

Water and otolith chemistry identify exposure of juvenile rockfish to upwelled waters in an open coastal system

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ABSTRACT: We present a novel approach to examine the relationship between pelagic juvenile fish and their environment in an open coastal system using a geospatial technique to relate water and otolith chemistries. We compared the chemistries of water and pelagic juvenile rockfish otoliths *Sebastes jordani* collected from a coastal upwelling system off central California during May and June 2009. To determine the presence and composition of an upwelling chemical signature, the elements Ba, Sr, and Mg expressed as ratios relative to Ca were quantified in the water and outer otolith margin using inductively coupled plasma mass spectrometry. Recently upwelled water, as indicated by a strong inverse relationship with water temperature, had an elevated Ba:Ca concentration. Using all 3 element ratios, cluster analysis and multivariate analysis of variance were used to identify 3 distinct chemical signatures for otolith and water samples. When mapped, these signatures displayed marked geospatial variability that we attributed to mesoscale upwelling dynamics. Canonical discriminant function analysis results indicated that the relative contribution of each element to the 3 signatures was similar between water and otoliths. Interestingly, otoliths containing upwelling signatures (high Ba:Ca) did not match spatially with upwelling signatures in the water. A directional-dependence analysis (spatial cross-covariance) revealed the highest covariance between water and otolith chemistries at a distance between 50 and 100 km apart, suggesting southern movement or transport of fish.

KEY WORDS: Upwelling · Central California · Barium · Multivariate statistics · Geospatial statistics · ICP-MS

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INTRODUCTION

Spatial and temporal variation in the replenishment of fish populations by larval recruitment has long been recognized as a fundamental driver of the structure and dynamics of marine populations, ecosystems, and fisheries (Rothschild 1986, Sinclair 1988, Caley et al. 1996, Carr & Syms 2006, Tapia & Pineda 2007, Cowen & Sponaugle 2009). Temporal and spatial variability in larval recruitment to adult populations is

driven by many factors, including transport by ocean currents (dispersal) and differences in larval growth and survival linked to variation in the quality of ocean conditions (e.g. temperature and food availability; Cury & Roy 1989, Wilson et al. 2008). Therefore, the more we know about how oceanographic processes and conditions affect larval supply, the better are our chances of understanding marine populations and successfully managing the fisheries they support (Ralston & Howard 1995, Sale 2004, Cowen et al. 2007).

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Off central California (USA) we currently lack sufficient knowledge to fully address the effects of ocean variability on pelagic fish larvae, largely due to the difficulty of observing and tracking pelagic larvae in the open ocean. Otolith microchemical analysis offers a promising approach with which to examine the effects of oceanographic variability on larval transport, growth, and survival of fish (DiBacco & Levin 2000, Campana & Thorrold 2001, Elsdon et al. 2008, Sponaugle 2010). Otoliths are paired calcified structures used for hearing and balance in fish. These structures accumulate calcium carbonate (CaCO_3) in daily growth layers that can be used to infer fish age and growth rate. Other minor elements present in the environment become incorporated via ion substitution for Ca. Consequently, the water chemistry to which a fish is exposed is permanently recorded within the otolith layers (Campana 1999, Bath et al. 2000, Walther & Thorrold 2006). As fish are exposed to different water masses, the elemental compositions are recorded as sequential elemental signatures within these layers, producing a daily record of a fish's past environment. By constructing maps of both the water chemistry of the environment and otolith chemistry of the fish residing there, one can explore and define relationships between the 2 (Elsdon et al. 2008).

Chemical differences in coastal oceanic water masses can arise by oceanographic processes, natural biogeochemical cycles, and anthropogenic inputs. We focused on wind-driven upwelling, a circulation pattern that brings cold, deep waters to the surface, along with characteristic nutrients and elements. An element thought to be an indicator of upwelled water is barium, Ba (Lea et al. 1989, Patterson et al. 1999, 2004, Elsdon et al. 2008, Kingsford et al. 2009). Ba in the ocean has a nutrient profile, meaning it is found in highest concentration in deeper waters and is depleted in surface waters. Dissolved Ba, the form available for uptake by the otolith, will precipitate as barite (BaSO_4) and becomes unavailable in microenvironments of decaying organic matter, such as dying phytoplankton and zooplankton fecal material which are enriched with sulfate. As the plankton sinks out of the surface water, so does the available Ba, sinking as barite along with the decaying matter. Barite can then re-dissolve at depth when it comes into contact with anoxic environments, and possibly through other processes that are not yet well understood (Dehairs et al. 1980, Esser & Volpe 2002). Thus, in many systems, dissolved Ba is present in high concentrations in surface waters and available for uptake by the otolith, but only when it is delivered to the surface during active upwelling.

Coastal upwelling systems support some of the most productive and economically valuable marine ecosystems in the world (Ryther 1969, Bakun et al. 1974). Temporal and spatial variability of coastal upwelling is thought to directly affect larval fish survival and transport within these systems (Parrish et al. 1981, Cury & Roy 1989, Ralston & Howard 1995, Wilson et al. 2008). Upwelling provides a boost of nutrients that fuels primary production and may contribute to enhanced larval survival by increasing food availability. Unlimited by food, larvae can accumulate larger lipid reserves (Hamilton 2008), lower their chances of mortality due to starvation, and better evade predation (Miller et al. 1988). Upwelling events play an important role in the success of larval recruitment to adult populations of marine demersal fishes, including rockfishes (Ralston & Howard 1995, Wilson et al. 2008, Morgan et al. 2009, Caselle et al. 2010). Hence, there is great value in better understanding the direct effects of upwelling on pelagic larvae.

Here we compared water and pelagic juvenile rockfish otolith chemistries from the open coastal upwelling system along central California to determine the presence or absence of an upwelling chemical signature. The spatial relationship between the locations of fish carrying the chemical upwelling otolith signature and the locations of upwelled water sources was assessed using a geostatistical approach. Identifying the chemical signature associated with upwelling exposure would give insight into the influence that upwelling has on interannual growth, transport, recruitment, and survival of rockfish in central California and possibly allow for a retrospective analysis of otoliths from successful cohorts of adult fish. To our knowledge, this is the first study of its kind, where both water and otolith chemistries are examined in an open active upwelling system using a pelagic species.

