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ABSTRACT: Shortspine thornyhead Sebastolobus alascanus are widely distributed along the eastern Pacific coast and are assessed as a demographically homogeneous stock off the coasts of California, Oregon and Washington. The validity of this assumption has been questioned because data suggest complex ontogenetic movements. Otolith microchemistry applied to immature fish was used to determine the age of settlement and the population structure of S. alascanus. Samples were collected from 2011–2013 from bottom trawl surveys in 5 areas along the coasts of (1) Washington, (2) northern Oregon, (3) southern Oregon and northern California, (4) central California and (5) southern California. The Ba:Ca ratio, measured from the primordium to the margin of otoliths, corresponds to the pelagic and benthic phases of immature fish. This ratio decreased steadily before the formation of the first annulus and increased thereafter. Water masses along the bathymetric gradient mostly correlated with the distribution of otolith trace element (Mg:Ca, Ba:Ca) and stable isotope (δ^{18} O and δ^{13} C) ratios on the continental shelf. These results indicate that the population of immature fish belongs to 2 distinct groups, distributed north and south of Cape Mendocino. Accordingly, the performance of a k-nearest-neighbor classifier was highest when structured based on these 2 regions, with 94 and 69% cross-validated classification accuracy, respectively into the northern and southern groups. These results confirm that S. alascanus settle after nearly 1 yr of pelagic life and show that otolith chemistry can be used to test critical hypotheses regarding the migration and population structure of this species.

KEY WORDS: Otolith trace elements \cdot Stable isotopes \cdot Shortspine thornyhead \cdot Fish settlement \cdot Population structure

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1. INTRODUCTION

Understanding the structure and connectivity of exploited fish populations is critical to sustainable management. This process requires adequate information on the demography and movement of these stocks in space and time and the identification of habitats that may contribute most to their productivity. Traditional mark-recapture methods have long been used to understand the dynamics of marine and freshwater fish, but premature tag losses and decreasing tag recovery rates over time combined with potential effects on fish physiology and behavior often limit the application of these techniques in inferring the dynamics of long-lived fish (Lucas & Baras 2000, Bridger & Booth 2003, Black et al. 2005, Jepsen et al. 2015). Natural chemical tags, such as otolith chemistry, do not suffer from such limitations and thus can be applied to study the migration of fish irrespective of their life span and scale of geographical distribution. Because otoliths are primarily made of aragonite, a calcium carbonate polymorph (Gauldie 1993, Campana 1999), and because they grow by accreting layers, they can record spatial and temporal variation in the physical and chemical properties of environmental waters throughout the life of individual fish. Once deposited in otoliths, the concentrations of trace elements are metabolically stable (Campana 1999), although some of these elements can be tracers of physiology rather than environment (Hüssy et al. 2021). In addition, all fish born in a given habitat and within the same cohort are similarly marked, and this chemical fingerprint will remain in the population throughout a fish's lifetime.

Laboratory and field studies have demonstrated strong relationships between otolith and water trace element concentrations for both estuarine-dependent fish (Bath et al. 2000, Elsdon & Gillanders 2002, Dorval et al. 2007) and marine resident fish (Martin & Wuenschel 2006, Miller 2009, Nishimoto et al. 2010, Limburg et al. 2011). Similarly, oxygen stable isotopic composition of water and otoliths negatively correlates with temperature in both laboratory-reared fish (Kalish 1991a, Milton & Chenery 2001, Dorval et al. 2011, Sakamoto et al. 2017, Shirai et al. 2018) and wild-caught fish (Dorval et al. 2007, Geffen 2012, Javor & Dorval 2014). Therefore, otolith chemistry can be a powerful tool to reconstruct the life history of fish, track fish movement and infer stock structure (Campana 1999, Thorrold et al. 2001, Elsdon & Gillanders 2003, Dorval et al. 2011, Sebatian et al. 2021). Otolith chemistry may be particularly suitable for understanding the dynamics of long-lived fish such as the shortspine thornyhead Sebastolobus alascanus.

S. alascanus are extensively distributed along the shelf and deep water slopes off the North American Pacific coast, from northern Baja California, Mexico, to the Bering Sea (Miller & Lea 1972, Eschemeyer et al. 1983). They are commercially exploited off California, Oregon and Washington by a deep-water, multi-species bottom trawl fishery on the continental shelf and slope (SAFE 2014). They are managed as a homogeneous stock in this region, although they exhibit complex spatial dynamics in their ontogeny (Piner & Methot 2001, Taylor & Stephens 2014). They can live over 80–100 yr (Butler et al. 1995, Kastelle et al. 2000) and grow to a maximum length over 70 cm (Taylor & Stephens 2014). Females generally mature at 18 cm off the US west coast (Pearson & Gunderson 2003), but their minimum size at maturity is highly variable (Love et al. 2002, Orlov & Abramov 2010, Taylor & Stephens 2014). Larval and juvenile stages have prolonged pelagic phases that can extend up to 14–15 mo (Moser 1974, Wakefield 1990, Wakefield & Smith 1990), but *S. alascanus* remain benthic throughout their late juvenile and adult stages. Although mtDNA sequencing patterns of genetic divergence of *S. alascanus* appeared to be related to geographic distance from southern California to Alaska, a clear delineation of fish stocks could not be proposed, even between fish collected at the geographic end-members of the distributional range (Stepien et al. 2000, Taylor & Stephens 2014). The latest stock assessment models (Hamel 2005, Taylor & Stephens 2014) assumed a single stock, with no significant structure from the USA–Canada to the USA–Mexico borders, but whether this is, in fact, the most appropriate stock delineation is yet to be determined.

