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Natal trace-elemental signatures in the otoliths of an open-coast fish

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Abstract

We show that chemical differences found along the open coast are sufficiently strong to leave a readable natal signature in fish otoliths. Laser ablation inductively coupled plasma mass spectrometric (LA-ICPMS) analysis of individual larval otoliths taken from near-term females of the primitively viviparous rockfish *Sebastes atrovirens* indicates detectable levels of several trace elements. Although these larvae show considerable between-brood differences in elemental concentrations for females taken at the same site, there was significant between-site discrimination of natal signatures for regions only tens of kilometers apart in the waters off of Santa Barbara, California. For several (but not all) elements, differences between regions were consistent between 2 yr. We also explore three different possible proxies (edges of otoliths of adult fishes, resin-based elemental accumulators, and samples of seawater) that might be used to predict geographic differences in natal signatures. Although these proxies all showed significant regional differences in the concentration of some elements, only manganese showed some congruency in the spatial patterns seen in the larval otoliths. Consequently, currently available proxy measures cannot accurately predict the elemental composition of otolith cores.

One of the least understood and most fundamental processes in the determination of population abundance and community structure in marine ecosystems is dispersal, or transport, of pelagic larvae. To what extent are local adult population dynamics influenced by the dispersal of larvae from neighboring source populations? Do self-recruiting populations exist? How far do larvae disperse? The sources and destinations of larvae have been unknown, and are seemingly unknowable, for nearly all commercially important species, and yet any model of resource management or biodiversity preservation depends on certain assumptions about transport and connectivity between marine populations.

The small size and long development time of the larvae of most marine animals have made studies of dispersal difficult. Small animals usually cannot be burdened with tags, and high mortality and potentially widespread dispersal of larvae make the recovery of tags problematic. Fortunately, fish and some marine invertebrates carry their own internal environmental recorders in the form of otoliths (fish) or statoliths (invertebrates), used for hearing, balance, and orientation (Campana 1999; Thorrold et al. 2001; Zacherl et al. 2002).

Otoliths are composed of calcium carbonate crystals (aragonite) within a protein matrix. They are formed at birth and grow daily as new crystal layers are deposited around the existing core by the precipitation of calcium ions from the surrounding endolymph. Trace elements from the surrounding waters substitute for calcium in the matrix of the otolith, and their concentration may reflect differences in the physical characteristics and elemental composition of the water mass in which they were formed (Secor et al. 1995; Bath et al. 2000; Elsdon and Gillanders 2002). Thus otoliths formed at the source of production potentially carry a natural record of the site of origin. The dynamics of incorporation of trace elements into the hard parts of organisms is poorly known (Campana 1999), and otolith chemistry is not necessarily a strict reflection of the physical and chemical properties of the seawater surrounding the fish (Campana and Thorrold 2001). In some cases, incorporation problems can be avoided by microchemical evaluation of the structures of young while still resident in source populations, and subsequent evaluation of the same structure in the same cohort after dispersal (e.g., Thorrold et al. 2001). It has been known for

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many years that the composition and amounts of trace metals change from place to place in the coastal ocean, and otolith trace-element content has been used for stock identification for decades (Campana and Thorrold 2001). In the present study, we ask whether trace element content might be used to identify natal sources of larvae in fishes that live along open coastlines.

Recent studies attempting to identify the natal sources of individual fish by using trace elements in the cores of otoliths were on species in which the young spend considerable time in freshwater or in estuaries, such as salmon (Zimmerman and Reeves 2000, 2002), shad (Gillanders 2002*a*), and weakfish (Thorrold et al. 1998, 2001). These analyses are relatively straightforward because the otoliths are large and the environmental differences are great. Unfortunately, most marine fishes spend their entire lives in salt water and are present in their natal environment for a very short time before beginning their pelagic larval life. The challenge has been to show that chemical differences in water found along the open coast are sufficiently strong to leave a discrete natal signature in the otolith (Campana et al. 1994; Patterson et al. 2001).

Many open-coast dwelling marine animals begin forming their otoliths or statoliths while still in the egg, before they begin their pelagic phase (Jones et al. 1999). Thus the core of the otolith or the statolith should contain a natal signature. Zacherl et al. (2002) recently showed evidence for geographical variation in elemental signatures from statoliths from encapsulated veliger larvae of the open-coast mollusk *Concholepas concholepas.* In the present study, we demonstrate that natal signatures can be read from the otoliths of larvae of the rockfish *Sebastes atrovirens* before these larvae enter the pelagic phase.

We also explore whether the otoliths of larvae captured at their site of origin can be distinguished microchemically from larval otoliths from other sites. First, we describe our methods to isolate and prepare preparturition larval otoliths in the kelp rockfish. Next, we ask whether these mineralized hard parts could serve as natural tags of natal origin by analyzing individual larval otoliths with laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to see if they contain consistently different levels of trace elements among geographically separated sites. Finally, because the sampling required for drawing a spatial map of natal signatures is likely to be prohibitive even for a small stretch of coastline, we investigated the possibility that more easily collected elemental samplers might act as proxies for the larvae. We explore the utility of three possible proxies for specific information on local natal signatures: adult otoliths, resin-based films that accumulate trace elements, and samples of seawater.

The coastal marine environment can be exceptionally heterogeneous in terms of its physical, chemical, and biological properties (see Donat and Bruland 1995; Hutchins et al. 1995). If an environmental marker is obtained continuously by a larva, it may be possible to use the marker to retrace an individual's geographic history, and it can be compared directly with contemporaneous physical data.

Along coastal margins, concentrations of many particle-reactive trace elements are significantly higher than are those in the open ocean because these elements have coastal inputs and are quickly removed from surface waters through deposition to the sediments or deep ocean layers (Bruland 1983). In addition, alongshore differences in trace element concentrations among coastal water masses are more likely to occur nearshore as a result of variation in river inputs, sewage and industrial effluents, upwelling intensity, and offshore oil drilling operations. Thus small spatial-scale differences in trace element composition may occur for elements that have a short residence time in the surface ocean.

Mass spectrometric analysis of trace element inclusions in otoliths may then be used to identify past environments of fish larvae. Mass spectrometry itself is several orders of magnitude more sensitive in detecting trace elements than is any other readily available technique. Laser ablation allows fine-scale assessment of microchemistry at specific locations on the otolith. We chose to use laser ablation to analyze preparturition larval otoliths (rather than more traditional solution-based methods) because the same laser technique can be applied to the core areas of otoliths of postlarval recruits. This allows the possibility of assigning recruits to sites of origin.

Materials and methods

Study organism—The kelp rockfish (*S. atrovirens*) (Jordan and Gilbert 1880) is a relatively common member of the shallow rocky subtidal community of the temperate eastern Pacific, ranging from northern California, U.S.A. to central Baja California, Mexico (Love et al. 2002). Larval release occurs from February–June, and settlement peaks in July and August in the Santa Barbara Channel. Rockfish (genus *Sebastes*) are primitively viviparous, with larvae developing within the mother for 1 to 2 months before they are released (Love et al. 2002). The preparturition region of the larval kelp rockfish otolith is ~30 μm in diameter, providing a sufficient amount of material to quantify the source environmental signature by using LA-ICPMS (see following).

Field collections and site characterization—Preparturition larvae were obtained from near-term females collected by spear in the field from five locations in the Santa Barbara Channel and Santa Cruz Island (Fig. 1) from March–May in 2001 and 2002 (Table 1). Broods were extracted from females by dissection, and larvae were preserved frozen for subsequent otolith extraction and preparation (see following). Additional adult kelp rockfish were also collected from the same sites to increase the sample size for the analysis of otolith edges (Table 1). Adult otoliths (sagittae and lapillae) were extracted by dissection and stored dry until preparation for LA-ICPMS analysis.