MATERIALS AND METHODS

Study region

The central California coastal study region is defined here as Point Reyes ($38^\circ 10' \text{ N}$ latitude) down to Monterey ($36^\circ 35' \text{ N}$ latitude) and extending to approximately 60 km offshore (Fig. 1). Within this region, coastal upwelling is concentrated at 2 upwelling centers, Point Reyes and Point Año Nuevo. Between these 2 upwelling centers is the retentive Gulf of the Farallones and a plume of lower-saline

water emanating from San Francisco Bay. At the southern end of the study region is Monterey Bay, within which circulating upwelled waters are often retained in a feature called an 'upwelling shadow' (Schwing et al. 1991, Graham & Largier 1997).

Upwelling along central California is driven by intermittent northwesterly winds that, in combination with the Coriolis Effect, drive Ekman transport of surface waters in a net offshore direction (Mann & Lazier 2006). These surface waters are replaced by cold, nutrient-rich waters that are upwelled from depths of 50 to 300 m. Upwelling within the central California system is most intense in the late spring and summer months (Strub et al. 1987). Within the upwelling season exist upwelling events, which are intense periods of upwelling lasting 5 to 10 d, interspersed with periods of wind relaxation (Send et al. 1987, Mann & Lazier 2006). Upwelling is highly spatially variable along the California coast; this variability is driven by changes in wind stress as well as bottom bathymetry and the structure of the coastline (Mann & Lazier 2006). While the net transport is 90° offshore, the surface layers in which pelagic rockfish reside are closer to a 45° and primarily southward deviation. This does not account for upwelling jets (Schwing et al. 1991, Roughan et al. 2006) or areas of

entrainment where upwelled water becomes trapped and circulates such as in the Gulf of the Farallones (Wing et al. 1998, Roughan et al. 2006) and in northern Monterey Bay (Graham & Largier 1997). These features associated with coastal upwelling likely have a large impact on larval and juvenile dispersal, in addition to possible entrainment in nearshore fronts which would limit movement offshore (Woodson et al. 2012) and behavior or active swimming which may facilitate transport.

Sample collection

We collected water and juvenile rockfish samples as part of an annual mid-water trawl survey conducted by the National Marine Fisheries Service, Southwest Fisheries Science Center in 2009 (Sakuma et al. 2006). This survey takes place from May through mid-June to target the time at which juvenile rockfish are most abundant in the pelagic environment and also coincides with the peak spring upwelling season off central California. The sampling stations of this survey were arranged to capture the spatial and temporal variability of oceanographic features along central California (Fig. 1). Water and juvenile rockfish samples were collected at these stations from 20 May to 12 June. We attempted to sample each station at least once and up to 3 times over this time period.

Seawater samples were collected with a 1080 General Oceanics GO-FLO sampler, which is a non-metallic, Teflon-coated sampling bottle designed to avoid both sample contamination at the surface and exchange of water from different depths. We obtained samples at 25 m depth, as this is typically above the thermocline and in the mixed layer where pelagic juvenile rockfish can be found (Ahlstrom 1959, Lenarz et al. 1991). Water samples were immediately filtered and preserved with Optima pure hydrochloric acid (resulting in 0.2% acid) under HEPA-filtered laminar flow conditions and stored frozen. The salinity and temperature associated with each sample were recorded via CTD (Sea-Bird Electronics, SEACAT 19plus). Water samples were collected ($n = 117$) both at night, coinciding with fish trawls, and during the day to improve spatial coverage.

We focused our study on pelagic juvenile shortbelly rockfish *Sebastes jordani*, which is typically the most abundant juvenile rockfish species collected in the survey. Like many rockfishes, shortbelly rockfish have a prolonged pelagic duration (approximately 3

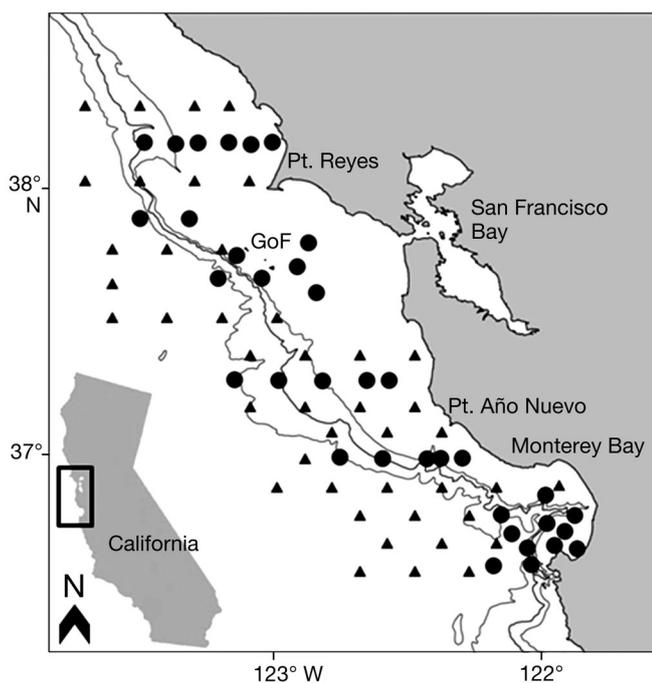


Fig. 1. National Marine Fisheries Service mid-water trawl survey core sampling area off central California. ●: stations where both trawling and water sampling were conducted. ▲: stations where only water samples were collected. GoF: Gulf of the Farallones

to 6 mo) that includes both larval and post-larval pelagic juvenile phases. Rockfish young disperse as larvae in the pelagic environment and remain offshore for the duration of both the larval and pelagic juvenile phase (Love et al. 2002). In these early life history stages, rockfish typically inhabit depths of 10 to 100 m but are most commonly found above the thermocline in the mixed layer (Ahlstrom 1959, Lenarz et al. 1991).