Bottom trawl surveys conducted in the 1990s followed by the ongoing NMFS West Coast Groundfish Bottom Trawl Survey in 2003 (Keller et al. 2017), which covers both the continental shelf and slope (covering depths 55-1280 m), have revealed large latitudinal and bathymetric differences in S. alascanus size distribution off the West Coast of the USA (Taylor & Stephens 2014). Juveniles settle on the shelf at about 100-200 m (Moser 1974, Vetter & Lynn 1997), and small fish (<10 cm) occur most frequently between 200 and 600 m. Smaller fish are found in large concentrations off Oregon from 44-46°N (see Fig. 2), forming a spatially explicit juvenile hotspot that is consistent across years (Tolimieri et al. 2020). In contrast, larger fish are more common in deeper water below 40°N in central and southern California (Jacobson & Vetter 1996, Taylor & Stephens 2014). The largest fish are found in deep water off northern California and southern Oregon. Alongshore movement is unknown, but Piner & Methot (2001) hypothesized that these distributional patterns resulted from S. alascanus moving southward and deeper from a recruitment area off the Columbia River region. More specifically, they believed that fish <25 cm off the Columbia River region moved southward to form the bulk of 26-60 cm fish that occupied Southern California. They also hypothesized that the largest fish collected off northern California and southern Oregon were a continuation of this movement. Since the 1990s, these patterns in S. alascanus size distribution have been consistently observed from survey data and have been conceptualized as a 'J-shape' migration hypothesis (Piner & Methot 2001, Taylor & Stephens 2014). However, the extent to which this hypothesis may have resulted from natural variability in recruitment patterns or spatial differences in fishing exploitation rates is not yet understood.

The ontogeny and behavior of S. alascanus are not conducive to large-scale latitudinal migrations. Video observations from submersible and remotely operated vehicles have characterized this species as a sit-and-wait predator that remains motionless at the bottom for prolonged periods (Jacobson & Vetter 1996). However, the sit-and-wait predator hypothesis that is widely accepted for most rockfish species cannot fully explain either the current distributional patterns in S. alascanus size or the mechanisms used by this species to maintain their reproductive capacity and a viable population. Because large mature fish reside mostly off central-northern California, oceanic currents could have played a role in transporting their offspring back to northern habitats (i.e. Washington and Oregon), where juveniles and young of the year are most abundant. A northward transport mechanism for larvae has not been proposed for this species, but the California Undercurrent (Agostini et al. 2006) is associated with northward movement of Pacific hake in this region.

Latitudinal patterns in S. alascanus size distribution may be also influenced by chemical properties emerging from along coast barriers, such as oceanic fronts and river plumes. Differing physical and biochemical processes have been hypothesized to limit the dispersal of larvae and juveniles between southern and northern biogeographic regions delimited by the California and Alaska Current divergent front (Stepien 1995). The Columbia River, the largest river on the US Pacific coast, supplies nearly 77-90% of the freshwater to the eastern Pacific Ocean from the Strait of Juan de Fuca to San Francisco, California (Barnes et al. 1972, Naik & Jay 2005), regulating coast-wide oceanic processes that influence the life history of fish populations. The river has a buoyant plume that delivers freshwater, dissolved and particulate organic matter, trace metals and pollutants to the coastal shelf (Aguilar-Islas & Bruland 2006, Bruland et al. 2008, Hickey et al. 2010). Stratification caused by the freshwater and nutrient-rich inputs not only enhances productivity within and at the margins of the plume but also influences the trace elemental chemistry of the plume and its underlying water masses.

Trace element concentrations are temporally variable within the Columbia River plume and show spatial patterns in surface water along the northeastern Pacific coast. Mn concentrations in the plume vary between neap and spring tides, ranging from 0–250 nmol kg⁻¹ (Aguilar-Islas & Bruland 2006, Fig. 1A). On average, ⁹⁰Sr concentration changes only from 0.4–0.75 micromicrocurie (µµc) kg⁻¹ from the

mouth of the Columbia River to offshore surface waters but is directly proportional to freshwater inputs (Park et al. 1965, Fig. 1B). The Columbia River plume also generates southward flows of recently upwelled waters from the Washington shelf, leading to increasing productivity on the shelf (Banas et al. 2009) and, therefore, elevated Ba in regions of upwelling. Southward of the plume, Ba concentration also shows spatial heterogeneity depending on the presence or absence of upwelled waters. For example, off Point Reyes and Point Año Nuevo in California, Woodson et al. (2013) found that the Ba:Ca ratio was significantly higher in recently upwelled waters $(6.54 \pm 0.41 \,\mu\text{mol mol}^{-1})$ than in non-upwelled waters (as low as $5.83 \pm 0.14 \mu mol mol^{-1}$). On a larger spatial scale, from San Diego, California, to Alarcon Basin, Mexico, Esser & Volpe (2002) reported depletions of surface Ba greater than 60% in warn and intermediate chlorophyll waters (Fig. 1D); whereas Sr varied only from about 83–87 mmol kg⁻¹ with salinity over the same region (Fig. 1C).

The variability of surface Ba in coastal waters of the northeastern Pacific Ocean contrasts with the concentration of this element in deep waters, which shows increasing Ba concentration with depth regardless of latitude (e.g. Brass & Turekian 1974, Chan et al. 1976, Fig. 1G). This vertical distribution of Ba is commonly referred to as a nutrient-like pattern (Lea & Boyle 1989, Lynch-Stieglitz & Marchitto 2014) and differs from that of Sr, which varies little with depth (Fig. 1F), or Mn, which is highly variable in the water column (Fig. 1E) (Bernat et al. 1972, Brass & Turekian 1974, Sunda 2012). As otoliths record the chemistry of environmental waters, it could be expected that S. alascanus otoliths would also reflect the vertical distribution of Ba and potentially the spatial heterogeneity of trace element chemistry observed along the northeastern Pacific coast.

Our primary objective was to investigate whether otolith chemistry could be used to infer the population structure of immature fish that settled on the continental shelf in waters of <500 m depth from Washington to California; and by corollary, to determine whether otolith chemistry could be a viable method to study *S. alascanus* movement in space and time and test the J-shape hypothesis proposed by Piner & Methot (2001). Because no ageing methods have been developed to consistently assign age to all possible length classes or life stages of *S. alascanus*, a secondary objective of this study was to determine whether otolith chemistry can help in identifying the first otolith annulus and the timing of settlement of immature fish. We focused on 4

1000

3000

5000

1000

3000

5000

1000

3000

5000

0

80

0.0

0.2

82

50

0.4

84

0.6

86

Sr (µmol kg⁻¹)

100

Ba (nmol kg⁻¹)

Mn (nmol kg⁻¹)