At the same locations in 2002, over the same sampling period (March–May), we directly sampled seawater and deployed diffusive gradients in thin films (DGT(c), Windsor Scientific Limited). The DGT (an open-pore hydrogel layer backed with a Chelex binding agent) measures labile metals by accumulation over time (Zhang and Davison 2000). In the DGT, trace metals are bound to the resin after passing through the hydrogel, which serves as a well-defined diffusion layer. DGT collectors were deployed for an average of

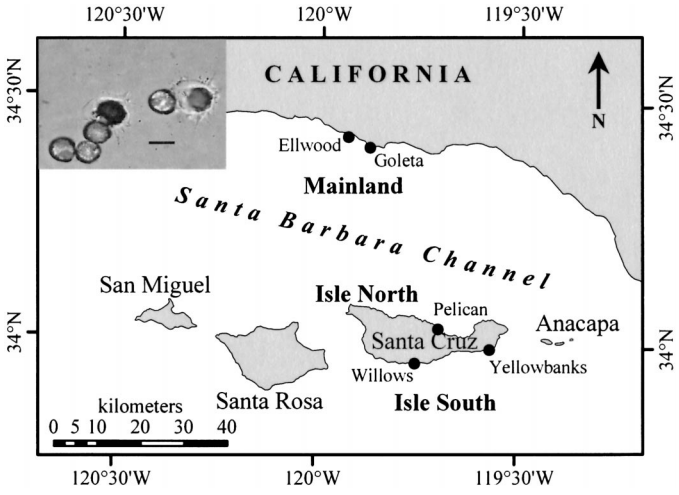

Fig. 1. Collection locations for kelp rockfish (*Sebastes atrovirens*), DGT samples, and seawater samples, showing regional designations used in the analyses. (Inset) Larval kelp rockfish otoliths prepared for analysis, with laser pits from previous sampling. Scale bar, 20 μm.

14 d, with an aim of integrating ambient elemental concentrations over time at a particular site. Seawater samples were also collected on average every 14 d, over the same time period as the DGT collectors (Table 1).

Collection sites (Fig. 1) were grouped by regions. The Mainland sites (Goleta and Ellwood) are 12 km apart. The IsleNorth region had a single site, Pelican, which is located directly across the Santa Barbara Channel from the Mainland sites. The IsleSouth region consists of two sites (Willows and Yellowbanks, 18 km apart) on the southern shore of Santa Cruz Island.

Preparation for ICPMS analysis—Larvae: To determine if otoliths of prerelease larvae bore a distinctive natal signature, we compared element/calcium ratios of otoliths from the larvae within broods from different sites and regions. Otoliths were extracted by suspending the larvae in an equal-volume mixture of 30% H_2O_2 and 0.1 mol L^{-1} NaOH (the buffering reagent). We rinsed otoliths five times in ultrapure water (resistivity $>$ 18.1 MΩ cm) and then pipetted them onto a clean 20 $\times$ 20 mm plastic slide, drawing off excess water and allowing the remaining water to evaporate. We mounted the otoliths for ICPMS analysis on double-sided tape (Scotch™ 3M Double-Coated Tape, Linerless, No. 665). We performed all of the isolation steps in a clean laboratory equipped with HEPA-filter class 100 laminar flow hoods. All of the glassware used in the isolation steps was first cleaned by using Citranox soap, rinsed five times with distilled water (resistivity $>$ 2 MΩ cm), soaked in 1 N mol L^{-1} trace-metal–grade HCl overnight, and then rinsed 10 times with ultrapure water.

Adults: Adult kelp rockfish sagittal otoliths were analyzed for the chemical composition of their most recent calcium carbonate deposition (i.e., the outer edge), coinciding with the period of preparturition larval development. By using acid-leached plastic forceps and multiwell tissue culturing trays, the right sagitta from adult specimens was cleaned of organic material by soaking in an equal-volume mixture of 30% H_2O_2 and 0.1 mol L^{-1} NaOH for 1 h. Each otolith was then rinsed with ultrapure water and individually soaked in five separate 5-min baths of ultrapure water. After the fifth bath, otoliths were rinsed again with ultrapure water and air-dried in a HEPA-filtered class 100 laminar flow hood. Whole otoliths were placed on double-sided tape just before LA-ICPMS analysis.

DGT: On retrieval from the field, DGT samples were immediately rinsed with ultrapure water, sealed in clean polyethylene bags, and refrigerated at 4°C. We followed preparation procedures described by Zhang et al. (1995) for the elution of the DGT samples. All preparation steps took place in a HEPA-filtered class 100 laminar flow hood. For each DGT, the resin gel was separated from the molding, filter, and diffusive gel layer. By using acid-leached plastic forceps, we transferred the resin gels to acid-leached 1.5-ml Eppendorf centrifuge tubes and added 0.6 ml of 1 mol L^{-1} HNO_3. We vortexed each sample and allowed it to elute for at least 24 h. Just before ICPMS analysis, samples were vortexed again and centrifuged to concentrate the resin gel. In acid-leached 0.6-ml Eppendorf tubes, 100-μl aliquots of DGT elute were diluted to 600 μl by the addition of ultrapure water. We then vortexed the sample solution and prepared two final dilutions with the addition of 1% HNO_3 and an internal standard (see ICPMS analysis section), a 1 : 2900 dilution for the more highly concentrated iron (Fe) and zinc (Zn) and a 1 : 138 dilution for all other analytes.

Seawater: At each site, we collected seawater ~2 to 3 feet above the benthic substrate in acid-leached 125-ml HDPE

Table 1. Sampling scheme for broods (which yield larval otoliths) and adult otolith samples. The number of larvae sampled per brood was 10 unless shown otherwise.

Year	Region	Site	Date	No. broods (No. larvae)	No. adults
2001	IsleNorth	Pelican	08 May–19 May	2 (6, 8)	8
	Mainland	Goleta	17 May	1 (8)	11
	Mainland	Ellwood	18 May–31 May	3 (10, 10, 9)	11
2002	IsleNorth	Pelican	11 Apr	2	2
	IsleSouth	Willows	25 Feb–12 May	1	4
	IsleSouth	Yellowbanks	25 Feb–09 Apr	5	12
	Mainland	Ellwood	08 Apr–09 May	6	11

bottles. Samples were immediately placed in airtight bags, on ice, and in the dark. We preformed all preparation steps in HEPA-filtered class 100 laminar flow hoods. Within 12 h of collection, we filtered seawater samples through 0.45-μm polycarbonate membrane filters using an acid-leached vacuum filter apparatus into a second acid-leached 125-ml HDPE bottle. Samples were then acidified with 1 μl ml^{-1} trace-metal–grade 12 mol L^{-1} HCl to a target pH of 2–3. Samples were stored in airtight bags in the dark until further preparation for ICPMS analysis.

We prepared our seawater samples in accordance to the direct analysis method described by Field et al. (1999). On the day of ICPMS analysis, seawater samples were diluted and then spiked with an internal standard (see ICPMS analysis section). In acid-leached 0.6-ml Eppendorf centrifuge tubes, seawater samples were prepared to a final 1 : 10 dilution for trace element analysis and to a final 1 : 312 dilution for minor element analysis. We prepared samples for minor element analysis diluting with 0.1% HCL, consistent with the high chloride matrix of the samples. The trace element analysis was found to be more stable and precise when diluted with 10% HNO_3.

ICPMS analysis—Larval and adult otoliths: Otoliths were analyzed on a Finnigan MAT Element 2 sector field ICPMS. The abundance of each trace element was expressed as a ratio relative to the amount of calcium to control for differences in amount of material analyzed per sample. We introduced samples into the ICPMS by using a VG UV microprobe Nd : YAG (Neodymium-doped Yttrium Aluminum Garnet) laser ablation system, frequency-quadrupled to 266 nm with a nominal beam width of 20 μm. The element menu included magnesium (Mg), calcium (Ca), manganese (Mn), iron, zinc, strontium (Sr), barium (Ba), cerium (Ce), and lead (Pb). Mg, Ca, Mn, Fe, and Zn were determined by using medium resolution mode (R = 3000) and Mg, Ca, Sr, Ba, Ce, and Pb were determined in low resolution mode (R = 300). Although low resolution provides the benefit of greatest signal intensity, medium resolution is required for elements that have molecular interferences. Isotopes collected were ^{24}Mg, ^{48}Ca, ^{55}Mn, ^{56}Fe, ^{66}Zn, ^{86}Sr, ^{138}Ba, ^{140}Ce, and ^{208}Pb. Mg was initially analyzed in medium resolution to avoid the doubly charged ^{48}Ca interference, but was subsequently analyzed by using low resolution to take advantage of the higher signal strength. The doubly charged ^{48}Ca interference was estimated in low resolution by collecting doubly charged ^{44}Ca at mass 22 and correcting for the relative abundances of ^{48}Ca and ^{44}Ca. The estimated contribution of the doubly charged ^{48}Ca to the mass-24 signal was subtracted to provide a corrected ^{24}Mg intensity. For all elements, the ratio of analyte isotope intensity to ^{48}Ca intensity was used to estimate the analyte : Ca ratio. These ratios were converted to molar ratios based on the isotope ratio mass bias correction calculated from calibration solutions with known analyte : Ca ratios. The solutions were aspirated into the ICPMS spray chamber using a low-flow nebulizer (20 μl min^{-1}). During the ablation process, a blank 1% HNO_3 solution was aspirated to maintain constant conditions in the plasma. The particulates resulting from the ablation process are swept into the spray chamber by using helium carrier gas, in which the wet and dry aerosols are thoroughly mixed before entering the plasma.