Juvenile rockfish were collected using a modified Cobb mid-water trawl with a 26 m headrope, 9.5 mm codend mesh, and a 14 × 14 m opening. Trawls were conducted at night to limit net evasion. The net fished for 15 min at a target headrope depth range of 25 to 30 m (Sakuma et al. 2006). Juvenile rockfish were measured (standard length) and stored at -80°C. In total, 333 juvenile rockfish were collected, of which 200 were selected for elemental analysis. Juvenile rockfish standard lengths ranged from 19 to 56 mm.

Sample preparation

All glass- and plasticware was acid leached prior to use. Otoliths were removed using glass probes, placed on a clean glass slide, and rinsed twice with ultrapure Milli-Q water (>18 M resistivity), followed by a 5 to 10 s rinse in Suprapur® hydrogen peroxide to remove any adhering tissue. Otoliths were then given a final rinse using Milli-Q water and dried in a HEPA-filtered laminar flow clean hood. Dry otoliths were stored in clean microcentrifuge tubes. An otolith from each pair was chosen at random to be mounted. We affixed the otoliths to a clean glass slide using Crystalbond™ resin. Using a progression of lapping films (Mark V A/O films, 9 µm, 3 µm, and 1 µm grits), otoliths were polished to a level plane exposing the margin (outer edge). We rinsed and sonicated slides containing individual otoliths in Milli-Q water for 10 min, followed by 3 rinses with Milli-Q water, and let them dry for 24 h in the laminar hood. Once dry, slides were stored in individual clean plastic bags. Water samples were thawed and diluted 1:20 with an internal standard in 1% trace metal grade nitric acid.

Sample analysis

The seawater and otolith samples were analyzed at the Marine Analytical Laboratory at the University of California, Santa Cruz (USA). The isotopes

¹³⁸Ba, ⁸⁸Sr, ²⁴Mg, and ⁴³Ca were quantified using inductively coupled plasma mass spectrometry (ICP-MS). Isotopic values were expanded to nominal elemental values and were expressed relative to Ca. These elements were chosen for analysis because each was consistently above detection limits in both the water and otolith samples (defined as 3 times the standard deviation of the blank values) and were possible indicators of upwelling. The majority of otolith Ba comes from the ambient environment (Walther & Thorrold 2006), and in general, positive relationships have been shown between Ba levels of the ambient water and the Ba in the otolith (Bath et al. 2000, Elsdon & Gillanders 2002, 2003b, 2005a, de Vries et al. 2005, Dorval et al. 2007, Miller 2009). Sr and Mg are not expected to vary much in seawater but may vary in the otoliths due to a possible positive or negative uptake relationship with temperature (Kalish 1989, Townsend et al. 1992, Secor et al. 1995, Bath et al. 2000, Elsdon & Gillanders 2002, Bath-Martin & Thorrold 2005). We predicted Ba would be the main element indicating upwelling exposure; however, the addition of Sr and Mg could allow for a stronger multi-element upwelling signature in the otolith. Water samples were analyzed on a Thermo Element XR magnetic sector ICP-MS. The standard used was a matrix similar to that of seawater created in the laboratory and of known elemental concentrations. Standards and blanks (1% nitric acid) were run every 12 samples to account for instrument drift and to perform blank-correction.

Otoliths were analyzed on a Thermo XSeries II quadrupole ICP-MS in concert with a Photon Machine Analyte193 excimer laser ablation system. A 300 µm transect was run around a portion of the otolith margin at 10% power, 1.93 J cm⁻², a repetition rate of 10 Hz, and a speed of 10 µm s⁻¹. A 30 µm diameter laser beam was centered on the otolith margin ablating 15 µm of otolith edge. The 15 µm sample is an average over the last 5 d of ambient exposure, as the average increment width at the otolith edge is ~3 µm. Ablation of less than 15 µm of material resulted in an unstable signal. Prior to sample ablation, a pre-ablation was performed to remove any surface contaminants (10% power, 1.93 J cm⁻², 69 µm diameter spot size, a repetition rate of 3 Hz, and a speed of 10 µm s⁻¹). Prior to each otolith sample, 20 s of background gas blank were recorded in order to determine limits of detection and to perform blank-corrections. The glass standards NIST 610 and NIST 612 (Pearce et al. 1997) were run between every 12 samples in

Table 1. Precision estimates (%RSD: percent relative standard deviation) and instrument limits of detection (LOD) for element to calcium ratios

Sample	Element	%RSD	LOD
Seawater	Mg:Ca	0.84	0.003 mol mol ⁻¹
	Sr:Ca	1.28	0.007 mmol mol ⁻¹
	Ba:Ca	1.78	0.12 μmol mol ⁻¹
Otolith	Mg:Ca	4.30	4.94 × 10 ⁻⁴ mmol mol ⁻¹
	Sr:Ca	4.10	4.6 × 10 ⁻⁵ mmol mol ⁻¹
	Ba:Ca	4.60	0.007 μmol mol ⁻¹

order to correct for instrument drift over the course of each session (300 μm transects, 25% power, 4.82 J cm⁻², 34 μm diameter spot size, a repetition rate of 10 Hz, and a speed of 10 μm s⁻¹). Precision was calculated as the mean relative standard deviation (%RSD) using the solution standard and NIST 612 for seawater and otoliths, respectively (Table 1). Limits of detection (LOD) were calculated as 3 SD of the blanks and expressed as ratios of the element to mean otolith Ca (Table 1). To test for any possible contamination resulting from the Crystalbond™, ablation transects were run using the otolith sampling parameters. All 4 elements (i.e. Ca, Sr, Mg, Ba) were indistinguishable from the background and were below detection limits. Therefore, we found Crystalbond™ to be an adequate mounting medium for this chemical analysis.

Statistical analysis

The water and otolith values used in the following analyses were averaged across replicate fish by station. Otolith elemental ratios were log-transformed to meet normality assumptions. Relationships between element accretion and body size or growth rate are frequently documented and thought to be associated with variability in the rate of protein synthesis to that of the crystallization rate of the otolith (Campana 1999). Significant correlations were identified between fish standard length and otolith Sr:Ca ($p < 0.001$, $R^2 = 0.40$) and Mg:Ca ($p = 0.002$, $R^2 = 0.29$). Therefore, standardized residuals were used.

