Original Article

Determining natal sources of capelin in a boreal marine park using otolith microchemistry

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The effectiveness of marine conservation areas are influenced by the structure of the food webs they encompass, including the population dynamics of potentially important forage species that occupy trophic positions between those of zooplankton and higher trophic levels. Capelin is a key forage species for marine mammals, seabirds, and fish in the Saguenay-St. Lawrence Marine Park (SSLMP, Canada), yet knowledge on its population dynamics is incomplete. In particular, the natal sources sustaining this critical forage species within the conservation area remain unknown. Otolith microchemistry as an index of natal habitat was investigated, including measures of boron, barium, iron, magnesium, manganese, and strontium over the protracted spawning season of capelin. Otolith microchemistry indicated that the principal natal source of 1+ capelin found within the SSLMP was located in the St. Lawrence estuary, outside the conservation area boundaries. To ensure the sustainability key forage species within the conservation area, larger scale long-term management strategies are necessary to encompass ecological processes related to capelin that extend beyond the conservation area boundaries.

Keywords: conservation areas, *Mallotus villosus*, microchemistry, natal origin, otolith, Saguenay Fjord, source sink dynamics, St. Lawrence, temporal variation.

Introduction

Marine conservation areas such as marine parks aim to protect biodiversity and to maintain the functions and services of marine ecosystems. Conservation networks—including *inter alia* marine protected areas, parks, and reserves—have been expanding worldwide since the 1970s. Specific objectives are established for each area that is designed (e.g. as a large single area or as a network of small areas) to address ecological and economic goals as well as socio-cultural, educational, and policy issues (Lubchenco *et al.*, 2003; Palumbi *et al.*, 2009; Costello, 2014; Pater and Oxley, 2014). One important challenge related to fulfilling the conservation area's ecological objectives is the ability to assess ecosystem dynamics that occur both inside and outside of the area, including the composition of species assemblages, food web structure, the importance of dispersal and migration processes, and the extent of connectivity between habitats (Anadón *et al.*, 2013; Lovvorn *et al.*, 2014; Magris *et al.*, 2014).

The Saguenay-St. Lawrence Marine Park (SSLMP) is one example of a large single marine conservation area. Located in eastern Canada (Figure 1), this boreal park protects biodiversity and several charismatic species (e.g. Beluga *Delphinapterus leucas* St. Lawrence population). The park is an important feeding area for numerous marine animals including whales, seals, seabirds, and fish. Thus, an important ecological objective for park managers is

the understanding and adequate management of the key species of the SSLMP food web.

Within the North Atlantic Ocean, capelin (*Mallotus villosus*) is an important predator of lower trophic level zooplankton and important prey for higher trophic level species (Trenkel *et al.*, 2014). This circumpolar species complex is genetically divided into four reproductively isolated clades (Dodson *et al.*, 2007; Colbeck *et al.*, 2011). Capelin in the SSLMP is part of the Northwest Atlantic (NWA) clade. Spawning occurs from May to July in the NWA on fine gravel mainly on beaches and, to a lesser extent, in adjacent deeper waters (Davoren *et al.*, 2007). The St. Lawrence system is an important spawning area for capelin within the NWA. High densities of capelin larvae have been observed in spring and early summer in the St. Lawrence system where it is the dominant ichthyoplankton species (Jacquaz *et al.*, 1977; Able, 1978; Sirois *et al.*, 2009; Bui *et al.*, 2012). Furthermore, juveniles and adults are frequently observed near the mouth of the Saguenay Fjord in spring (Bailey *et al.*, 1977; Simard, 2009), suggesting that significant recruitment occurs in the SSLMP.

The natal sources of 1+ capelin in the SSLMP are uncertain, complicating management of this critical forage species within the Marine Park. More specifically, managers need a clear understanding of the natal sources sustaining capelin within the marine conservation area and the importance of the SSLMP in sustaining regional capelin stocks beyond the boundaries of the SSLMP. In this context, we aimed to identify the natal sources of capelin located in the SSLMP and adjacent regions.