0.8

88

150



Fig. 1. (A–D) Relationship between salinity and trace elements (Mn, Sr, Ba) measured in surface waters and (E–G) depth profiles of these elements at different locations along the northeastern Pacific coast. (A) Mn data collected by Aguilar-Islas et al. (2006) during neap (open blue diamonds) and spring tide (blue crosses) in the Columbia River plume; (B) ⁹⁰Sr measured by Park et al. (1965) from the mouth of the Columbia River plume to offshore waters; (C) Sr and (D) Ba measured by Esser & Volpe (2002) from San Diego, CA, to Alarcon Basin, Mexico; (E) Mn data reported by Sunda (2012) at 32° 42′ N, 145° 0′ W; (F) Sr profile presented by Brass & Turekian (1974) (red triangles) at 28° 29′ N, 121° 38′ W and Bernat et al. (1972) (open red cir cles) at 9° 13′ N, 119° 18′ W; (G) Ba concentrations reported by Chan et al. (1976) (open red circles) at MIT Stn 204 and Brass & Turekian (1974) (filled red circles) at 28° 29′ N, 121° 38′ W

Е

1.0

F

90

G

2. MATERIALS AND METHODS

2.1. Field sampling and study design

Sebastolobus alascanus otoliths analyzed in this study were extracted from small, immature fish col-



lected from 2011–2013 off the US Pacific west coast from Washington to California (Fig. 2). Fish were sampled during the West Coast Groundfish Bottom Trawl Survey (WCGBTS) conducted annually from May–October by the Northwest Fisheries Science Center (NWFSC) (Keller et al. 2017). *S. alascanus* are sampled in every haul where they occur (51% of all hauls and 90% of hauls from waters >250 m). For purposes of this study, the existing collection was subset to focus on data from immature fish collected in waters of <500 m depth within 5 different areas: Washington (1-N, >47°N); northern Oregon (2-N, 44–46.25°N); southern Oregon and northern California (3-N, 40–44°N); central California (4-S, 36–40°N); and southern California (5-S, <36°N) (Fig. 2, Table 1). The number of samples selected by year in



Fig. 2. (A) Distribution and latitudinal variability in the density of immature shortspine thornyhead *Sebastolobus alascanus* (1–17 cm, number of individuals ha⁻¹, green bubbles) collected by the NWFSC bottom trawl survey from Washington to California during the 2011–2013 period. Black cross symbols: location of each haul. (B) purple, orange and yellow dots: samples used in this study, collected on the continental shelf in <500 m depth in the geographic areas delimited by horizontal black dash lines along the latitudinal gradient, except in the area between latitude 46.5–47° N. In (A) and (B), 1-N indicates that the first geographic area was located in the northern region (N), and so forth

Region	Area	—— Fisl 2011	h sample by y 2012	2013 vear	Total sample	Length range (cm)	Temperature (°C)	Salinity (ppt)
N	1-N	6	5	5	16	9–17	6.23 (0.09)	33.97 (0.01)
	2-N	12	17	1	30	9–17	6.13 (0.05)	34.04 (0.00)
	3-N	10	12	8	30	10–17	6.51 (0.07)	34.07 (0.01)
S	4-S	6	9	8	23	8–17	7.20 (0.12)	34.13 (0.01)
	5-S	13	10	7	30	6–17	7.34 (0.08)	34.24 (0.01)

Table 1. Fish samples and sizes selected from the bottom trawl survey by region (N: northern; S: southern), area and year of collection. Water temperature and salinity (mean ± SE) were measured at the depth of capture between 171 and 498 m

a given region ranged from 1–17, reflecting annual availabilities of fish-at-size to the bottom-trawl survey. Within each area, salinity- and temperatureat-catch varied little across the 3 yr of sample collections (Table 1), and depth-at-capture ranged from 171–498 m across areas and years.

2.2. Otolith sectioning and cleaning

From each individual fish, one sagittal otolith was randomly selected, mounted on a glass slide using Crystalbond 555 (SPI[®]) glue and then sectioned using a low-speed Isomet saw (Buehler 11-1280-160). Otolith thin sections were cut transversally, through the primordium, to expose their core and possibly their growth increments. Thin sections measured approximately 1 mm in thickness after being slightly polished using lapping films. Remaining glues were completely removed from otolith sections after immersion in warmed distilled water.

Otolith thin sections were further prepared for analysis in a class-100 clean room at the Southwest Fisheries Science Center (SWFSC). Otoliths were cleaned on acid-washed glass slides using glass probes to remove all possible remnant materials, and then triple-rinsed in MilliQ-water (MQ- H_2O) and soaked for 3-5 min in 30 % H₂O₂ (J.T. Baker UltrexII Ultrapure). All otoliths were again triple-rinsed in MQ-H₂O before drying in a laminar flow hood for at least 24 h. After drying, otolith sections were mounted on petrographic slides using Krazy Glue (Elmer's Products). Each slide contained 10-12 otoliths distributed in 2 columns and 5 or 6 rows depending on their size. Regional fish samples were equally represented in most slides and their otoliths were randomly selected and allocated to rows and columns. Finally, the petrographic slides were rinsed and then sonicated in a bath of MQ-H₂O for 10 min. After sonication, all otolith slides were dried in a laminar flow hood for at least 24 h.

2.3. Trace element analysis

Otolith thin sections were analyzed by laser ablation inductively coupled mass spectrometry (LA-ICP-MS) at the University of Massachusetts (UMASS) Boston Environmental Analytical Facility (EAF). A CETAC LSX-213 laser ablation system (Cetac Technologies) connected to a Perkin Elmer Elan® DRC II ICP-MS (Perkin Elmer) was used to quantify trace element concentrations along each otolith section from the primordium to the edge. The spot method was used, setting the energy level at 50%, pulse rate at 20 Hz, burst at 200 shots, which resulted in spot diameters of 100 µm. Distance between the spots was 150 µm in all otolith sections. In this study, 5 isotopes were quantified: ²⁴Mg, ⁴⁶Ca, ⁵⁵Mn, ⁸⁸Sr and ¹³⁸Ba, to detect regional differences in otolith trace element composition. The limit of quantification was 10 times the concentration of an analytical gas blank (helium) and set to below 1 ng kg⁻¹ for each analyte measured in otoliths. Calcium is the most abundant element in otolith aragonite and therefore was used to standardize the concentration of the other elements. Because larvae spend nearly 1 yr in pelagic waters before settling as juveniles on rocky bottoms (Moser 1974, Wakefield 1990, Wakefield & Smith 1990), the behavior of trace elements was also profiled across otolith sections to track fish movements across water masses and to ground-truth the area containing the first annulus and the region of post-settlement (RPS) in all samples. Otolith trace elements were necessary to identify the RPS because the first annulus and subsequent increments are not always well defined in S. alascanus otoliths, and consistent ageing methods have not yet been developed for all lengths and/or life stages of this species. Therefore, in each otolith, the first annulus was identified using a light microscope and/or the trace element profile from the primordium to the margin. Further, as Table 1 shows, only otoliths extracted from small-size immature fish (6-17 cm) were selected from bottom trawl catches

and analyzed in this study, assuming (1) these fish have moved little between their times of settlement (Moser 1974, Vetter & Lynn 1997) and capture and (2) otolith chemistry measured in the RPS would be strongly related to the chemical and physical characteristics of the bottom-water depth at which fish samples were captured, accordingly to established relationships between water and otolith chemistry (Bath et al. 2000, Hoie et al. 2003, Martin & Wuenschel 2006, Dorval et al. 2007, Sakamoto et al. 2017).