Linear regressions were used to detect relationships between water chemistry and water temperature, which is inversely related to upwelling strength. These regressions indicated which elements (Ba:Ca, Sr:Ca, Mg:Ca) identify upwelled water.

To determine the likely number of distinct chemical signatures present in the water, we used Ward's hierarchical clustering method (hclust function in package *stats*, R Development Core Team 2011). Ward's hierarchical clustering is an agglomerative or 'bottom up' method that creates groupings based on similarity of multiple variables. Each observation starts as its own cluster, and at each step, the 2 clusters with the smallest Euclidean distance are merged. This process continues until the final 2 clusters merge to complete the resulting dendrogram. Unlike K-means clustering, Ward's method does not require specification of the number of clusters in advance. However, when determining clusters, we used a height value that resulted in all clusters having at least 3 stations. Once the clusters were identified, multivariate analysis of variance (MANOVA) was used to determine which clusters were significantly different from one another. We then used a canonical discriminant function analysis (CDFA) in the SAS (SAS Institute 2009) CANDISC procedure to determine the contributions of individual elements to each signature. These analyses were also applied to the otolith chemistries.

We used a spatial analysis of cross-covariance (Arc GIS geostatistical analysis tools; Isaaks & Srivastava 1989) to examine spatial lags between water and otolith chemistry that may be related to on-offshore and alongshore transport. The spatial analysis of cross-covariance provided an assessment of the coherence between water and otolith chemistry. Moreover, this procedure allowed us to address fish movement over the 5 d of otolith chemistry that were averaged within each otolith. Based on the average speed of the California Current (10 cm s⁻¹; Roughan et al. 2006), a passive particle could travel 40 km over a 5 d period. In addition, speeds during upwelling off California have been documented at between 20 and 30 cm s⁻¹ (Lynn et al. 2003) and even up to 50 cm s⁻¹ (Huyer & Kosro 1987); therefore, transport out of the upwelling center and into more relaxed regions within the 5 d recorded in the otoliths is likely. Without extensive sustained swimming and behavioral studies, it is impossible to know whether swimming ability limits or enhances juvenile rockfish dispersal. Spatial cross-covariance allowed assessment of the relationship between every possible pairing of water and otolith chemistry samples. Only stations where both water chemistry and otolith chemistry data existed were used in the analysis. Cross-covariance was examined in 8 compass directions to determine the direction of highest covariance before running the final analysis.

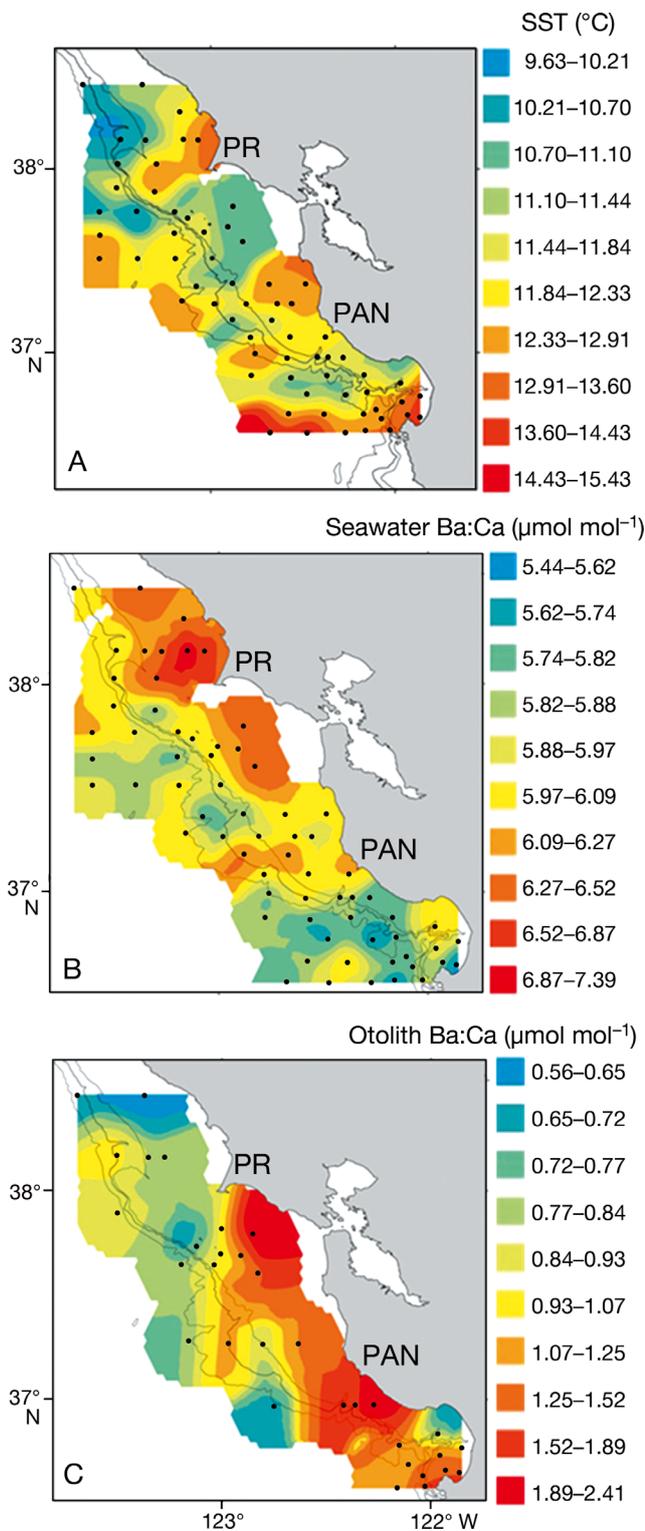


Fig. 2. Two major upwelling centers: Point Reyes (PR) and Point Año Nuevo (PAN). (A) Sea surface temperature in °C. (B) Seawater Ba:Ca $\mu\text{mol mol}^{-1}$. (C) Otolith Ba:Ca $\mu\text{mol mol}^{-1}$. All panels are average values from 20 May to 12 June, interpolated by inverse distance weighting. Black points indicate stations where samples were collected

RESULTS

Water chemistry

Both sea surface temperature and water chemistry were highly variable across the study region (Fig. 2). Cold upwelled waters were present around Point Reyes, indicating advected water from a recent upwelling event. Warmer water at the core of the upwelling center, immediately north of Point Reyes, indicated that the event had ended. We detected a significant negative relationship between Ba:Ca and sea surface temperature across sites ($R^2 = 0.2393$, $p < 0.001$). In contrast, we did not detect relationships between either Mg:Ca or Sr:Ca of the seawater and sea surface temperature.