The minor and trace element concentrations found in otoliths represent a promising approach to provide insights into early life history and to elucidate natal origin. Otoliths grow daily by the biomineralization of a carbonate layer within a protein matrix. Some minor (e.g. magnesium) and trace (e.g. barium and strontium) elements are incorporated in a quasi-permanent way into the carbonate structure during otolith growth (Campana, 1999). The concentration of these elements within the daily growth rings of otoliths depends on both environmental and physiological factors (Walther *et al.*, 2010; Miller, 2011; Reis-Santos *et al.*, 2013; Sturrock *et al.*, 2014). As elemental incorporation depends on environmental factors such as salinity, temperature, and the elemental concentrations in the surrounding water mass, otolith chemical composition can be used to recreate ecological processes such as population connectivity, migrations, and natal origin (Standish *et al.*, 2011; Cook *et al.*, 2014; Engstedt *et al.*, 2014).

Here, we use otolith microchemistry to explore capelin natal sources in the SSLMP and adjacent regions. We hypothesize that the capelin sources are located both inside and outside the SSLMP. The contribution from each natal source region was assessed to evaluate the importance of the SSLMP contribution to its own capelin stock.

Material and methods
Study site and data collection
The study site was located in eastern Canada (Quebec) and encompassed the St. Lawrence Estuary, the north-western part of the Gulf of St. Lawrence, and most of the Saguenay Fjord, including the SSLMP (Figure 1). Spawning had occurred progressively from May to July in the upper estuary, the fjord, the lower estuary, and the north-western gulf region in 2009 (Ouellet *et al.*,

2013), a pattern previously observed in several studies (Jacquaz *et al.*, 1977; Fortier and Leggett, 1982; Sirois *et al.*, 2009).

Capelin larvae were sampled at 31 stations during three missions that took place during the period of capelin spawning in 2009 (at the ends of May, June, and July, respectively, referred to as missions 1, 2, and 3). The sampling protocol followed that described by Ouellet *et al.* (2013). Briefly, a 0.5-m bongo sampler equipped with two 333-μm mesh nets was towed obliquely for 10 min (3–4 knots) in the surface layer of the estuary (0–50 m) and the fjord (0–15 m). When larvae were present, five larvae per station and sampling time were selected ($n = 280$) for otolith analysis. All selected larvae were 1–3-d old to reduce bias induced by the variation of the elemental concentrations at the hatching site as well as by the transport of larvae from other sites. The mean total length was 6 ± 1 mm. Juveniles (age 1+ years; $n = 35$) were caught 1 year later, in June 2010. They were sampled through tows lasting for 10 min using a midwater trawl (mesh 6–76 mm) at four stations close to the mouth of the fjord (Figure 1). The 1+ category was verified by the presence of a single winter zone on the otolith. The mean total length of selected juveniles was 72 ± 5 mm and they were considered as recruits for the local capelin stock within SSLMP. Samples were preserved in 95% ethanol (Greenfield Inc., Brampton, ON, Canada) as this mode of storage does not affect otolith microchemistry (i.e. for Ba and Sr; Hedges *et al.*, 2004).

Sample preparation and analytical analyses
The left sagittal otolith of each larva was removed and fixed randomly on a petrographic slide (see methodology in Lazartigues *et al.*, 2014). Juvenile otoliths were removed and sanded to the midplane with different grades of aluminium oxide lapping films (3M, St. Paul, MN). Aluminium was monitored in each sample, in both the otolith and glue, to attest to the absence of contamination due to the lapping process. The otoliths were then randomly fixed to a slide using Crystalbond glue (AREMCO Inc., Valley Cottage, NY). The slide containing juvenile otoliths was sonicated in ultrapure water for 5 min, triple rinsed, and then dried in a class-100 laminar flow hood for 24 h.

