & Gillanders (2004) attempted to determine the relative effects of temperature, salinity, ambient Sr and Ba concentration and the interactions among these factors on otoliths of juvenile black bream. They used a backwards step-wise regression, which allowed the elimination of salinity from the model because it did not significantly affect otolith elemental signatures. Their analysis demonstrated that of the 3 factors, temperature and ambient elemental concentration influence otolith chemistry, but did not provide an estimate of the magnitude of effect of each factor.

Effect-size analyses are used infrequently in ecological literature, yet such analyses can be useful for estimating statistical fit in complicated factorial studies (Graham 2001). Here we must point out 2 caveats

regarding the use of effect-size estimates. First, the effect-size measure η^2 tends to overestimate effect sizes, thus comparing effect sizes across elements must be done with caution. Second, effect-size estimates are of limited utility in studies that do not explore the full range of variation in the factors tested. To see if the effect-size estimates were true for this study we calculated a second measure of effect size for the main effects as follows:

$$\frac{\text{Large Mean} - \text{Small Mean}}{\sqrt{MS_{\text{error}}}}$$

Where Large Mean is the largest mean in a group (i.e. egg source, culture seawater or temperature) and Small Mean is the smallest mean in the same group. In fact both measures of effect size were exactly correlated and yielded the same ranking of importance; consequently we only report η^2 because it is the most appropriate for estimating the relative importance of each factor. The field temperature results show that we successfully bracketed the range of temperatures experienced by *Kelletia kellestii* at the collection sites (Table 1), and in fact we bracketed the full range of temperatures experienced by *K. kellestii* throughout its range. However, we only collected one sample of seawater at each site and probably did not bracket the full range of variation in seawater elemental concentrations across *K. kellestii*'s range. We have only begun to discover egg-source effects and we certainly do not know the full extent of variation produced by these effects, although the fact that egg source was the most important factor affecting Pb signatures in this study (Table 3) indicates that it can potentially be powerful.

Within the constraints of the variation measured in this study for each factor we found that for Pb:Ca the most influential factor was egg source, while effects sizes for Ba:Ca showed that temperature was the most influential factor. While these effect-size estimates are only applicable to the variation measured in the parameters of this particular study, the results show that elements responded differently to the same factors. Consequently it seems that predicting elemental signatures will require an understanding of what elements are bio-available, what factors in the area can affect each element and how those elements are influenced by each factor, all at an appropriate scale.

CONCLUSION

We demonstrated that egg source affects statolith elemental signatures in *Kelletia kellestii* larvae, that elements may be influenced by a number of factors, as

was the case for Ba and Pb (Table 2), and that the most influential factor on the statolith signature of one element may not be the most influential factor for another element (Table 3). Our results also support past research showing that Ba:Ca and Pb:Ca tend to increase in *K. kellestii* larval statoliths with increasing concentration in seawater (Fig. 4a,b), and that Sr:Ca and Ba:Ca in *K. kellestii* larval statoliths tend to decrease with increasing temperature (Fig. 5a,b). Finally, we provided the first evidence that Pb may also decrease with increasing temperature (Fig. 5c).

The use of seawater samples as a proxy for elemental signatures in calcified structures seems less promising given the number of factors and their possible interactions that can influence element uptake. This (Table 3) and past studies (Bath et al. 2000, Elsdon & Gillanders 2002, 2003) have clearly demonstrated that elements in calcified structures respond differently to various factors. We now know that not only environmental variables affect element signatures, but egg source can too. In addition, endogenous factors such as ontogenic effects (e.g. De Pontual et al. 2003, Chittaro et al. 2006), growth rate (Bath Martin & Thorrold 2005) and the type of CaCO_3 crystal present in various regions of the otolith (Brophy et al. 2004, Ruttenberg et al. 2005) can affect elemental signatures and further complicate the use of seawater samples as a proxy for elemental signatures in calcified structures. Finally, because so many factors can influence the chemistry of calcified structures, it may not be appropriate to use statoliths or otoliths to reconstruct environmental histories in the field unless the influential factors have been validated.

Use of statoliths for larval tracking remains a promising prospect, however, because site-specific natal signatures can be used as long as they are spatially distinct and temporally stable over the time period of interest. Given that egg-source effects exist, this tool might someday be applied to larval tracking studies using larval statoliths or shells of planktonic spawners among whose representatives are some that support the most commercially important invertebrate fisheries (e.g. abalone, mussels, oysters).

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