We identified 3 chemical signatures present in the seawater using Ward's hierarchical clustering method (Table 2, Fig. 3). The 3 signatures were significantly different (MANOVA, $p < 0.001$). Following the significant MANOVA, univariate ANOVA demonstrated that all 3 elements (Ba:Ca, Mg:Ca, and Sr:Ca) contributed to the difference (Table 3). One of the 3 signatures (Signature 3) exhibited very high Ba:Ca values (mean \pm SD: $6.54 \pm 0.41 \mu\text{mol mol}^{-1}$) compared to the other 2 (Table 2) and is likely that of recently upwelled water.

Otolith chemistry

Otoliths with highest Ba:Ca were collected south of Point Reyes, and around and to the south of Point Año Nuevo (Fig. 2). Ward's hierarchical clustering method identified 3 distinct otolith chemistry signa-

Table 2. Means (SD) for clusters as determined by Ward's hierarchical cluster method for both water and otolith samples. Values are expressed as ratios to Ca

Cluster	Ba:Ca ($\mu\text{mol mol}^{-1}$)	Mg:Ca (mol mol^{-1})	Sr:Ca (mmol mol^{-1})
Seawater			
1	5.83 (0.14)	4.93 (0.05)	9.54 (0.13)
2	6.06 (0.16)	5.00 (0.03)	9.37 (0.11)
3	6.54 (0.41)	5.01 (0.22)	9.43 (0.59)
	Ba:Ca ($\mu\text{mol mol}^{-1}$)	Mg:Ca (mmol mol^{-1})	Sr:Ca (mmol mol^{-1})
Otoliths			
1	0.76 (0.12)	0.11 (0.02)	1.57 (0.19)
2	1.10 (0.27)	0.17 (0.03)	1.62 (0.07)
3	1.63 (0.43)	0.13 (0.02)	1.67 (0.12)

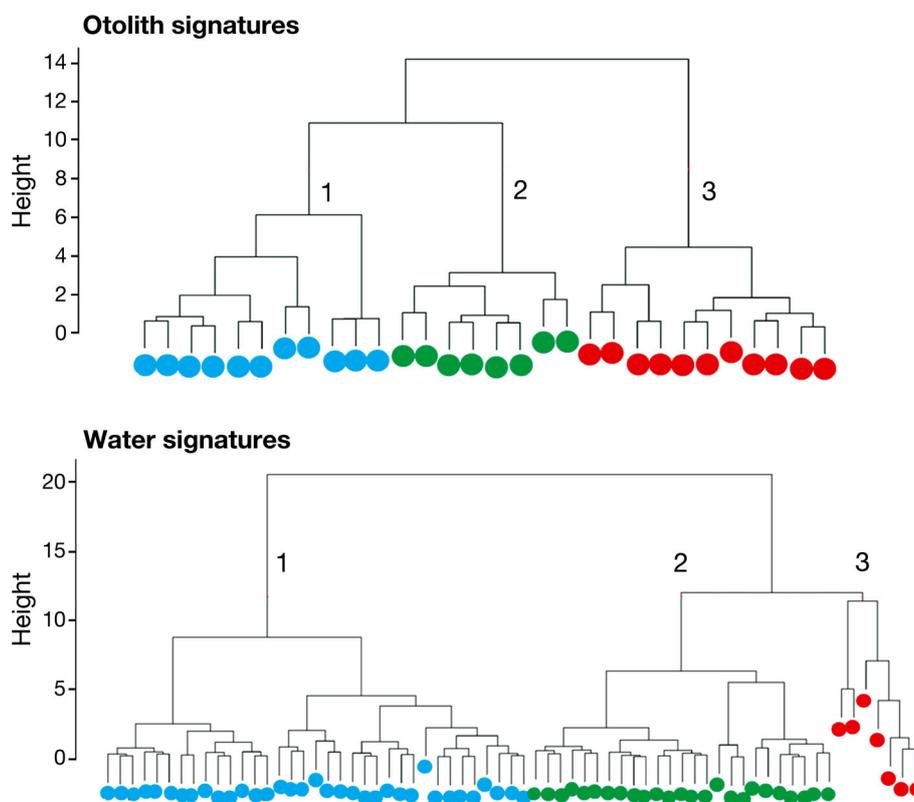


Fig. 3. Clusters produced by Ward's hierarchical cluster method. The height scale on the y-axis reflects the relative similarity between different samples. Three clusters were identified both in water and otolith chemistries. Water signatures were matched up with otolith signatures based on their similarity, i.e. Signature 3 for water and otoliths were clusters with the highest Ba:Ca ratio. These assignments were later supported by the canonical discriminant function analysis results

Table 3. Univariate analysis of variance (ANOVA) results following significant multivariate ANOVA comparing element concentration across the 3 clusters as defined by the hierarchical cluster method. ANOVAs were run for all 3 elements across water and otolith clusters

Source	df	SS	MS	F	p
Water chemistry					
Ba:Ca	2	3.09	1.54	42.39	<0.001
Error	64	2.33	0.04		
Mg:Ca	2	0.09	0.04	7.56	<0.001
Error	64	0.37	0.01		
Sr:Ca	2	0.48	0.24	5.20	0.010
Error	64	2.94	0.05		
Otolith chemistry					
Ba:Ca	2	0.13	0.06	19.67	<0.001
Error	28	0.09	0.00		
Mg:Ca	2	0.24	0.12	25.65	<0.001
Error	28	0.13	0.00		
Sr:Ca	2	0.03	0.01	10.37	<0.001
Error	28	0.04	0.00		

tures (Table 2, Fig. 3). The 3 clusters were significantly different (MANOVA, $p < 0.001$), and based on univariate ANOVAs, all 3 elements (Ba:Ca, Mg:Ca, and Sr:Ca) contributed to the differences among clusters (Table 3). One of the signatures (deemed Signature 3 for consistency) exhibited elevated levels of Ba:Ca (mean \pm SD: $1.63 \pm 0.43 \mu\text{mol mol}^{-1}$). Fish with signature 3 are likely those that experienced upwelled water over our sampling period.