Because of their small size (~30 μm in diameter), larval otoliths afforded only enough material for a single LA-ICPMS acquisition. Therefore, regardless of resolution mode, each larval otolith was completely consumed in ablation. For adult otoliths, we ablated a series of six pits (approximately 28 μm in diameter, 8 μm in depth) along the postrostral edge of the right sagittal otolith. Three ablations were analyzed in medium resolution mode, and three were analyzed in low resolution. Before any pit was ablated, the surface of the otolith was first preablated to remove any potential surface contamination. To characterize the chemical composition of the surface of the adult otolith more precisely, we averaged the values of the three pits for each resolution type. There was no variation in laser aperture, attenuation, or frequency rate for juvenile or larval otoliths. The focus was adjusted for every run, always focused on the surface of the sample.

Blanks of the double-sided tape were ablated to verify a lack of signal intensity when the laser hit tape only. The average signal intensity of the blank tape was <2% of the average signal intensity from ablated otolith material for all elements except Fe, which averaged only 17.8% of the levels typically seen in otoliths. Other methods of mounting otoliths (such as embedding in resin) resulted in the larval otoliths sitting at variable depths, and this decreased the recovery efficiency of an already small amount of material. Adhering the larval otoliths to the two-dimensional surface of the tape produced a better recovery of material from laser acquisition.

DGT: After element extraction and sample dilution, we used solution-based ICPMS to measure concentration levels in the extract. External standardization using calibration solutions was used to estimate the concentrations of all analytes in the extracts. As currently available, ICPMS analysis of the DGT can detect trace amounts of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb. Ni levels were generally below detection limits. Indium (In) was the internal standard used for Cd and Pb; scandium (Sc) was used for Cr, Mn, Fe, Co, Ni, Cu, and Zn. The ICPMS data were collected by using the following isotopes: ^{45}Sc, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{58}Ni, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{110}Cd, ^{115}In, and ^{208}Pb. Conversions of concentrations in extracts to concentrations in seawater were based on published conversion formulae that are specific for each element and depend on exposure time and temperature (Zhang et al. 1995).

Seawater: Elements analyzed were Al, Ca, Cr, Mn, Fe, Co, Cu, Zn, Sr, Ba, Ce, and Pb. Al and Fe were occasionally detectable but were generally below detection limits. Isotope dilution was used for the ^{138}Ba analysis by using a standard enriched in ^{135}Ba. The method of standard additions was used for all other analytes, with Sc as the internal standard for Al, Ca, Cr, Mn, Fe, Co, Cu, and Zn; yttrium (Y) for Sr; and In for Ce and Pb. We collected the following isotopes by ICPMS: ^{27}Al, ^{45}Sc, ^{48}Ca, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{89}Y, ^{115}In, ^{135}Ba, ^{138}Ba, ^{140}Ce, and ^{208}Pb.

For both solution-based analyses (seawater and DGT samples) and laser-based analyses (larval and adult otoliths), the

Finnegan Mat Element 2 was operated with a RF power of 1200 W by using nickel sample and skimmer cones with typical auxiliary and cooling gas flows of 0.8 L min^{-1} and 14 L min^{-1}, respectively. For solution-based analyses, we typically used a sample gas flow rate of 0.9 L min^{-1}. For laser-based analyses, the sample gas was a mixture of 0.8 L min^{-1} of argon through the nebulizer and 0.5 L min^{-1} of helium through the laser sample cell. The laser was operated at a repetition rate of 3 Hz and an energy setting of 0.1 mJ. Precision estimates and detection limits for all sample analyses are listed in Web Appendix 1 at http://www.aslo.org/lo/toc/vol_50/issue_5/1529al.pdf.

Statistical analyses—Elemental concentrations in otoliths are expressed as Me : Ca in mmol mol^{-1} or μmol mol^{-1}. DGT elemental concentrations are expressed as mol L^{-1} of seawater, and concentrations in seawater are expressed as mol L^{-1} of Me divided by mol L^{-1} of Ca to facilitate comparison with the otolith data. Elements that consistently had values below detection limits were not included in statistical analyses. For those elements that had occasional values below detection limits, those values were included in analyses as representations of extremely low levels of that particular element in the sample.

To detect regional differences in the concentrations of particular elements and multielement fingerprints, we performed analyses of variance (ANOVA) and multivariate analyses of variance (MANOVA). Before statistical testing, residuals were examined for normality and homogeneity among regions. To meet model assumptions, all analyses were performed on natural log transformed data. For adult edges, DGT samples, and seawater, we used one-way ANOVA models, testing the effect of region (fixed factor) for each year separately. For larval otoliths, to test the effect of each element separately, we used a nested ANOVA (broods nested in region). Tukey's HSD test was used to detect a posteriori differences among means ($\alpha = 0.05$). For larval, DGT and seawater concentrations we used MANOVA models to test for spatial differences in multielement fingerprints. Region was used as a fixed factor in separate MANOVA models for each year. Pillai's trace was chosen as the test statistic because it is more robust to small sample sizes, unequal cell sizes, and situations in which covariances are not homogeneous (Scheiner 1993).

We also used canonical discriminant function analyses (DFAs) on larval data to visualize spatial differences between sites (2001) or regions (2002), and to examine classification success for larvae from different sites or regions. Cross validations were performed by using jackknife (''leave one out'') procedures in SPSS (SPSS Inc.).

Because prerelease larval otoliths are very small, we could ablate a single otolith from a brood in either low-resolution mode (detecting Sr, Ba, Pb, and Zn), or medium-resolution mode (detecting Fe, Mg, and Mn), but not both. Although this makes no difference for single-element analyses across regions, it precludes some multielement analyses by individual because the brood-processing technique prevented us from knowing whether two otoliths were from the same fish. Therefore, we performed multielement analyses only on the low-resolution mode data. These elements (Sr, Ba, Pb, and Zn) provided the most consistent differences among regions, and were more than sufficient to perform multivariate analyses.

Results

Microchemistry of prerelease larval otoliths—*S. atrovirens* near-term larvae averaged 7 mm in total length. Otoliths from the larvae measured ~30 μm in diameter, and no clear banding pattern was observed (Fig. 1, insert).

For larval otoliths from the two regions in 2001 and the three regions in 2002, we measured the metal : Ca ratios for seven elements (Fig. 2) and tested whether their concentrations were different among regions (Table 2). In 2001, nested ANOVA results showed significant among-region differences for Mg, Mn, Sr, Ba, and Pb ratios (all elements except Zn). The values for Mainland (M) samples were significantly higher for Mg, Mn, Sr, and Pb, whereas IsleNorth (IN) samples were significantly higher for Ba (Fig. 2). There were also significant differences among broods within regions for all elements except Sr (Table 2).