LA-ICP-MS analysis was performed using an ArF 193 nm RESOlution M-50 laser ablation system (Resonetics, Nashua, NH) coupled to an Agilent 7700X ICP-MS (Agilent, Mississauga, ON, Canada). Juvenile otoliths were ablated using the line scan mode (5 mJ, 15 μm, 20 Hz, 2.5 μm·s^{-1}), with the line crossing the core. Elemental signatures from larval otoliths were obtained in raster scan mode (3 mJ, 5 μm, 10 Hz, 2.5 μm·s^{-1}). The target elements were ^{11}B, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{29}Si, ^{39}K, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{66}Zn, ^{86}Sr, ^{87}Sr, ^{88}Sr, ^{136}Ba, ^{137}Ba, and ^{138}Ba. Seven values were recorded per element every second. The calcium content was assumed to be 40.0% for analysis normalization. Some of the target elements (Co, Cu, and Zn) were not useful to efficiently discriminate groups, due in part to values below limits of detection (LOD). Some others were used solely to verify the absence of contamination (Al) and for calibration (Ca). These elements were excluded from the statistical analyses. Boron (B) was above the LOD for all larvae caught in the C3, C4, and C5 groups (i.e. in the estuary and at the mouth of the fjord) and below the LOD (or at levels approaching LOD) for most larvae caught in C1 and C2 (i.e. in the fjord). This suggests non-random causes and merits inclusion in the analyses. The other elements were geographically structured and above LOD and were thus
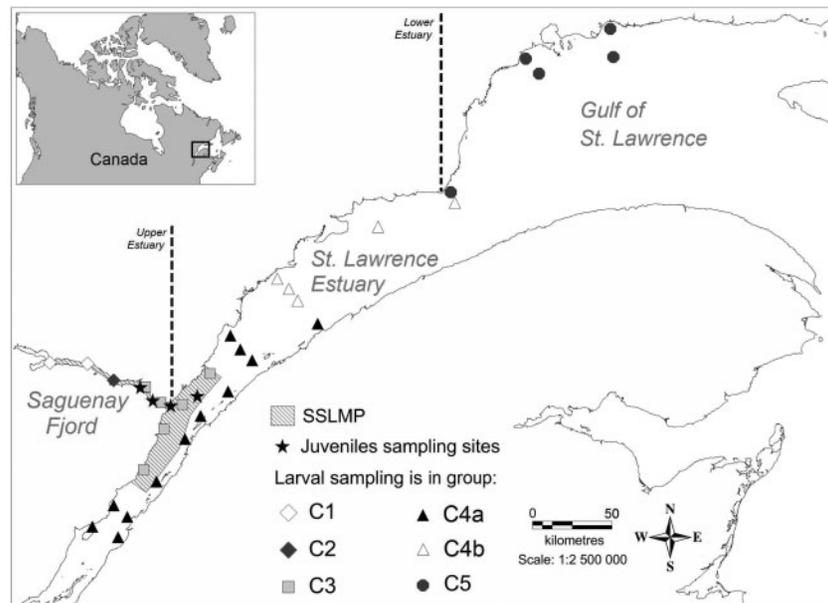
Figure 1. Sampling sites for larvae and juveniles within the Saguenay Fjord, St. Lawrence Estuary, and Gulf of St. Lawrence, including the SSLMP and the separation of larval sites into the groups C1–C5. C1, SSLMP—fjord upstream, white diamonds; C2, SSLMP—fjord middle portion, grey diamond; C3, SSLMP—fjord mouth and estuary, grey squares; C4, remaining estuary, triangles, including C4a (black triangles) and C4b (white triangles); C5, north-western gulf, grey circles. Juveniles were caught in SSLMP (black stars).

included. However, some of them (Na and K) were then excluded from the final elemental signature because these elements were redundant with the variance ascribed to other elements. Reference materials were NIST SRM 610 (calibration) and USGS MACS-3 and GP-4 (quality control). The ICP-MS conditions (gas fluxes, power, etc.) are described by Lazartigues *et al.* (2014). Analytical performances were briefly presented in Supplementary Data S1.

Data reduction and analysis

Data reduction of the ICP-MS signal was performed using Iolite (Paton *et al.*, 2011) in Igor Pro (Wavemetrics Incorporated, Portland, OR). The integration (Trace Elements IS procedure in Iolite) was performed on the largest ablation line for larval otoliths (ablating about 50–100 μm^2 area). For juveniles, the signal was equally integrated on either side of the core, during 2 s to match a 100 μm^2 surface ablated. The methodology to localize the core of juvenile otolith is explained in Supplementary Data S2. Data from both larvae and juveniles were $\log_{10}$ transformed to meet the assumptions of statistical analyses.

Statistical analyses

General statistical model based on geography

The ecosystem was divided into five geographic groups (Figure 1). Groups C1–C3 represented larvae hatched in the SSLMP. The larger portion of the St. Lawrence Estuary formed group C4 (triangles in Figure 1). Finally, the group C5 corresponded to the larvae from the north-western Gulf of St-Lawrence. SAS 9.2 and JMP 10.0 (SAS® Institute, Cary, NC) packages were used to process analysis of variance (ANOVA), Tukey–Kramer's honestly significant difference (HSD) tests, multivariate ANOVA (MANOVA, Pillai's trace), and linear discriminant function analyses on larval data. The objective was to evaluate the concordance between hatching sites (geographic

groups) and elemental signatures in larval otoliths. As eggs are adhesive, the spawning areas are assumed to be similar to hatching areas for 1–3-d-old larvae.