LA-ICP-MS was performed on petrographic slides, each containing 2 columns of otolith thin sections. The USGS certified Microanalytical Carbonate Standard (MACS-3) was used to calibrate the concentration of trace element measurements from otoliths. In addition, the concentration of ⁴³Ca was monitored as an internal standard in all otolith samples, blanks and the MACS-3 standard. Prior to analyzing each otolith column, 2 quality check standards (MACS-3) were analyzed, and they were again measured every 2-3 otolith samples. Collected data were used to correct for instrument drift and matrix effects and to compute the relative standard deviation (RSD) after the analysis of each otolith column. Parameters and element concentration (in ppm) were automatically calculated for each analytical run using the GeoPro Macro version 2.1 (Cetac Technologies). Mean precision (% RSD) was <10% for all the trace elements analyzed. Concentrations of all elements were normalized to Ca and expressed in molar ratios to Ca.

2.4. Stable isotope analysis

Following LA-ICP-MS, all otolith thin sections were photographed, allowing the identification of all ablated spots along the transect line from the otolith primordium to the edge. An image analysis system was then used to delineate 3 regions on all ablated otolith sections: (1) the core region, here defined by the area inside the first annulus, which was generally located within 600 µm of the primordium; (2) the RPS, located immediately after the core between 750 and 1350 μ m; and (3) the outer margin of the otolith, containing the region >1350 µm. The maximum diameter of 1350 µm was chosen for the RPS based on the smallest otolith lengths observed across all 5 geographic areas of this study and also to allow the collection of enough milled powder to conduct the stable isotope analysis.

Ablated otolith sections were milled to collect aragonite powder, using a Micromill sampling prep device

(Electro Scientific Industries [ESI], New Wave Research, Serial no. MM-000-163) at the UMASS-EAF. For the purpose of this study, sampling was performed within the RPS, around the ablated transect line. We assumed that the RPS was the best region that could reflect regional differences in small fish that settled in shallow waters. Data from the core region were not used because they most likely recorded pelagic water conditions experienced by larvae and juveniles during the first year of life. Because the primary spawning locations are not well identified and because of the duration of the pelagic stage (~1 yr), the natal origin of larvae and early juveniles cannot be known, and hence their otolith core is not suitable for testing spatial differences (see Fig. 3). The process of milling each otolith yielded between 120 and 248 µg, and all samples were collected using ESI 0.008 drill bits.

Otolith powder samples were analyzed for $\delta^{18}O$ and $\delta^{13}C$ at the UMASS-EAF, using a Thermo Delta V⁺ Isotope Ratio Mass Spectrometer with a Thermo Gas Bench II. Powder samples were stored in Wheaton vials and then spiked with concentrated phosphoric acid at 90°C. The same order of samples randomized within slides during LA-ICP-MS was used, and 10 otolith powder samples were sequentially analyzed after measuring 4 reference standards (NSB-19 and IARO22); i.e. 2 samples of each standard. Mean SEs, computed from replicated NSB-19 and IARO22 samples during parameter acquisition, were 0.22 and 0.17‰, respectively, for δ^{18} O and δ^{13} C. Accordingly, the raw data were corrected and reported in ‰ relative to the PDB (Pee Dee belemnite) reference standard, using conventional δ notation.

2.5. Statistical analyses

A total of 100 otolith thin sections were ablated, providing ratio estimates of each trace element from the primordium to the margin. For Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca ratios, the magnitude of their variation along the ablated transect line was determined by estimating the median of elemental ratios at each ablated spot. Mean trace element ratio was estimated for each otolith section within the RPS (750–1350 µm from the primordium). These mean ratio estimates were combined with δ^{18} O and δ^{13} C measured within the RPS and with 3 physical variables measured during bottom trawl sampling: temperature-, salinity-and depth-at-capture. This data set was first used to conduct multidimensional scaling analysis (MDS) to

detect major patterns associated with physical factors in the otolith chemistry data collected across the 3 yr. The isotonic MDS, a non-metric MDS developed by Krustal and Shepard in the 1960s (Cox & Cox 2001, Ripley 1996), was used, and the analysis was implemented using the MASS library of Venables & Ripley (2002) in R version 4.1.1 (R Core Team 2020). Prior to running MDS, all variables were standardized to the same scale (mean \pm SD, 0 \pm 1) across the 3 yr (2011– 2013). Dissimilarity matrices were created based on Euclidian distances, and distance between individual fish was projected in 2 dimensions. Goodness-of-fit was measured using the convergence criterion 'stress', with a level of stress between 0 and 1 considered adequate.

Secondly, to evaluate whether otolith chemistry can be used to study S. alascanus movement and to test the J-shape hypothesis, the data set was categorized into 2 main regions: (1) the northern region (N), containing the 3 areas that are located north of Cape Mendocino including Washington, northern and southern Oregon and northern California; and (2) the southern region (S), including central and southern California areas located south of Cape Mendocino (Table 1). Based on the MDS results, each of these regions was further divided in 2 depth strata, containing samples collected in <350 and ≥350 m respectively. As normality and homogeneity of variance-covariance matrices within each stratum could not be met, we used the non-parametric method k-nearest-neighbor (k-NN) discriminant function analysis for classifying individual fish based on trace element and stable isotopes ratios (Hand 1981). The k-NN method does not assume normality or homogeneity of the variance-covariance matrices among strata and is less sensitive to the presence of outliers than traditional parametric methods. Using this method, classification rates may be also computed for an unspecified area (or 'other' group) into which individual fish with a low probability of belonging to any of the pre-determined groups are assigned. Final classification rates were calculated after cross-validation based on the jackknife leave-one-out method. This method consists of building classification models based on training sets containing n - 1 observations; each one of these models was then used to allocate the sample left out (test set) of a given group. We ran 2 types of models; the first one (M1) was based on the 2 regions (N and S) and the second (M2) was based on the 2 regions and 2 depths (+ means deep), namely (N, N+, S, S+). Classification accuracies were computed using proportional priors and pooled data across the 3 yr (2011–2013). All model runs were conducted using SAS software version 9.4.