For 2002, significant among-brood, within-region differences in trace element concentrations existed for every element examined (all $p < 0.0001$) (Table 2), but in most cases there were clear and significant differences between regions as well. For regional differences (Fig. 2), Zn and Ba showed the same pattern, with IN samples having the highest levels, followed by M, and then by IsleSouth (IS). Pb levels were also distinctive across regions, but in a different pattern (IN>IS>M). For Sr, the IN and IS sample levels were not distinguishable from one another, but both were higher than the M samples. Fe levels were higher in the IS regional samples than in the M or IN samples. Although Mn levels showed a significant regional effect ($p = 0.035$), the Tukey HSD procedure indicated no region that had levels significantly different from the other. Finally, despite good resolution, Mg levels in larval otoliths were nearly identical across all three regions.

By combining information from the elements detectable in low-resolution mode (Zn, Sr, Ba, Pb) in a MANOVA, regions were distinctly different from one another in 2001 (Pillai's trace = 0.883, $F_{4,36} = 67.80$, $p < 0.0001$), and 2002 (Pillai's trace = 0.713, $F_{8,264} = 18.27$, $p < 0.0001$). These differences can be visualized using canonical DFA (Fig. 3). For this analysis, we present data for site groupings for 2001 and region groupings for 2002. The 2001 samples show a strong pattern of discrimination among sites of origin (82.9% of cross-validated [jackknifed] cases correctly classified), with Ba and Pb driving function 1 and Zn and Sr affecting function 2 (Fig. 3A). A similar pattern of discrimination among regions of origin occurred in 2002 (70.1% of cross-validated [jackknifed] cases correctly classified), driven primarily by Sr and Pb in function 1, and by Zn and Ba in function 2 (Fig. 3B).

Although the natal regions could be clearly separated on the basis of otolith elemental concentrations in both 2001 and 2002, the pattern of differences was not the same. Note that between-year comparisons are only possible for the IN and M regions. Some elements tended to show the same

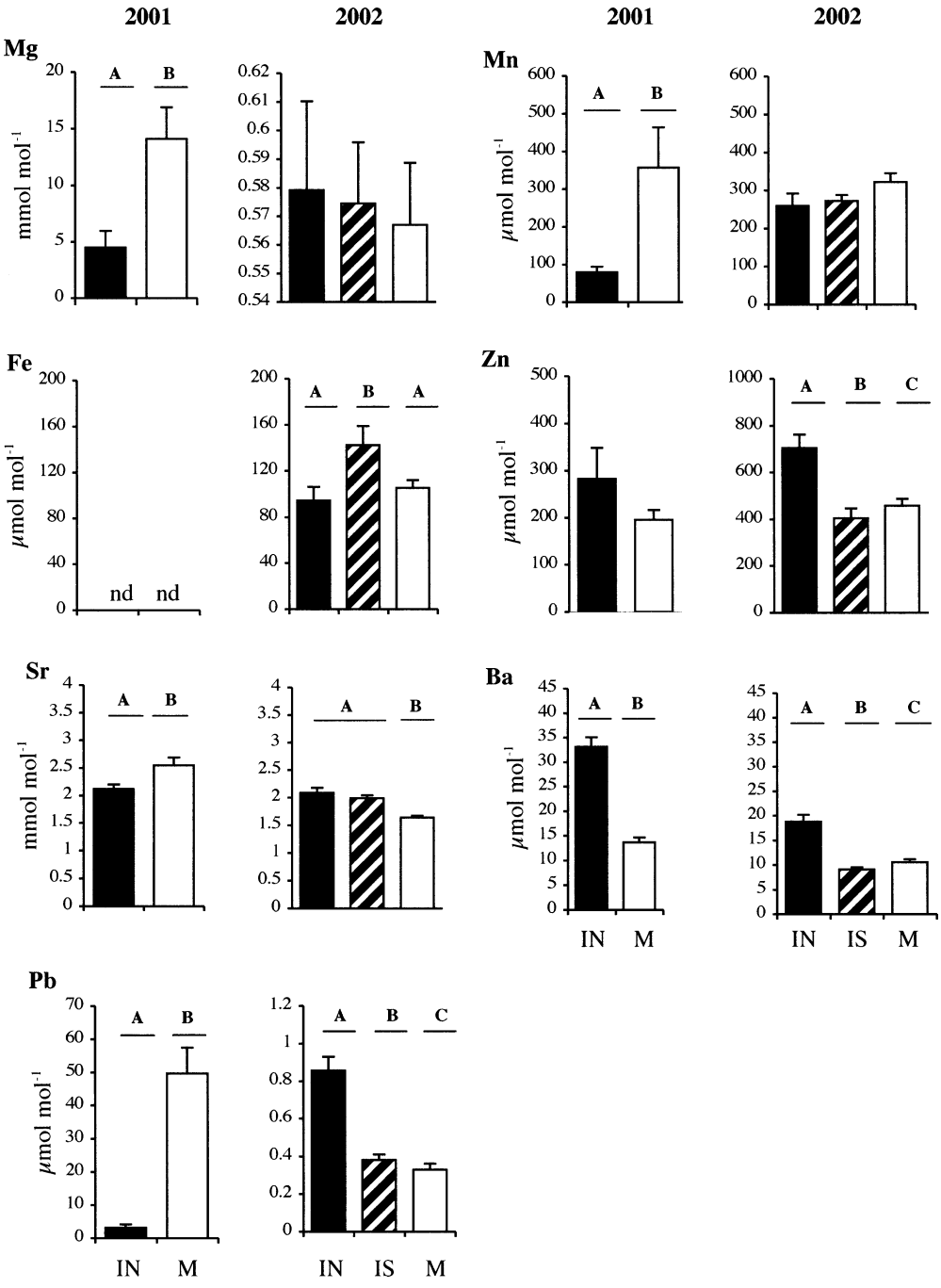

Fig. 2. Trace element concentrations (expressed as Me Ca^{-1} ± 1 SE) in larval otoliths of *Sebastes atrovirens* among regions (IsleNorth, IN; IsleSouth, IS [2002 only]; and Mainland, M). Separate plots are shown for each year of sampling. Significance values are from nested ANOVA (Table 2) testing variation in region and brood. Significant regional differences (Tukey HSD) are shown by different letters (e.g., A, B). nd indicates no data. Note different scales on y-axes.

pattern of differences in both years (Mn, Zn, and Ba), but other elements (Mg, Sr, and Pb) clearly did not (Fig. 2).

Microchemistry of the edges of adult otoliths—Concentrations of most trace elements (expressed as Me : Ca ratios) at the edges of otoliths of adults (Fig. 4) were considerably lower than were levels found in preparturition larvae captured at similar times and places. Less than 15% of the 59 individuals examined had detectable levels of Mn, Fe, Zn, and Pb in the outer edges of their otoliths, precluding any spatial or temporal analysis based on these elements (means are shown in Web Appendix 2 at http://www.aslo.org/lo/toc/vol_50/issue_5/1529a2.pdf). Levels of Sr and Ba were similar in larval and adult otoliths, whereas Mg and Fe in adults

Table 2. Nested ANOVA comparing trace element concentrations (expressed as Me Ca^{-1}, natural log transformed) in larval otoliths of *Sebastes atrovirens* among broods and regions (Mainland, IsleNorth, IsleSouth [2002 only]).

		2001			2002		
Me Ca^{-1}	Source	df	SS	*F* ratio	df	SS	*F* ratio
Sr Ca^{-1}	Region	1	0.28	6.31*	2	1.36	36.00***
	Brood (region)	4	0.32	1.84 ns	11	1.92	9.23***
	Error	35	1.54		126	2.39	
Ba Ca^{-1}	Region	1	7.01	167.00***	2	8.63	50.15***
	Brood (region)	4	0.96	5.69**	11	10.85	11.46***
	Error	35	1.47		126	10.84	
Pb Ca^{-1}	Region	1	65.87	652.45***	2	27.19	82.83***
	Brood (region)	4	47.08	116.58***	11	83.57	47.40***
	Error	35	3.53		125	20.51	
Mg Ca^{-1}	Region	1	19.34	41.59***	2	0.02	0.18 ns
	Brood (region)	4	74.94	40.29***	11	4.10	6.78***
	Error	41	19.06		124	6.82	
Mn Ca^{-1}	Region	1	33.72	22.85***	2	1.27	3.36*
	Brood (region)	4	33.74	5.71**	11	10.36	4.98***
	Error	28	41.32		124	23.43	
Zn Ca^{-1}	Region	1	0.28	2.72 ns	2	7.61	54.48***
	Brood (region)	4	10.82	25.90***	11	28.83	37.52***
	Error	35	3.66		124	8.66	
Fe Ca^{-1}	Region				2	2.31	7.17**
	Brood (region)				11	15.37	10.66***
	Error				24	19.97	

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns $p>0.05$. Broods were nested within regions. Separate analyses were done for each year of sampling.

occurred at levels considerably lower than the lowest average levels seen in larvae.