Natal origins were attributed by assigning the elemental signature measured in the core of the juvenile otolith (representing the hatching period) to a larval group (C1–C5) using the HISEA method (Millar, 1987, 1990). Briefly, the otolith chemical composition of young larvae sampled 1–3 d after hatching, form the baseline (resampling procedure) of elemental signatures for the five regions of origin (groups C1–C5). Each elemental signature from the mixed sample of juveniles was assigned to one of the five source groups when the classification-likelihood estimator (Θ4) of their signature value was greatest. It is important to note that the bias in the model increases when some stocks have little or no contribution (i.e. when the contribution was less than 20%). This bias sometimes leads to standard errors that are higher than the contribution estimate. When the HISEA bootstrap procedure was applied to assign natal origin, the means (±SD) of the composition estimator were obtained over 1000 simulations. An alternative classification subdivided region C4 into two parts (C4a and C4b; Figures 1 and 2 and Supplementary Data S3). One group was close to the SSLMP in the St. Lawrence Estuary (C4a) and the other close to the north-western Gulf of St. Lawrence, in the northern portion of the lower estuary (C4b, Figure 1).

Quantifying the impact of spatio-temporal variations of otolith elemental signatures

In the general model (based on geography, see the section above), most reclassification errors occurred between C4 (estuary excluding the SSLMP) and C3 (estuary portion of the SSLMP). A potential cause of a lower classification success is that otolith signatures can vary over a protracted spawning season (Cook, 2011). The

influence of the depth of spawning (deep water vs. beach spawning) was not assessed but may also play a role in misclassification of capelin larvae (Davoren *et al.*, 2015). Here, we attempted to quantify the impact of the horizontal spatio-temporal variations in otolith signatures on the performance of the discriminant analysis based on geographical groups. We compared the model based on geography to a new model based on otolith chemistry. In this new model, a "virtual moving-boundary" group (C_{VMB}) was created to replace C3 in each mission (the new C4s correspond to the remaining portion of the estuary). Each station that presented the same signature as the initial C3 (for a given mission) was included in C_{VMB}. Thus, the geographical boundaries of C_{VMB} varied among missions according to the otolith composition of the larvae. In May (mission 1, effect [M1]), C_{VMB} included the initial C3 and one southern station from the upper estuary. In June (mission 2, effect [M2]), C_{VMB} boundaries included C3 and four stations downstream in the lower estuary. In July (mission 3, effect [M3]), C_{VMB} presented the same boundaries as C3 (effect [M3] = 0). Reclassification success was calculated using the model based on otolith chemistry for each mission and compared with the reclassification success obtained using the model based on geography. This process allowed us to quantify the portion of misclassification that could be attributed to the global spatiotemporal effect on the model, including the effects [M1] and [M2] as well as their interaction ([M1] x [M2]).

Results
Elemental concentrations
All of the target elements had significant differences between sites when assessed using univariate analysis (ANOVA and HSD Tukey–Kramer, $p < 0.05$, Figure 2). The highest number of between-site differences was observed for Mn, Ba, and Mg. Mn concentrations showed a decrease from C1 to C2 and then an increase from C2 to C5 (min. C2, 120 ± 22 ng·g^{-1}; max. C5, 3984 ± 341 ng·g^{-1}). The highest concentrations of Ba (72 ± 6 ng·g^{-1}) and Mg (914 ± 91 ng·g^{-1}) were recorded for larvae in the surface water of the upstream portion of the fjord (C1) that is notably influenced by freshwater input. Both elements presented the same pattern: the concentrations decreased from C1 to C3 and then increased from C3 to C5 (min. C3, 11 ± 1 and 236 ± 10 ng·g^{-1} for Ba and Mg, respectively). Boron was not detected in C1 and its concentration remained low in the fjord (< 10 ng·g^{-1} in C2 and C3) although it was high in both the estuary (C4, 24 ± 2 ng·g^{-1}) and the gulf (C5, 17 ± 4 ng·g^{-1}). Concentrations of Sr, which ranged from 4670 to 5207 ng·g^{-1}, did not present a clear trend nor did Fe concentrations, which ranged from 309 ± 21 ng·g^{-1} to 1159 ± 397 ng·g^{-1}. The other elements, Na and K—known to be highly impacted by physiology—also showed differences between sites. Concentrations of K were higher in the estuary and in the gulf than in the fjord, with a pattern rather similar to Mn (i.e. minimum concentrations in C2). The highest concentration of Na was observed in C5 ($12\,495 \pm 1703$ ng·g^{-1}) and the Na pattern among sites was quite similar to Ba and Mg patterns (i.e. minimum was C3).