3. RESULTS

3.1. First annulus and the RPS

The profile of trace element ratios was used to identify the first annulus and determine the RPS from each otolith section. The smallest transect line, between the primordium and the edge, measured 1350 µm and the longest was 5000 µm in length. In most individuals, the first annulus formed at approximately 600 µm from the primordium (Fig. 3). Estimated medians showed the change in the ratios of Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca along the otolith transect lines (Fig. 4). The median of the Mg:Ca ratio steadily declined from the primordium down to 2700 µm and then increased up to the otolith margins (Fig. 4A,B). Both Mn:Ca and Sr:Ca medians varied little across the otolith sections (Fig. 4C,D), although the median of Mn:Ca did slightly decrease from 0.46 to 0.81 µmol mol^{-1} between the primordium and 750 μ m. The median of Ba:Ca decreased from the primordium to its lowest value at 600 µm and then steadily increased up to 1650 µm, with small fluctuations thereafter (Fig. 4E,F). The trends in the median estimates showed that the trajectory of Ba:Ca was the most consistent among the 4 elemental ratios, and hence it could be used to not only identify the location of the first annulus but also to define the RPS in otoliths of immature fish. Accordingly, in this study, the RPS was defined as the otolith region located at 750-1350 µm from the otolith primordium (Fig. 4F).



Fig. 3. Immature *Sebastolobus alascanus* otolith thin section showing first annulus and ablated spots along a transect line from the primordium to the otolith margin. Distance between spots: 150 µm



Fig. 4. Trace element profiles for 4 selected ratios (Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) based on the median of these ratios estimated at each spot (red open circles) from *Sebastolobus alascanus* otolith thin sections collected in 2011–2013. (A,C,D,E) Blue open circles: observed ratios, with a maximum of 100 measurements spot⁻¹; for clarity, (B) and (F) show median trends of Mg:Ca and Ba:Ca on reduced ranges of the *y*-axis. In (F), arrows on the otolith section indicated laser ablation spots and the portion of this trend that was used to define the region of post-settlement (750–1350 μm) in each otolith

3.2. Spatiotemporal variability and structure in otolith chemistry

The distributions of trace element ratios showed considerable patterns related to bathymetry. Across

years, depth-at-capture of individual fish was a major factor controlling the patterns of otolith chemistry on the continental shelf (Fig. 5A). The MDS data generally showed 2 major groups of samples along Dimension 1 at about 0, separating fish that were



Fig. 5. Spatial relationships based on non-metric multidimensional scaling of otolith chemistry measured in the region of postsettlement of immature *Sebastolobus alascanus* otoliths and environmental data collected during bottom trawl surveys in 2011–2013 (stress = 0.21). In (A), number labels indicate the depth-at-capture (m) of fish samples for 2 depth strata: <350 m (blue circles) and \geq 350 m (red circles). In (B), data points from each of the 5 areas of collection are shown in colored circles: red: 1-N; blue: 2-N; green: 3-N; purple: 4-S; orange: 5-S

mostly collected at depths \geq 350 m from those sampled in shallower waters. However, there was no clear separation among samples collected in the 5 areas, although fish collected from north of the Columbia River (1-N) to northern California (3-N) were mostly grouped above the 0-line along Dimension 2 (Fig. 5B).

Within each geographic area, depth-at-capture had distinct effects on elemental ratios measured in the RPS, although to variable extents, depending on the area and trace element considered. The median of the Mg:Ca ratio was lowest in fish collected in shallow water (<350 m) in southern California (5-S). In this geographic area, Mg:Ca showed a gradual but 20-fold increase from shallow to deep water, ranging from 0.04 to 0.8 mmol mol⁻¹ (Fig. 6A). In the other areas, otolith Mg:Ca ratios between shallow and deep waters showed little difference, although from Washington to central California the median of Mg:Ca was consistently higher in otoliths collected at <350 m than in those sampled at \geq 350 m (Fig. 6A).

From northern Oregon (2-N) to central California (4-S), the median of Mn:Ca consistently decreased in deeper waters (Fig. 6B). Otoliths collected off Wash-



Fig. 6. Variation of trace element ratios (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) measured in the region of post-settlement of immature Sebastolobus alascanus otoliths across 5 geographic areas (1-N, 2-N, 3-N, 4-S, 5-S) and 2 depth strata (white: <350 m; gray: ≥350 m). Each box represents the interquartile range of values; bold horizontal line: median; whiskers: 1.5 times the interquartile range from the upper and lower quartiles; open dots: extreme outliers that are within 3 times the interquartile range

ington in shallow and deep waters had similar median Mn:Ca, but fish caught at <350 m had a smaller range in Mn:Ca than those collected at ≥ 350 m. Off Washington, deep water otoliths showed more variability in Mn:Ca than in any other geographic areas or depth strata, while showing continuity with Mn:Ca measured in otoliths at <350 m. The gradual increase, but nearly 140-fold in otolith Mn:Ca, from 0.03 μ mol mol⁻¹ at <350 m to 4.32 μ mol mol⁻¹ at ≥350 m (Fig. 6B) might directly result from the variability of

Mn concentration in the Columbia River plume (see also Fig. 1A).

The Sr:Ca ratio in the RPS was highly variable within and across areas, regardless of depth-at-capture. From Oregon to central California, the median of Sr:Ca was higher in shallower than in deeper waters, but this pattern reversed off Washington and southern California (Fig. 6C). The lack of difference in otolith Sr:Ca among geographic areas potentially reflected the low variation of Sr measured along depth profiles and in surface waters of the northeastern Pacific Ocean (see also Fig. 1B,F).

Within all areas except southern California, the median of Ba:Ca was higher in otoliths collected at \geq 350 m than at <350 m (Fig. 6D). In deeper waters, the median increased from 2.5 µmol mol⁻¹ off Washington to 3.5 µmol mol⁻¹ off central California. The latter area showed, however, the most variability in Ba:Ca measured within the RPS, ranging from 1.33–8.45 µmol mol⁻¹. Otoliths collected in southern Cali

fornia (5-S) had the lowest median observed among areas (Fig. 6D): $1.8 \ \mu mol \ mol^{-1}$ in shallow water and $1.93 \ \mu mol \ mol^{-1}$ in deeper water.