Only two elements (Sr and Ba) showed any significant differences across regions in concentrations, and these only in 2002 (ANOVA Sr: $F_{2,26} = 9.84$, $p < 0.001$; Ba: $F_{2,26} = 6.66$, $p < 0.001$) (Web Appendix 2). The pattern for Sr in adult otoliths (IN=IS>M) was identical to that seen in the prerelease larvae for the same year (Fig. 2). For Ba, both larval and adult otoliths were notably enriched in samples from IN in 2002 relative to other regions; the larval samples also showed a difference between the remaining two regions (M>IS), but this was not reflected in the adult samples.

Possible physical proxies for larval natal signatures—DGTs: Because of the nature of the binding agent, not all elements detectable in seawater or the otoliths are extractable from DGT samples. Levels of Cr, Mn, Fe, Co, Cu, Zn, Cd, and Pb were generally above detection limits (Fig. 5). Levels of Fe and Zn were an order of magnitude higher than other elements (Fig. 5), and the extracts required further dilution and separate analysis. Mn, Fe, Co, and Cd showed significant between-region values (see Web Appendix 3 at http://www.aslo.org/lo/toc/vol_50/issue_5/1529a3.pdf). For those elements, the mainland samples were enriched relative to the island samples (Fig. 5), but values from the northern and southern shore of Santa Cruz Island overlapped extensively. By combining information from the detectable elements in a MANOVA, regions were distinctly different from one another in 2002 (Pillai's trace = 0.99, $F_{16,66} = 4.01$, $p < 0.001$).

Seawater: Elemental concentrations in the seawater samples were expressed as a ratio to Ca concentrations to facilitate comparisons with the larval otolith data (Fig. 6). Of all the detectable elements (Fig. 6), only Mn, Co, Zn, Ce, and Ba showed significant regional differences (see Web Appendix 4 at http://www.aslo.org/lo/toc/vol_50/issue_5/1529a4.pdf). For those elements (except Ce), the mainland samples were enriched relative to the island samples, but values from the northern and southern shore of Santa Cruz Island overlapped extensively (Fig. 6). By combining information from the detectable elements in a MANOVA, regions were distinctly different from one another in 2002 (Pillai's trace = 1.016, $F_{18,34} = 1.95$, $p < 0.045$).

Discussion

To clarify comparisons, Table 3 provides a summary of the qualitative differences reported in the present study in elemental concentrations by region, across all techniques and years.

Larval otoliths—Samples from both 2001 and 2002 indicate that there are distinct regional differences in natal elemental signatures in the otoliths of prerelease kelp rockfish (*S. atrovirens*). Although there were significant between-brood differences within local areas for nearly every element analyzed, regional differences in elemental concentrations were detectable for five of six elements in 2001, and for five of seven elements in 2002. Although differences might be

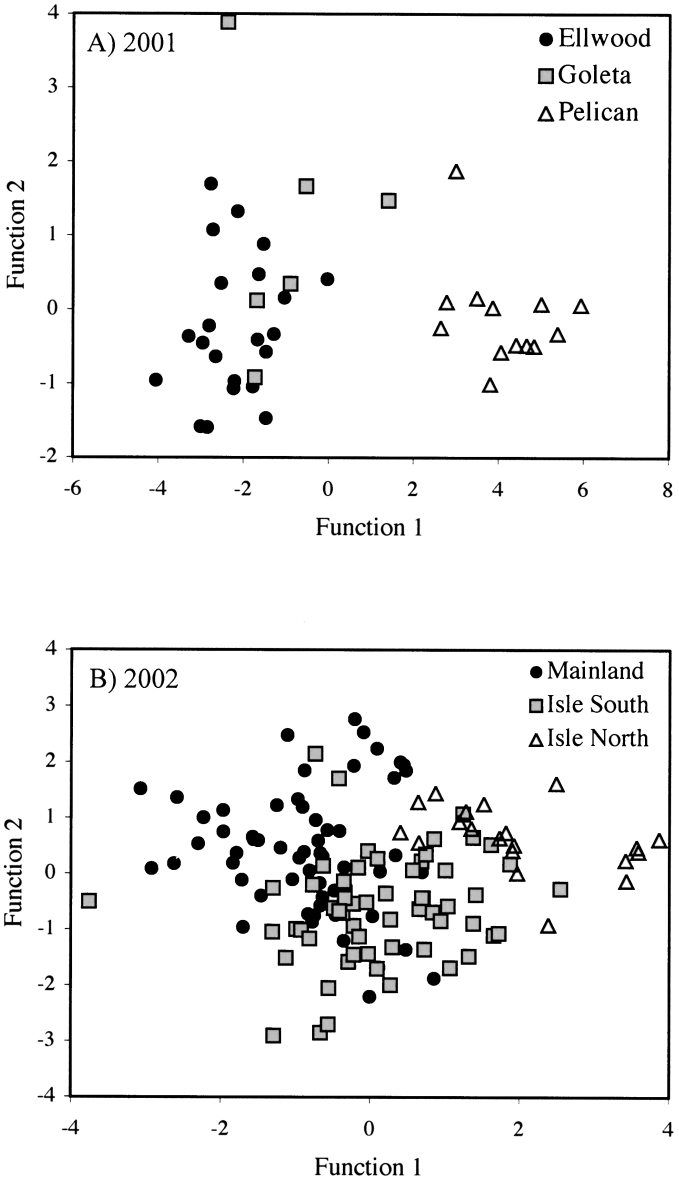

Fig. 3. (A) Canonical discriminant function analysis (DFA) of prerelease larval otoliths, by site, for 2001. Function 1 weights are 0.840 Ba, −0.974 Pb, −0.229 Sr, and −0.693 Zn. Function 2 weights are 0.168 Ba, 0.008 Pb, 0.934 Sr, and 0.164 Zn. (B) CDF analysis of prerelease larval otoliths, by region, for 2002. Function 1 weights are 0.818 Sr, 0.509 Ba, 0.685 Pb, and −0.042 Zn. Function 2 weights are −0.436 Sr, 0.606 Ba, −0.373 Pb, and 0.491 Zn.