Discrimination of geographic sources and relations to otolith signature
In the general model based on geographic groups (including the three missions and five geographic groups), Ba, Mg (axis 1, 74.0%), and Mn (axis 2, 20.5%) were the most important

elements leading to hatching site discrimination (Figure 3a and b). The multivariate analyses were in agreement with the univariate results. The group C1 mostly differed from the other groups by high content of both Ba and Mg in the larval otoliths. C2 was mainly discriminated by low levels of Mn. The groups C3, C4, and C5 represented a gradient of increasing amounts of Ba, Mg, and Mn. The bootstrap reclassification success ranged from 71 to 100% for larvae (Table 1). Nearly 17% of larvae were misclassified, mainly because of the similarity between groups C3 and C4.

There were significant differences in otolith elemental signatures between geographical groups (MANOVA, Pillai's trace value: 0.95, $F_{18, 792} = 20.38$, $p < 0.0001$), missions (MANOVA, Pillai's trace value: 0.54, $F_{12, 526} = 16.36$, $p < 0.0001$) and group × mission interactions (MANOVA, Pillai's trace value: 0.88, $F_{42, 1602} = 6.55$, $p < 0.0001$).

Discriminant analyses were also conducted separately for each mission to highlight the robustness of the group structure against temporal effects. Success of reclassification to the groups C1, C2, C3, C4, and C5 ranged from 79 to 94% for classifications based on bootstrapping the same single mission (Supplementary Data S4). Classification success ranged from 74 to 85% if bootstrapping excluded the classified mission.

Within the general model based on geographic groups, spatiotemporal effects ([M1] + [M2] + [M1 × M2], Figure 4) contributed 46% of the total misclassification of larvae by our geography-based model (i.e. it accounted for 8% of the 17% of misclassified larvae). The main effect was also observed in June ([M2]) accounting for 33% of the total bias.

Overall, the temporal variations in otolith elemental signatures along the protracted spawning season were less important than the differences observed among groups (Figure 3c). As a consequence, despite the chemical similarity between C3 and C4, we assumed that each geographic group could be distinguished from each other due to their high classification success (i.e. 83%) and the low temporal effects obtained with our general geography-based model. As such, each geographic group presented a "natal source signature" in which juvenile capelins were assigned.

Natal sources
We used the assignment of natal origin of juveniles captured in the SSLMP to estimate the contribution of each geographical hatching source. We found that the C4 group was the main source, contributing $74 \pm 12\%$ of juveniles caught in this study (Table 2). The upstream region closest to the SSLMP (C4a) was the main production source of the whole contribution from C4 (Supplementary data S3). The other groups were relatively low contributors and showed the largest relative standard errors (error $\geq 50\%$ estimate). The north-western gulf (C5) contributed about 16% of juveniles and the SSLMP (C1 + C2 + C3) contributed about 10%. There were no fish assigned to the natal origin C1; suggesting that 1+ juvenile caught at the mouth did not originate from the upstream portion of the fjord.