The CDFA illustrates the relative contribution of each element to the 3 signatures as defined by the hierarchical analysis for both water and otoliths (Fig. 4). Water and otolith signatures showed similar patterns. The upwelling signature (Signature 3) was consistently high in Ba:Ca and moderate in Mg:Ca and Sr:Ca. Signature 2 was consistent as well, being the highest in Mg:Ca and moderate in Ba:Ca and Sr:Ca. Signature 1 was characterized by the lowest values of Ba:Ca and Mg:Ca. Signature 1 was inconsistent between otolith and water chemistries with regard to Sr:Ca. Specifically, Sr:Ca in the otolith for

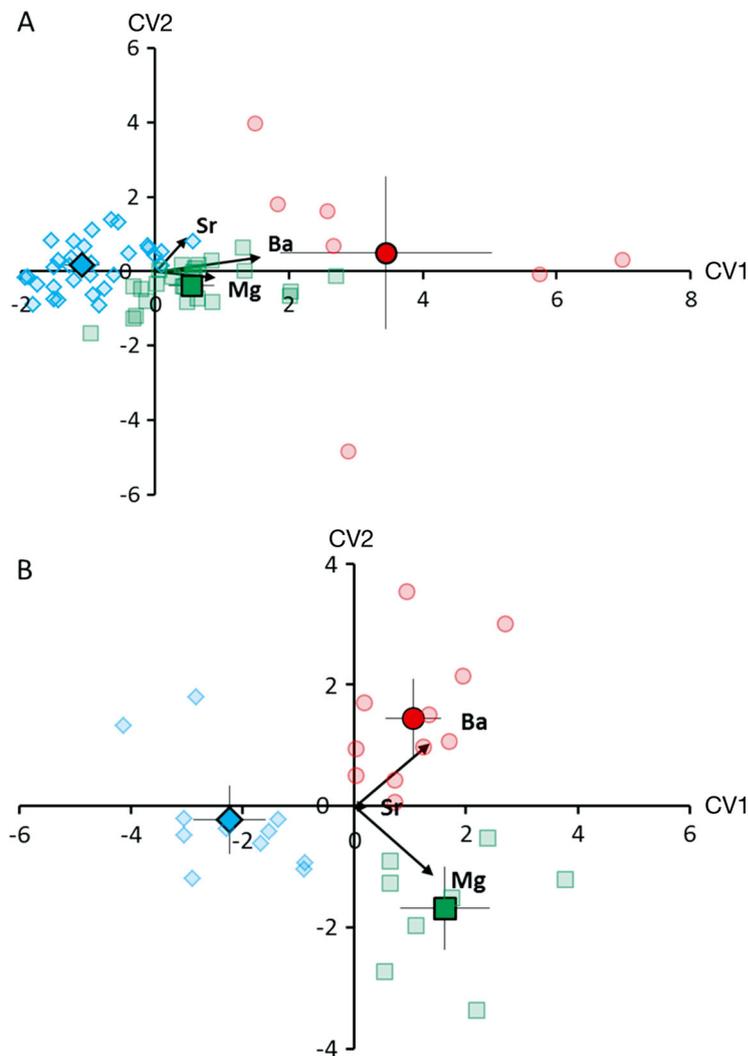


Fig. 4. Canonical discriminant function analysis of the chemical signature clusters, illustrating the relative influence of each element on the 3 signatures as defined by the hierarchical analysis for (A) water samples and (B) otolith samples. \diamond : Signature 1, \square : Signature 2, \circ : Signature 3. Large icons represent the means and SD of each signature cluster. Arrowheads represent standardized weight coefficients of each element:Ca for the first and second components (CV1: Canonical Variate 1, CV2: Canonical Variate 2)

Signature 1 was the lowest of the 3 groups while in the water it was the highest (although the range in the water was small).

Individual water samples that made up Signature 3 (high Ba:Ca, upwelling signature) were found to occur around the upwelling center of Point Reyes (Fig. 5). Otolith samples associated with Signature 3 occurred to the south of Point Reyes (Fig. 5). This directional shift may be driven by transport over the 5 d of averaged otolith chemistry. To assess the offset, we compared water and otolith chemistries using spatial cross-covariance to determine the distance at

which covariance between water and otolith chemistry was greatest. After examining the covariance in 8 compass directions, the strongest covariance pattern (>2 SD) between water and otolith Ba:Ca was in the southeast direction (135°) and at a scale of about 50 to 100 km (Figs. 6 & 7; Legendre & Legendre 1998). This was not surprising, as this is the predominant orientation of the shelf break and the average direction of travel of surface waters in the region (Hickey 1979, Steger et al. 2000). Moreover, highest covariance was found between water samples from Point Reyes and otolith samples from the Gulf of the Farallones. Water samples from the Gulf of the Farallones and otoliths from Point Año Nuevo also exhibited high covariance at the same scale.

DISCUSSION

Understanding how the spatial and temporal variability of upwelling affects survival and recruitment success of pelagic fish larvae will greatly benefit the management of marine populations and ecosystems. Developing tools to identify fish that experience upwelling allows researchers and managers to better address the role it plays in larval transport, growth, survival, and eventual recruitment to an adult population. We present a novel application of a geostatistical method to examine the relationship between pelagic juvenile fish and their environment in an open coastal system.

Specifically, we confirmed the presence of a chemical signature in upwelled water that can be detected in pelagic juvenile rockfish otoliths and did so in a spatial context where fish were moving within the open coastal system of central California. It has been hypothesized that in this region, juvenile rockfish are transported southward from areas of high advection (driven by upwelling) to areas of relaxation (e.g. Gulf of the Farallones, Monterey Bay; Wing et al. 1998). Our results support this, as individuals carrying the upwelling signature were found in the retentive areas south of upwelling centers.