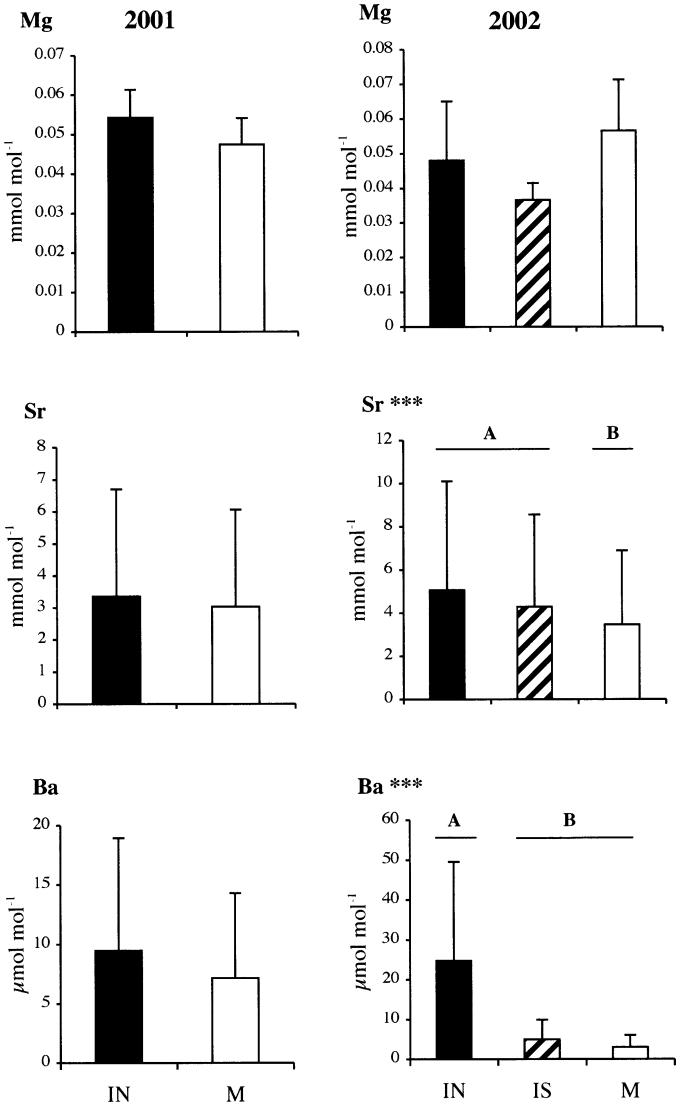

Fig. 4. Concentrations of Mg, Sr, and Ba in edges of adult otoliths of *Sebastes atrovirens*. Concentrations (mean ± 1 SE) are expressed as Me Ca^{-1} in mmol mol^{-1} or μmol mol^{-1}. Separate plots are shown for each year of sampling. Values for other elements were generally below detection limits, precluding statistical comparison (for values, see Web Appendix 2). Significance values are from one-way ANOVA testing variation in region for each year of sampling (*** $p < 0.001$). Significant regional differences (Tukey HSD) are shown by different letters (e.g., A, B). Note different scales on y-axes.

expected between sites on opposite sides of the Santa Barbara channel, it is important to note that samples from the north and south shores of Santa Cruz Island differed in five elements (Fe, Zn, Sr, Ba, and Pb) in 2002. Taken as a whole, the mix of elemental concentrations across regions produces a natal signature that allows good discrimination and assignment success (Fig. 3).

This strong geographic differentiation supports the future possibility of assigning recruits to source populations, given more complete knowledge of spatial geographic variation in source signatures along the Pacific coast. Such assignment is a simpler task when the number of potential source sites is limited, as occurs in species that are confined to estuaries while young (see Thorrold et al. 2001). At this time, a complete ''atlas'' of natal signatures does not exist for any open-coast species. Now that we have established that natal signatures exist, there is a strong need to characterize the scale of spatial and temporal variation in these signatures. The ideal elements comprising an assignable natal signature would vary over a spatial scale that is compatible with a reasonable sampling scheme, and this spatial variation

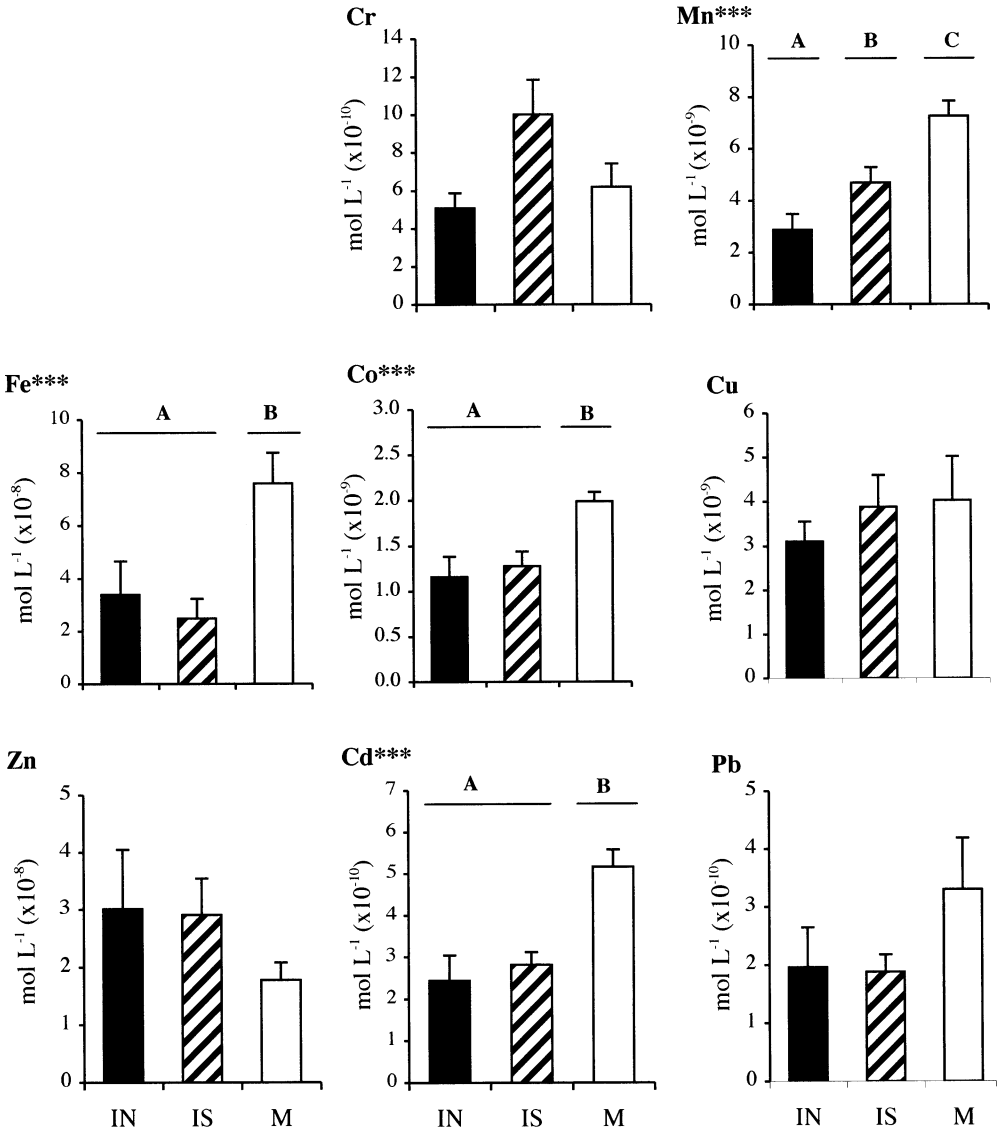

Fig. 5. Trace elemental concentrations in DGT sampled in 2002 in the period during which prerelease larval samples were taken (March–May). Concentrations (mean ± 1 SE) are expressed as mol Me L^{-1} of seawater using conversion formulae referenced in the Methods. Significance values are from one-way ANOVA testing variation in region (See Web Appendix 3; *** $p < 0.001$). Significant regional differences (Tukey HSD) are shown by different letters (e.g., A, B).

should be consistent over time. Fine-scale spatial variation would require an exhaustive sampling regime to document (especially if this finer-scale variation is not embedded in larger scale patterns). Temporal consistency in spatial patterns would reduce the necessity of resampling the natal signature pattern for each reproductive event.

At this point, we only know that most elements measured showed differences in concentrations in larval otoliths taken from sites tens of kilometers apart. (It is worthwhile to note that the two mainland sites in 2001, Goleta and Ellwood, are ~10 km apart along the same span of coastline, yet differed significantly in both Mn and Pb concentrations.) Although some elements (Mn, Zn, and Ba) remain good candidates for temporal consistency (in that they showed the same pattern of regional concentration differences in both 2001 and 2002), others clearly do not. Mg, Sr, and Pb were all enriched in the island samples in 2001 but had the opposite pattern in 2002, despite being good between-site discriminators in both years (Fig. 2). Gillanders (2002b) summarizes the considerable temporal variability in microchemical composition of otoliths from fishes taken at the same site over months or years.