The δ^{13} C composition of *Sebastolobus alascanus* otoliths showed latitudinal difference between otoliths collected in shallow and deep waters (Fig. 7A). The median of δ^{13} C in fish collected at depths ≥ 350 m increased from Washington (1-N) to southern Oregon and northern California (3-N) and then decreased off Central and southern California. The medians of δ^{13} C in fish collected in shallow water (<350 m) also increased, but from Washington up to southern California (5-N). Thus, fish collected at ≥ 350 m off the Columbia River plume had the lowest range of δ^{13} C among areas. In addition, δ^{13} C was positively correlated with δ^{18} O measured in *S. alascanus* otoliths ($\rho = 0.25$, p = 0.01).

As expected, the median of δ^{18} O was higher in fish collected in cooler (\geq 350 m) than those collected in warmer (<350m) water off northern Oregon (2-N) and central and southern California (Fig. 7B). The



Fig. 7. Variation of δ^{13} O and δ^{18} O measured in the region of postsettlement of immature *Sebastolobus alascanus* otoliths across 5 geographic areas (1-N, 2-N, 3-N, 4-S, 5-S) and 2 depth strata (white: <350 m; gray: ≥350 m). Boxplot parameters as in Fig. 6

1

0

-1

-2

-3

-4

δ¹³C (‰)

Table 2. Percent of classification of immature *Sebastolobus alascanus* otolith samples to their region (N and S) or region– depth (N, N+, S, S+; where '+' indicates depth \geq 350 m) of collection based on *k*-nearest-neighbor models after Jackknife cross-validation. Three trace elemental ratios (Mg:Ca, Mn:Ca, Ba:Ca) and 2 stable isotope ratios (δ^{18} O and δ^{13} C) were used in each model. Values in **bold** indicate correct classification of samples to their region or region-depth of origin. The group labeled 'other' contains fish otolith samples that could not be classified to either region or depth strata

Models ——— Cross-validation accuracy (%)—							
	Sample source	Ν	S			Other	
M1	N (n = 54)	94	6			0	
(k = 2)	S (n = 39)	31	69			0	
		Ν	N+	S	S+		
M2	N (n = 32)	72	3	3	3	19	
(k = 3)	N + (n = 22)	14	64	0	0	23	
	S(n = 18)	6	6	44	0	44	
	S+(n=21)	10	0	0	48	43	

median δ^{18} O of fish collected at <350 m slightly decreased from the mouth of Columbia River (1-N) to southern California (5-N). Using the relationship between salinity and [δ^{18} O] in water established by Craig & Gordon (1965) and the general model (otolith δ^{18} O vs. temperature) proposed by Campana (1999), temperature exposures measured in the *S. alascanus* otolith RPS were approximately 6.55 and 7.52°C for fish collected off southern California (5-S) and south of the Columbia River plume (2-N), respectively. These results showed that otolith δ^{18} O can accurately reproduce temperatures measured from the bottom trawl (see Table 1).

As shown in Figs. 6 & 7, both trace elemental and stable isotope ratios measured in the RPS showed spatial differences, but the magnitudes of these differences were strongly influenced by depths-at-capture. Further, the MDS plot (Fig. 5) showed that the data could be separated into 2 chemically distinct geographic regions, namely the N and S regions as defined in Section 2.5. Therefore, to evaluate whether these data could be used to test the J-shape hypothesis, we first classified fish samples based on these 2 regions and Mg:Ca, Mn:Ca, Ba:Ca, δ^{18} O and δ^{13} C, i.e. model M1 (k = 2; Table 2). Secondly, fish samples were classified based on region and depth strata using Model 2 (k = 3; Table 2), accounting for any effects that might be related to depth-at-capture of fish within each of the 2 regions (N and S).

The *k*-NN results showed that model M1 correctly classified 94 and 69% of *S. alascanus* otoliths collected from the N and S regions, respectively. With a

total error rate of 0.16, model M1 generally supported the existence of 2 distinct geochemical regions based on otolith chemistry measured in 2011-2013. In the multivariate space, the high classification rate from M1 in the N region seemed to be mostly controlled by regional differences in the trace element chemistry, for example the Ba:Ca and Mg:Ca ratios. Dropping either ratio from M1 reduced classification rates by 15-24% in the N region, while slightly increased classification rates in the S region by 2-5%. In contrast, the classification rate in the S region was highly sensitive to regional differences in the stable isotopes. Dropping δ^{13} C or δ^{18} O from M1 significantly decreased classification rates in the S region by 13–52%, but decreased classification rates in the N region by only 4-7%.

The *k*-NN model M2 accurately classified 72% of otolith samples collected into stratum N, 64% of N+ otoliths, 44% of S otoliths and 48% of S+s otoliths (Table 2). The total error rate of model M2 was estimated to be 0.40 and thus was much higher than model M1's estimate.

4. DISCUSSION

This study demonstrated that otolith chemistry can be a good tracer of settlement age of Sebastolobus alascanus collected along the northeastern Pacific continental shelf. Trends in Ba:Ca ratios were the most useful for identifying the first annulus and the RPS in otoliths. The trace element ratios (Mg:Ca, Mn:Ca and Ba:Ca) and stable isotope (δ^{18} O and δ^{13} C) composition in immature fish otoliths varied among areas of sample collection, but the spatial variability of these elements and isotopes was strongly influenced by the depth of capture along and across the continental shelf. The chemistry of S. alascanus otoliths revealed 2 distinct settlement regions of immature fish: one off the Columbia River plume expanding south to northern California and another off central and southern California. Classification accuracy of fish samples to their region of collection was highest when the classification model was based on these 2 regions, but moderate and fair when each of these 2 regions was subdivided in depth strata. These results show that otolith chemistry can be used as a natural tag to test critical hypotheses regarding the migration of fish that settle on the continental shelf from the Columbia River mouth through the southern California Bight, but refining classification models to account for depths-at-capture may require a significant increase in sample size.