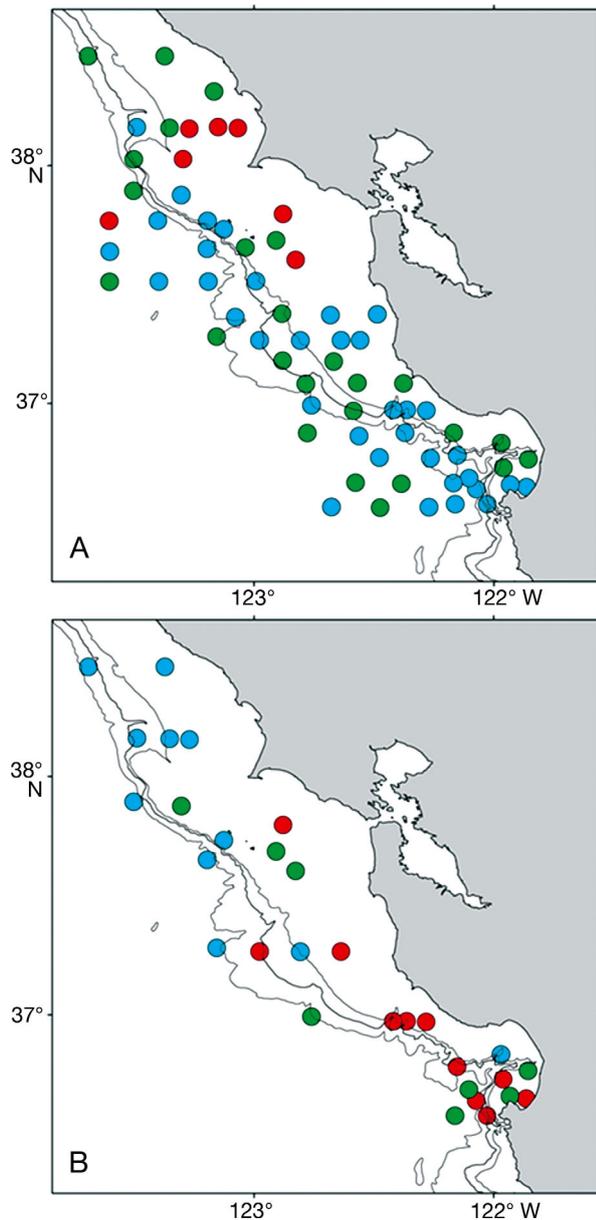


Fig. 5. (A) Water samples and (B) otolith samples coded by assigned signature cluster. ●: Signature 1, ●: Signature 2, ●: Signature 3

The coldest temperatures and highest levels of Ba:Ca appeared around the upwelling centers of Point Reyes and Point Año Nuevo. Ba was highest around the more intense of the 2 centers, Point Reyes. A significant relationship with cold temperatures indicated Ba as the primary indicator of upwelled water off central California, which agrees with observations made by Elsdon et al. (2008).

We quantified 3 chemical signatures in both the water and otoliths based on 3 elements (Ba, Sr, and Mg) expressed as ratios relative to Ca. The 3 signa-

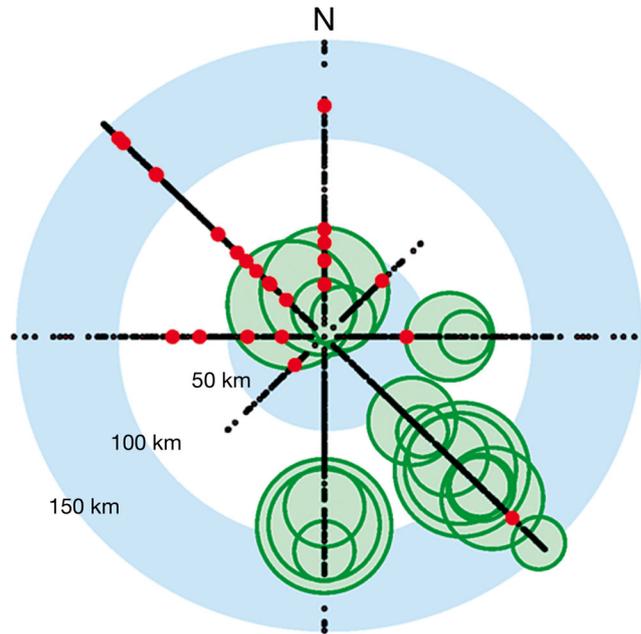


Fig. 6. Results of spatial cross-covariance, which examined the covariance between every possible water and otolith chemistry pairing (using Ba:Ca only), in all directions out to 150 km. Green circles denote significant (>2 SD of the mean covariance) positive correlations; the size of the circle demonstrates the strength of the correlation. Red dots are significant negative correlations. Blue and white background rings show distance in 50 km intervals

tures were similar in composition for water and otolith samples, which is expected, as signatures from the water are transferred to the otolith (Campana 1999, Bath et al. 2000, Walther & Thorrold 2006). The weak pattern observed in Signature 1 is driven by Sr:Ca and likely due to the very limited variability of Sr:Ca in the ocean (de Villiers 1999), and in fact, we observed this limited variability in our own water samples (range: 8.43 to 10.22 mmol mol^{-1} , SD: 0.24). We originally anticipated that Sr:Ca may show variation in the otoliths due to possible temperature differences as both positive and negative relationships between temperature and otolith Sr uptake have been shown (Kalish 1989, Townsend et al. 1992, Secor et al. 1995, Bath et al. 2000, Elsdon & Gillanders 2002, Bath-Martin & Thorrold 2005, Webb et al. 2012). However, this did not appear to be the case, as little difference was seen across Sr:Ca in the otoliths as well (range: 1.24 to 2.22 mmol mol^{-1} , SD: 0.20).