In addition to qualitative changes in the geographic pattern of elemental concentrations, for some elements, there were also striking between-year differences in the concentrations themselves. In 2001, measured levels of Mg and Pb were much higher than in 2002, whereas the opposite pattern held for Fe and Zn. Other elements (Mn, Sr, and Ba) were measured at similar levels in both years. Because there is no consistent pattern of between-year differences across ele-

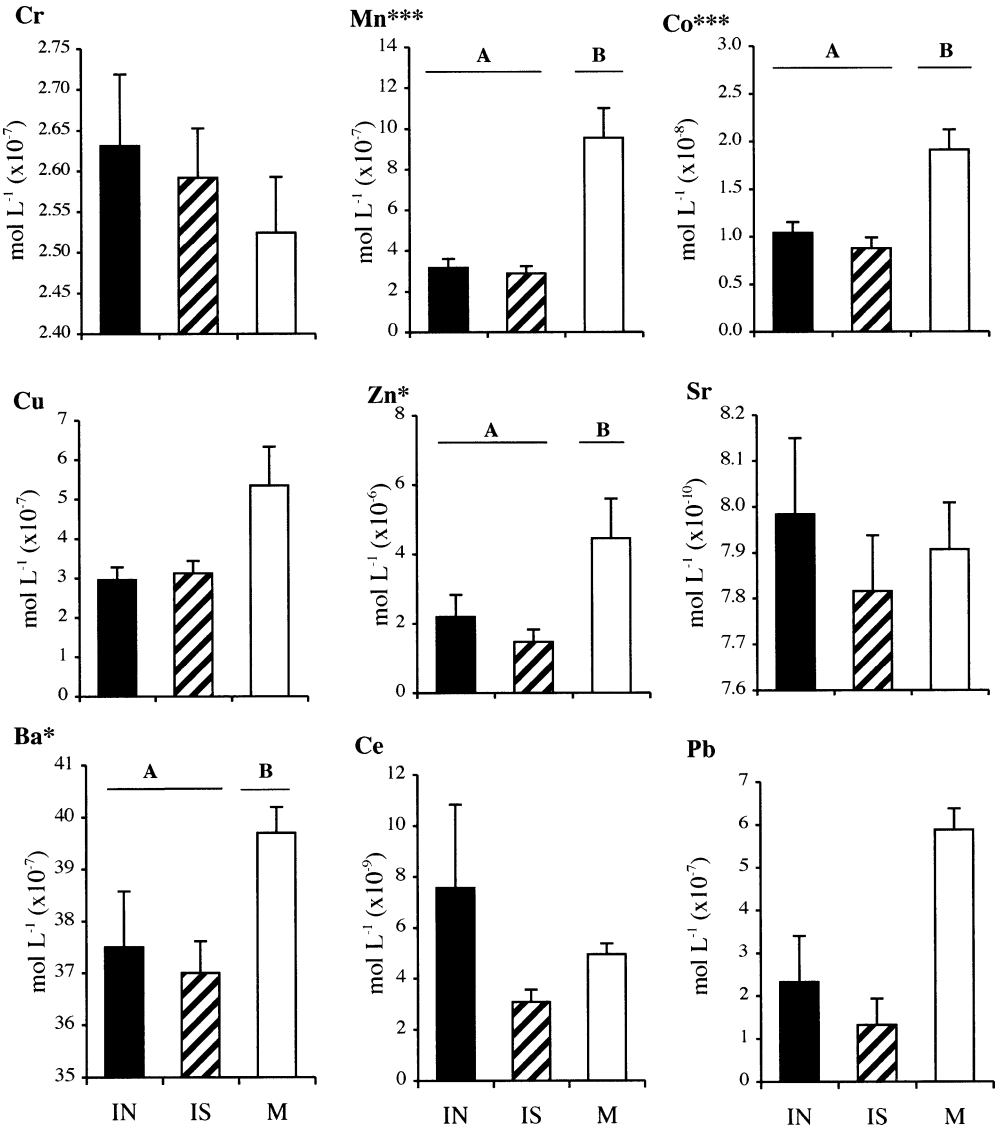

Fig. 6. Trace elemental concentrations in seawater sampled in 2002 in the period during which prerelease larval samples were taken (March–May). Concentrations (mean ± 1 SE) are expressed as mol L^{-1} of Me divided by mol L^{-1} of Ca to facilitate comparisons with the larval otolith data. Significance values are from one-way ANOVA testing variation in region (see Web Appendix 4; $*p < 0.05$, $***p < 0.001$). Significant regional differences (Tukey HSD) are shown by different letters (e.g., A, B).

ments, and because readings within years were relatively consistent for most elements, it is unlikely that these contrasts are due to procedural differences.

Strong maternal effects were seen for every element in both years (except Sr in 2001), in that there were significant between-brood, within-region differences (Table 2). This occurred even when regional differences were not apparent (e.g., Zn in 2001, Mg in 2002). The source of this brood effect is not clear. It could be a true maternal effect, reflecting differences in the environment or nutrients provided by the mother in utero, which in turn could reflect differences in maternal diet and/or physiology. There is also a possibility that these within-brood effects are due to handling differences in collection, preservation, or preparation for ICPMS analysis.

Potential proxies for larval otoliths—At the onset of any study of natal signatures, the spatial pattern of elemental natal signatures must initially be determined from the prerelease larvae (or from pelagic larvae sampled soon after release but before leaving the natal site). Although this is a necessary first step, sampling larvae at the natal site can be difficult and time-consuming. The broods sampled here represented an enormous amount of collection time, because only near-term females would yield a proper sample, and such females were rarely found. Species with benthic egg

Table 3. Overall comparisons of regional (Mainland, M; IsleNorth, IN; IsleSouth, IS [2002 only]) patterns of elemental concentrations for larval natal signatures, edges of adult otoliths (both for 2001 and 2002), and two physical proxies (2002 only).

Element	Natal signatures 2001	Adult edges 2001	Natal signatures 2002	Adult edges 2002	DGT 2002	Seawater 2002	Conclusions
Sr	M>IN	IN=M	IN=IS>M	IN=IS>M		IN=M=IS	Natal signature not temporally stable; adults may resemble larvae; seawater does not resemble adults or larvae
Ba	IN>M	IN=M	IN>M>IS	IN>IS=M		M>IN=IS	Natal signature temporally stable? Larvae do not resemble adults or seawater
Mg	M>IN	IN=M	IN=IS=M	M=IN=IS			Natal signature not temporally stable; larvae do not resemble adults
Mn	M>IN		M=IS=IN		M>IS>IN	M>IN=IS	Natal signature temporally stable? Larvae resemble physical proxies; physical proxies resemble each other
Pb	M>IN		IN>IS>M		M=IN=IS	M=IN=IS	Natal signature not temporally stable; larvae do not resemble physical proxies; physical proxies resemble each other
Zn	IN=M		IN>M>IS		IN=IS=M	M>IN=IS	Natal signature temporally stable? Larvae do not resemble physical proxies; physical proxies do not resemble each other
Fe			IS>M=IN		M>IN=IS		Larvae do not resemble DGT; mainland enrichment in proxy
Ce						M=IN=IS	Nonsignificant mainland enrichment
Cr					IS=M=IN	IN=IS=M	No match in physical proxies
Cu					M=IS=IN	M=IS=IN	Similar pattern in physical proxies
Co					M>IS=IN	M>IN=IS	Similar pattern in physical proxies, mainland enrichment
Cd					M>IS=IN		Mainland enrichment

Average concentrations are ordered, largest values on the left. Significant differences (by ANOVA) are indicated by an inequality sign (>).

masses may be better candidates for larval collection (e.g., the gastropod *Kelletia kelletii*) (see Zacherl et al. 2002). In the likely event that the spatial distribution of natal signatures is not temporally stable, the sampling required for establishing the spatial patterns each reproductive season is likely to be prohibitive. Because of this, we investigated the possibility that more easily collected elemental samplers might act as proxies for the larvae. In most cases, the results were not encouraging (Table 3). Although the physical samples often showed good ability to discriminate between regions, the qualitative spatial pattern of elemental abundance did not often match that seen in the larvae.

Adult otolith edges: Kelp rockfish are thought to be site-localized as adults, rarely moving far from a restricted home range (Love et al. 2002). Because of this, otoliths of resident adults could function as local environmental recorders, revealing regional differences in elemental concentrations over the same period of time that natal signatures are forming in larvae. This has the advantage of using the same species and the same structure to estimate geographic patterns of elemental concentrations in the core regions of larval otoliths. Although collection of any conspecific adult in a region of interest is by no means certain, they are considerably more common than near-term gravid females.