4.1. Post-settlement chemical fingerprint

Trace element chemistry measured from the primordium to the margin of otoliths reflected juvenile S. alascanus movement from surface to bottom waters and possibly ontogenetic movement after settlement. Previous studies have shown that the vertical distribution of Ba in the Pacific Ocean is correlated with nutrient concentrations (Brass & Turekian 1974, Chan et al. 1976). Ba is typically removed from solution in the upper water column through uptake by sinking particles, but it is regenerated in deeper waters as these particles slowly dissolve (Lea & Boyle 1989, Lynch-Stieglitz & Marchitto 2014, Grissom 2015). Several studies have also shown that the Ba: Ca ratio in oceanic and estuarine waters has a positive linear relationship with otolith Ba:Ca (Bath et al. 2000, Dorval et al. 2007, Miller 2009), and this relationship is not influenced by water temperature (Bath et al. 2000). Hence, the increase of otolith Ba:Ca ratios in immature fish after settlement is consistent with both water chemistry and patterns in the ontogenetic migration of S. alascanus predicted by previous studies (Jacobson & Vetter 1996); whereas lower levels of Ba:Ca prior to settlement are indicative of the pelagic life phase of larvae and early juveniles (Moser 1974, Wakefield 1990). Further, the timing of settlement of immature S. alascanus, as validated by otolith Ba:Ca ratios, showed similar patterns to those of juvenile snapper Chrysophrys auratus in the Hauraki Gulf, New Zealand Sebatian et al. (2021). Coincidentally, Sebatian et al. (2021) found that Ba decreased to its lowest level at about 620 µm from the otolith primordium when this species settled in bottom waters.

Although the profile of the Mg:Ca ratio steadily decreased across S. alascanus otoliths from the primordium to about 2700 µm, the relationship between otolith Mg:Ca ratios and environmental parameters has shown mixed results. Martin & Wuenschel (2006) found no significant effects of water temperature and salinity on the uptake of Mg in otoliths of juvenile gray snapper Lutjanus griseus. Similarly, Mg measured in otoliths of silver perch Bidyanus bidyanus did not significantly respond to changes in the concentration of Mg in water or diet (Woodcock et al. 2012), likely due to high regulation in the blood plasma (Dorval et al. 2007). In contrast, Javor & Dorval (2017) reported that the deposition of Mg in juvenile Sardinops sagax otoliths was affected by temperature, while (Tanner et al. 2013) found that the Mg:Ca ratio in otoliths of juvenile flounder Solea solea was correlated with salinity. These mixed patterns in the

behavior of Mg:Ca ratios have been also observed in other marine genera. The Mg:Ca ratio has been used as a paleo-thermometer to retrace the temperature of calcification of benthic foraminifera, but many studies have demonstrated that uncertainties associated with converting Mg:Ca to temperature might reduce the ability of this ratio to detect deep water temperature gradients that are typically used to discriminate water masses (Elderfield et al. 2006). To our knowledge, there are limited data on the distribution of Mg in surface and deep waters along the northeastern Pacific coast, and thus factors that control the concentration of this element are not well understood in this region. Nevertheless, in immature S. alascanus, this study detected a clear decrease of Mg:Ca ratios prior to an increase at the otolith margins. This result showed great potential for using the Mg:Ca ratio in reconstructing movement and habitat-use of individual fish from shallow to deeper waters.

The median of Mn:Ca and Sr:Ca varied little from the primordium to the margin of otoliths, likely due to the physical and chemical characteristics of bottom waters. Both water temperature and salinity can influence otolith Sr:Ca ratios (Elsdon & Gillanders 2002). Similarly, otolith Mn:Ca has been found to be positively correlated with water Mn:Ca ratios (Forrester 2005, Dorval et al. 2007, Limburg et al. 2011) but negatively correlated with water temperature (Fowler et al. 1995, Miller 2009). While Mn is highly variable in both surface and deep waters along the northeastern Pacific coast (Bernat et al. 1972, Brass & Turekian 1974, Aquilar-Islas & Bruland 2006, Sunda 2012), Mn concentration may also increase in bottom waters and surface sediments when anoxic conditions are predominant (Eaton 1979, Sholkovitz et al. 1992). Elevated Mn:Ca ratios in otoliths due to such anoxic conditions were documented for spotted seatrout Cynoscion nebulosus collected in the Chesapeake Bay (Dorval et al. 2007) and cod Gadus morhua sampled in the Baltic Sea (Limburg et al. 2011). Thus, environmental factors such as temperature, salinity and dissolved oxygen may have interacted to preclude any significant changes in Mn:Ca and Sr:Ca ratios across the otolith sections. Consequently, both Mn:Ca and Sr:Ca ratios of S. alascanus may not be good proxies for identifying ontogenetic movement of this species.

In sum, the profile of the Ba:Ca ratios measured across otoliths unequivocally confirmed that *S. alascanus* settle nearly 1 yr after larval and juvenile pelagic stages. Additionally, Ba:Ca may be used to delineate the location of the first annulus and the RPS, particularly in otoliths of older and larger fish where annual growth increments cannot be always identified.

4.2. Continental shelf gradient

Trace element and stable isotopic composition, except Sr:Ca ratios, measured within the RPS of S. alascanus were variable among areas, but their spatial distribution along and across the continental shelf was influenced by both the amount of freshwater inputs and the depth-at-capture of fish within a given area. For example, the otolith Mn:Ca ratio was more variable off Washington (1-N) than in any other areas, potentially a direct result of Mn concentration and variability in the Columbia River plume (Aguilar-Islas & Bruland 2006). Bathymetry was particularly important to elucidate along-shelf gradients in otolith chemistry and to distinguish the northern and southern regions of settlement. As in other studies, Mg:Ca and Ba:Ca ratios seemed to reflect complex dynamics in coastal systems, where otolith chemistry may not be directly related to physical parameters. For example, off Central California, Woodson et al. (2013) found that the Ba:Ca ratio measured in otoliths of a pelagic juvenile fish, Sebastes jordani, did not match spatially with upwelling chemical signatures in waters when fish movement or transport was not taken into account. Similarly, along the Patagonian shelf, Ashford et al. (2007) reported that the ability of otolith chemistry to discriminate water masses occupied by the Patagonian toothfish Dissostichus eleginoides improved when fish age- or depth-at-capture was accounted for. The northern region identified by S. alascanus otolith chemistry encompasses the Columbia River, which produces most of the freshwater inputs to the continental shelf up to San Francisco Bay (Naik & Jay 2005). In contrast, the southern region comprises the southern California Bight, a confluence zone of cold California Current waters with warmer waters from the south and very little freshwater inputs (Lynn & Simpson 1990, Bray et al. 1999). However, relatively few studies have been conducted on correlating otolith and water chemistry in these regions. Likewise, a full understanding of the mechanisms that control S. alascanus otolith chemistry may require not only the collection of water chemistry but also the use of geospatial techniques to track both water mass transport and fish movement, as Woodson et al. (2013) suggested for pelagic juvenile rockfish.