We found a spatial offset between water and otoliths of Signature 3, which was the high Ba:Ca upwelling signature. Spatial cross-covariance analysis quantified this spatial offset and revealed that otolith Ba:Ca concentration covaried positively with water Ba:Ca concentration that was 50 to 100 km to

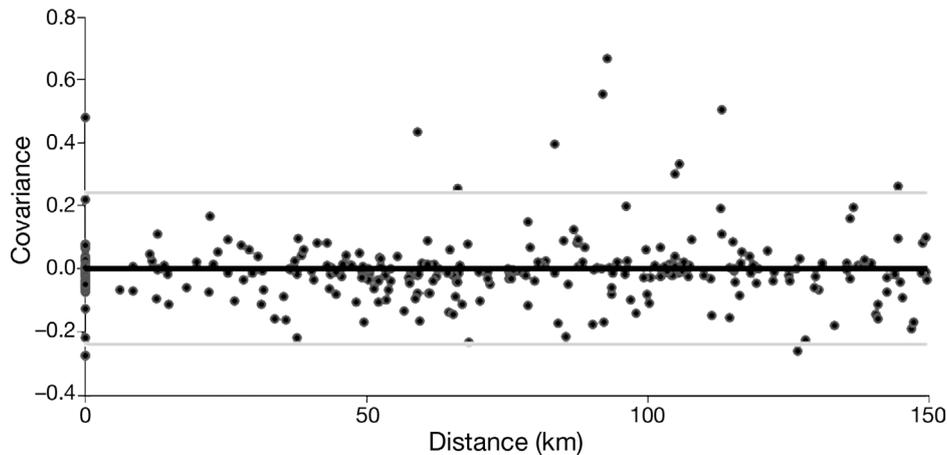


Fig. 7. Spatial cross-covariance between Ba:Ca of the water and Ba:Ca of the otoliths. Analysis was in the alongshore direction of 135°. Grey lines indicate 2 SD of the mean covariance (2 SD = 0.24). The highest and most significant covariance occurs at a distance between 50 and 100 km. These highest correlations are between water samples from Point Reyes and otolith samples from the Gulf of the Farallones, and water samples from the Gulf of the Farallones and otolith samples from near Año Nuevo

the north of where the otolith samples were collected. This distance is well within the range that a passive particle originating at the upwelling center of Point Reyes might travel in a 5 d period (Huyer & Kosro 1987, Lynn et al. 2003, Roughan et al. 2006). This directional shift in Ba:Ca pattern is likely driven by southern transport of pelagic juvenile rockfish in the open system of central California as the California Current moves equatorward along the coast (Hickey 1979, Parrish et al. 1981, Steger et al. 2000, Mann & Lazier 2006). We acknowledge that juvenile rockfish are not passive particles; however, without further research on sustained swimming and behavior, we are unable to say whether this may limit or enhance their dispersal distance. Juvenile rockfish of different species but similar size to those studied here (mean: 37 mm fork length) have been shown to exhibit critical swimming speeds ranging from 25.3 to 34.3 cm s⁻¹ (Kashef 2012). With critical swimming speeds close to observed current speeds, we find it unlikely that juvenile rockfish could resist prevailing currents for prolonged durations.

Disentangling the regulators of elemental uptake to the otolith is an ongoing obstacle in otolith science (Elsdon & Gillanders 2003a); however, it is not likely that the spatial patterns in the otolith chemistry we found are the result of sampling-derived or biologically-derived lags in response to ambient conditions. We reduced the likelihood of artificially creating a lag due to sampling method by reducing our laser diameter, reducing the laser power to minimize the ablation depth, and sampling with rasters that were parallel to the growth bands at the margin of the otoliths. Previ-

ous studies have suggested that there is a lag in the time to detect elemental saturation in otolith chemistry (e.g. Elsdon & Gillanders 2005b, Lowe et al. 2009, Miller 2011). Unfortunately, the temporal offsets between otolith and water chemistries they interpreted may be confounded by the analytical approaches they took. As an example, the diameter of the laser beam, the depth of the ablation, and the instrument acquisition rate lead to a moving average value between adjacent ablations (Hoover 2012). Thus, saturation is only detected when all increments ablated in 3 dimensions represent the region corresponding to the more recent chemistry. This apparent lag can also be extended by smoothing statistics used on the transect data. Therefore, any true biological lag would be hard to parse out of these analyses. Milton & Chenery (2001) used a sub-daily increment sampling diameter on the otoliths of a diadromous species. They demonstrated a lag before full saturation was detected in the otolith. This is unsurprising, as changes in osmoregulation occur between freshwater and marine systems that result in a change in the partition coefficients between otolith and water chemistries (Wells et al. 2003, Zimmerman 2005) that may not be immediate. However, we do not anticipate such a physiological response for a wholly marine species. Importantly, the majority of studies reporting a lag in saturation also reported that the otolith chemistry demonstrated an immediate response to a change in ambient chemistry. Therefore, otoliths should be a sensitive indicator to changing water chemistry in a system such as ours where upwelling dynamics lead to ephemeral exposure to upwelled water and associated Ba.

Another possible scenario that could create the observed pattern in otolith Ba:Ca is vertical migration. Since pelagic juvenile rockfish have the ability to migrate vertically in the water column, there is the potential that in some locations fish are going deeper than others, perhaps far below the thermocline where they might encounter higher levels of Ba. However, based on observations in the literature (Ahlstrom 1959, Lenarz et al. 1991), the behavior of making repeated migrations into deeper water is unlikely. Also, not enough is known about the vertical distribution of Ba and how deep a fish would need to go to encounter enough Ba to be mistaken for an upwelling signature.

Requirements for successful application of our methodology are knowledge of the oceanography and mesoscale structuring and the associated interannual variability of the system in question. Both water and otolith chemistries are also required to employ the appropriate geostatistics. It is important to note that the success of these analyses is likely year and ocean condition dependent. More work is needed to fully assess the interannual variability of the upwelling signature and the transfer of water chemistry to otoliths in the central California open coastal system.

A next step to this work should include controlled laboratory experiments to quantify the water chemistry and otolith chemistry relationships within the natural range and variability of temperatures experienced by the fish. Determination of specific partition coefficients would allow us to better link water and otolith chemistries. We then need to determine whether the upwelling signature can be detected in the juvenile portion of surviving adult otoliths. Given the results of this study, it appears that otolith microchemistry is a useful tool in identifying fish that experienced upwelling and eventually answering the question of how direct upwelling exposure affects juvenile growth, survival, and recruitment success.

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