On the other hand, adults may face quite different physiological challenges than do larvae, and there is no guarantee that they will sequester elements in the same pattern as reflected in the natal signatures. Several elements (Mn, Zn, and Pb) could be detected consistently in larvae but were generally below detection limits in adults. Concentrations of Mg, Mn, Fe, Zn, and Ba were much higher in larval otoliths. The extreme example of this pattern was seen in Mn, in which levels were about two orders of magnitude higher than that seen in the adults, similar to the pattern of Mn enrichment in the core areas of the otoliths of herring recently noted by Brophy et al. (2004). Not all elements were enriched in larval cores however; Sr levels tended to be higher in adults.

Only three elements (Mg, Sr, and Ba) occurred at high enough levels in adults to be useful for a geographic analysis and for comparison with larval patterns. Sr showed the same pattern of concentration (IN=IS>M) for both adults and larvae in 2002, but the patterns did not match in 2001. Neither Mg nor Ba showed any noticeable correspondence between adult and larval patterns.

These results suggest some caution in attempts to predict the pattern of natal elemental signatures based on patterns seen in juvenile or adult otoliths. In a related study, Patterson et al. (2004) found no correspondence between the natal and

postsettlement signatures of a tropical damselfish, and only the postsettlement signatures showed any geographic structure. Because the natal signatures were measured in postsettlement (and postlarval dispersal) fishes, it is difficult to determine whether there was a pattern of natal signatures that was subsequently blurred through dispersal, or whether there was no pattern to begin with.

Physical sampling: It is important to stress that elements that are incorporated into otoliths are subject to biological processes (e.g., active uptake or removal in the blood and endolymphatic fluid) before they are available to be included in the calcium carbonate matrix. These processes may be nonlinear responses to physical parameters (such as temperature, salinity, or pH), and heavily regulated elements may show little internal variation relative to availability in surrounding seawater (Campana and Thorrold 2001). Thus there is no a priori expectation that spatial differences in relative concentrations *across* elements in physical samples will be reflected in the patterns seen in otoliths. Because of regulation, even relative differences in concentrations of a single element in the environment may not be detectable in otoliths. Regardless of these potential confounds, the basic expectation is that the qualitative difference in elemental concentrations in otoliths across regions should reflect availability, and that relative availability is measured in the physical collectors.

DGTs: If local levels of elemental concentrations in seawater fluctuate over time, analysis of a single seawater sample could be misleading. As a potential proxy for natal otoliths, the DGT has the advantage of accumulating elements over time, integrating elemental concentration levels in seawater over approximately the same period of time that the natal otolith core is forming. As it turns out, the seawater and DGT samples showed similar regional differences in concentrations for about one half of the elements the two had in common (see Table 3), suggesting that local temporal fluctuations in elemental concentrations are not sufficiently strong to obscure the regional patterns.

Regional patterns in the DGT samples were also reasonably consistent over time, and elements that showed significant regional differences suggested a pattern of enrichment in mainland samples relative to the islands; only Mn showed a further significant difference between the north and south shores of Santa Cruz (IS>IN).

DGT samplers, as currently designed, do not accumulate Mg, Sr, or Ba, each of which was a useful discriminator for larvae. Among the elements also detectable in larvae, only Mn showed a regional pattern of concentration that was similar in DGT samples and larval otoliths (regional differences were not significant in the larvae, however). Although Fe, Zn, and Pb had clear, significant patterns of regional differences in the 2002 larvae, the patterns seen in the DGT samples did not resemble the larvae. For example, the Fe levels in larvae were significantly higher in samples from the IS region relative to M and IN, whereas the DGT samples showed a pattern of enrichment in Mainland samples (M>IN=IS).

Seawater: Chemical analysis of seawater has been ongoing for decades, providing a longer-term basis for predictions of geographic differences in the availability of elements that might be incorporated into the natal otolith signature. Although seawater samples provide only a snapshot of elemental availability at the time of sampling, a series of samples can be integrated to detect consistent larger scale differences. Analysis of seawater samples over the time of natal otolith formation in 2002 showed the same pattern of mainland enrichment as was suggested in the DGT samples. For Mn, Co, Zn, and Ba, the mainland levels were significantly higher than were levels seen in the island regional samples, and these were the only significant differences seen. Where comparisons could be made, the significant regional patterns seen in seawater were similar to those seen in the DGT samples for Mn and Co, but the pattern for Zn was not (Table 3). For elements with nonsignificant regional differences, seawater and the DGT samples showed similar trends in Cu and Pb, but not in Cr (Table 3).

Of the suite of elements detectable in both seawater and the larval samples (Mn, Zn, Sr, Ba, and Pb), only Mn showed a similar geographic pattern of concentration. Patterns in adult otolith edges for Sr and Ba also did not match that seen in seawater, even though there were significant regional differences in Ba for both otoliths and seawater.

Summary for proxies—Among the three elements (Mn, Zn, Ba) that showed some indication of temporal stability in the larval otoliths (in that they showed the same regional pattern of concentration in both 2001 and 2002), only Mn showed the same pattern in the physical proxies. Of course, with a limited set of times, regions, and elements, this single correspondence could have been due to chance, and we are continuing this study to ascertain whether Mn may be of use in predicting geographic patterns of natal signatures. It should be stressed, however, that the ability to discriminate among natal regions increases rapidly with an expanded menu of elements, each showing significant but distinct regional patterns (Fig. 2). At this point, it is clear that the set of potential proxies investigated here show little promise of direct utility, in the sense that even qualitative geographic patterns of elemental concentrations did not match those seen in the larval otoliths.

The approach attempted here asks whether environmental data on seawater chemical composition could be used to predict natal otolith microchemical composition. In contrast, most past studies have asked whether the otolith could be used as an indicator of past environmental conditions. Although these approaches differ, they both search for some correspondence in the qualitative patterns of geographic differences in trace metal concentrations in otoliths and in seawater. Results from both culturing studies and environmental surveys suggest that although Sr:Ca and Ba:Ca concentration ratios in otoliths were often related to water chemistry, the Me:Ca ratio for other elements was not (Bath et al. 2000; Campana and Thorrold 2001; Thorrold and Hare 2002). Interestingly, in this study we detected no correspondence between the geographic patterns of Sr and Ba concentrations in seawater and those patterns in either larval or adult otoliths.

Beyond straightforward (and time-consuming) sampling of the larvae themselves, what should the next steps be with regard to the development of proxies? With experimental culture and manipulations of elemental concentration in the laboratory, it may be possible to develop expressions relating elemental concentrations in seawater (measured directly or with a DGT sampler) to that seen in the natal signature, but the data presented here suggest that such relationships are likely to be complex. Alternatively, there are many other binding agents than Chelex resin, and some of these may be useful in (1) expanding the list of elements in common between the DGT and the larval otolith, and (2) taking up those elements in a manner more congruent with the larval otolith.

However, the successful development of such a larval otolith proxy depends critically on understanding the underlying mechanism(s) that control elemental incorporation in embryonic otoliths, which we currently lack. The apparent distinctness of the chemistry of otolith cores could be a result of any of the following: a large maternal contribution, higher incorporation rates (i.e., greater distribution coefficients), different matrix composition (i.e., higher protein content), or different crystal mineralogy (e.g., vaterite rather than aragonite). Identifying which of these mechanisms are driving the observed elemental characteristics of otolith cores is clearly the necessary first step in the development of such a proxy.

Microchemical composition of otoliths of fish larvae collected at their natal sites (before pelagic dispersal) shows significant spatial variation at scales of tens of kilometers. This is the first demonstration of the existence of natal otolith signatures in a species born along open coastlines, and opens the possibility of assigning postlarval recruits to natal areas through an analysis of the core of the otolith. Before that can be attempted, however, we need to know the spatial and temporal scales of variability in natal signatures—this will answer the question of whether the ''atlas'' of natal signatures would need to be redrawn each reproductive season.

Drawing the ''atlas'' would be considerably simplified if natal otolith signatures could be predicted from knowledge of local water chemistry or otolith chemistry of adults from the same area. Unfortunately, we found little correspondence between such potential proxies and the natal signatures, indicating that currently available proxy measures are inadequate to characterize natal otolith chemistry.

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