Although variable, in most areas the behavior of δ^{18} O was consistent both across and along the continental shelf. For example, otolith δ^{18} O increased with depth off northern Oregon and southern California, whereas fish collected in these 2 areas at a similar depth stratum had lighter δ^{18} O signatures in south-

ern California than off northern Oregon. Javor & Dorval (2014) reported similar spatial patterns in Pacific sardine *S. sagax*, which they attributed to a lesser amount of freshwater inputs in southern California compared to Washington and Oregon. This study also showed that otolith δ^{18} O can accurately reproduce temperatures measured from the bottom trawl, confirming the adequacy of the assumption that immature fish did not significantly move during the period corresponding to the RPS. Recognizing the limitations of this study, future research needs to focus on designing studies that can directly test this assumption.

As in other fish species, otolith δ^{13} C was positively correlated with $\delta^{18}O$ and varied with the depth of settlement of immature S. alascanus. Many authors have shown that otolith $\delta^{13}C$ is not related to water chemistry but rather is strongly dependent on fish metabolic rate and feeding habits (Kalish 1991a,b, Hoie et al. 2003). Although S. alascanus feed mostly on fish and crustaceans (Orlov 2001), large-scale spatial differences in their diets have not been documented. However, Anderson et al. (2013) showed that beta diversity, the variation or turnover in identities of demersal fish species, decreased with depth on the northeastern Pacific coast, exhibiting peaks around 400–600 m or at greater depths. As a major fish predator, considerable variations in the composition of S. alascanus diets over the depth range of this study could be expected as well. Therefore, $\delta^{13}C$ measured in S. alascanus otoliths may provide a good record of the relative change in the species composition of its prey from shallow to deep waters across the shelf.

4.3. Testing migration hypotheses

S. alascanus settled at different depths on the continental shelf, and as result, its otolith chemistry was strongly associated with bathymetric gradients. However, water mass distribution on the continental shelf, controlled by oceanic processes over this gradient, ultimately determined the deposition of trace elements and stable isotopes in *S. alascanus*, showing 2 geochemically distinct regions coast-wide.

The total classification error rate was much lower (0.16) in the 2-region model (M1) than in the 2-region, 2-depth model (0.40, M2). With classification accuracy varying from 69–94%, model M1 could be used to allocate adult fish to the northern and southern region settlement regions and test the J-shape hypothesis. Further, in model M1, classification accu-

racy in the N region was highly dependent on the trace element chemistry; whereas in the S region the rate of classification was dependent on stable isotopic composition. As previously reported by Thorrold et al. (2001) and Dorval et al. (2005), these results show the importance of using both trace elements and stable isotopes in discriminating fish species that display complex habitat use.

While model M2 attempted to control depth-atcapture effects, it resulted in a small sample size (18-32) in each of the strata, which potentially lowered the power of the k-NN in correctly classifying otolith samples to their stratum of collection. One of the major properties of the k-NN method is that under very fair conditions, the classifier error rate will be Bayes-optimal as the sample size tends to infinity (Ferri et al. 1999, Zhang et al. 2006). Therefore, classification error rate is highly dependent on sample size, with lower sample size yielding higher error rates. The accuracy of the k-NN classifier has also been shown to decrease as the ratio of sample size and the intrinsic dimensionality decreases (Ferri et al. 1999). Model M2 was based on a 4×5 design (4 strata × 3 trace elemental ratios and 2 stable isotopes), and thus may require an increase in sample size before achieving a similar performance as model M1. Note also that both models used pooled data across the 3 yr of collection to avoid further reduction in sample size, thus they could not account for potential year effects. Not accounting for these interannual variations may also decrease the power of the discriminant analysis because the bottom trawl does not sample the same depths each year, and sampling effort is not equally allocated across geographic areas (Tolimieri et al. 2020).

Despite annual variability in the physical oceanography on the shelf and the availability of immature fish to the bottom trawls, these results demonstrate that trace element and stable isotope composition recorded in S. alascanus may contain enough information to study fish movement north and south of Cape Mendocino and test the J-shape migration hypothesis (Piner & Methot 2001). Future studies could use 2 basic approaches to allocate adult fish to their natal region in order to test this hypothesis. First, more samples could be collected within year, region and depth strata to fully increase the power of the k-NN models or any alternative discriminant analysis methods. Second, k-NN classifier error rates may also decrease with the number of features up to an optimal level (Raudys & Jain 1991). This study included only 3 elemental ratios (Mg:Ca, Mn:Ca, and Ba:Ca) in the classification models, thus measuring

additional trace elements in the RPS may help better optimize model M1. Third, the allocation of fish to their location of collection could also be improved by measuring otolith chemistry in immature S. alascanus collected by the bottom trawls over more than 3 yr. This would greatly increase model sample sizes and the likelihood of representing most settlement depths in the otolith chemistry data. This is even more important because there is no standardized method to age S. alascanus across all life stages, and thus adult fish cannot be accurately assigned to their respective year-classes. Whether sample size could be increased in the future or more trace elements could be assayed in otoliths, this study shows that otolith chemistry can be a viable method to study population structure and movement of long-lived fish on the US continental shelf, and to test critical hypotheses for the improvement of their assessment and management.

5. CONCLUSIONS

This study demonstrates that otolith chemistry can be used to track the migration of Sebastolobus alascanus from surface to bottom waters on the eastern Pacific continental shelf, allowing the identification of the first annulus in individual fish otoliths. Elemental fingerprints of these otoliths can also be useful in tracking the ontogenetic movement of immature and adult fish from shallower into deeper waters. Further, combined with depth-at-capture, a proxy for salinity and temperature, S. alascanus otolith chemistry helped identify 2 geochemically distinct regions of settlement for shortspine thornyhead: a northern region spanning from the Columbia River plume through Cape Mendocino; and a southern region, encompassing central and southern California. The ability to use otolith chemistry to study the movement of S. alascanus may improve by having detailed information on depth-at-settlement of immature fish and by increasing sample size across years and/or the number of trace elements assayed in otoliths of fish within the 2 regions.

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