Discussion
Otolith signatures
In this study, our model was primarily driven by Ba and Mg, secondarily by Mn, and to a lesser extent by B, Sr, and Fe. Some previous studies, also based on otolith composition differences in Ba, Mg, and Mn were able to discriminate natal source sites (and
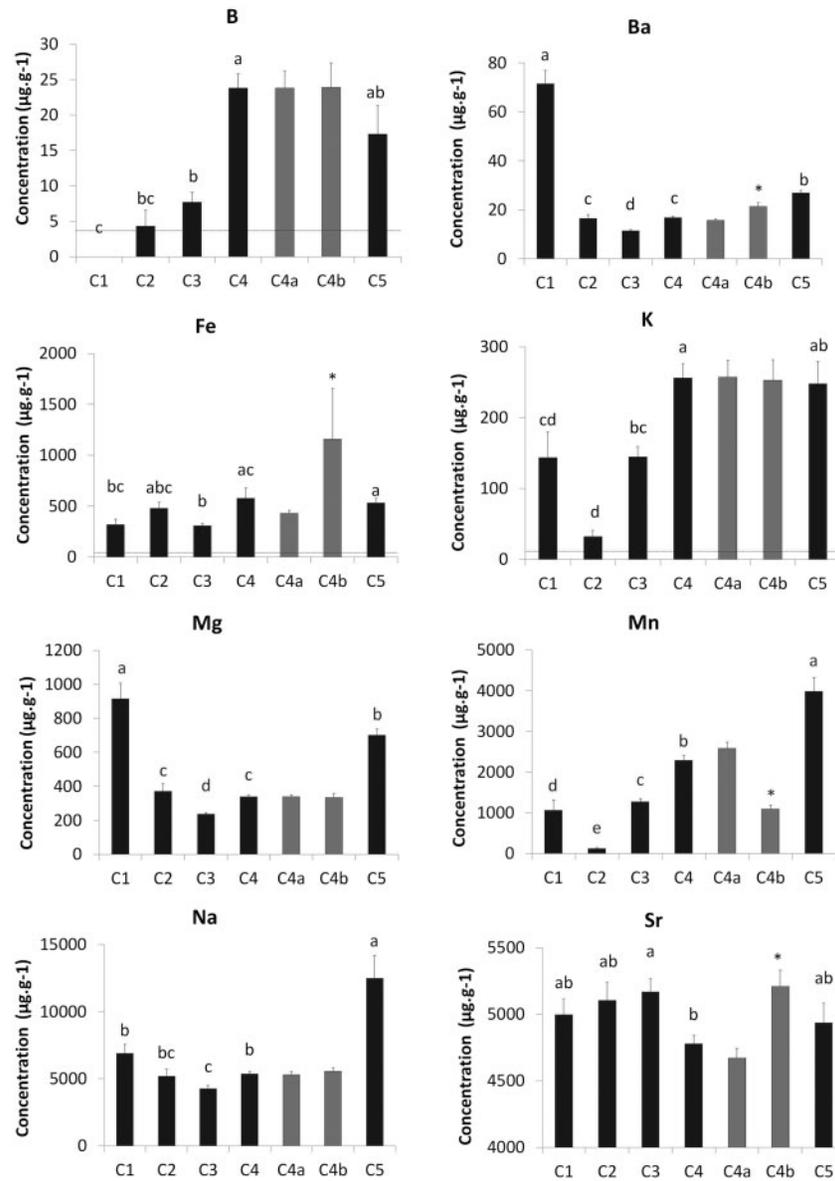
Figure 2. Concentrations of trace and minor elements in larval otoliths (µg·g⁻¹). For each element, relative concentrations averages (mean ± SE) having a common letter are not significantly different (ANOVA, Tukey–Kramer HSD performed on transformed variables for C1, C2, C3, C4, and C5; no difference if $p > 0.05$. *C4b was different than C4a; t test, $p < 0.05$). The dotted lines indicate the mean detection limit of the method. Values below the detection limit were approximated to zero. C1, SSLMP—fjord upstream; C2, SSLMP—fjord middle; C3, SSLMP—fjord mouth and estuary; C4, remaining estuary (divided in C4a, remaining estuary close to the SSLMP and C4b, remaining estuary close to the gulf); C5, north-western gulf. B, boron (^{11}B); Ba, barium (^{138}Ba); Fe, iron (^{56}Fe); K, potassium (^{39}K); Mg, magnesium (^{24}Mg); Mn, manganese (^{55}Mn); Na, sodium (^{23}Na); Sr, strontium (^{88}Sr).

interannual variation) in brackish water conditions for other species (Hamer *et al.*, 2003; Tanner *et al.*, 2012). These studies also showed that intrayear variations, although significant, were lower than intersite and interannual variability (Hamer *et al.*, 2003; Tanner *et al.*, 2012). Otolith elemental signatures generally vary through time, mostly among years and sometimes within years, months, or seasons (Gillanders, 2002; Hamer *et al.*, 2003; Cook, 2011; Reis-Santos *et al.*, 2012, Tanner *et al.*, 2012). In this study, interannual variability was not assessed although it would be important to undertake in order to obtain representative baseline data for assigning the natal origin for other year-class capelin.

Short-term temporal variations within a protracted spawning season could potentially confound temporal variation with spatial variation (Gillanders, 2002; Cook, 2011).

In this study, there were variations in otolith chemistry during the protracted spawning period (three months). Low discrimination occurred between C3 and C4 (especially C4a sector) during mission 2. During this mission (June 2009), the sampled area included the upwelling area of the estuary close to the head of the Laurentian Channel and was marked by a current at the surface (0–40 m) adequate to water mixing and larval drift in the C3 and C4a sectors (see current and transport context in Ouellet *et al.*,
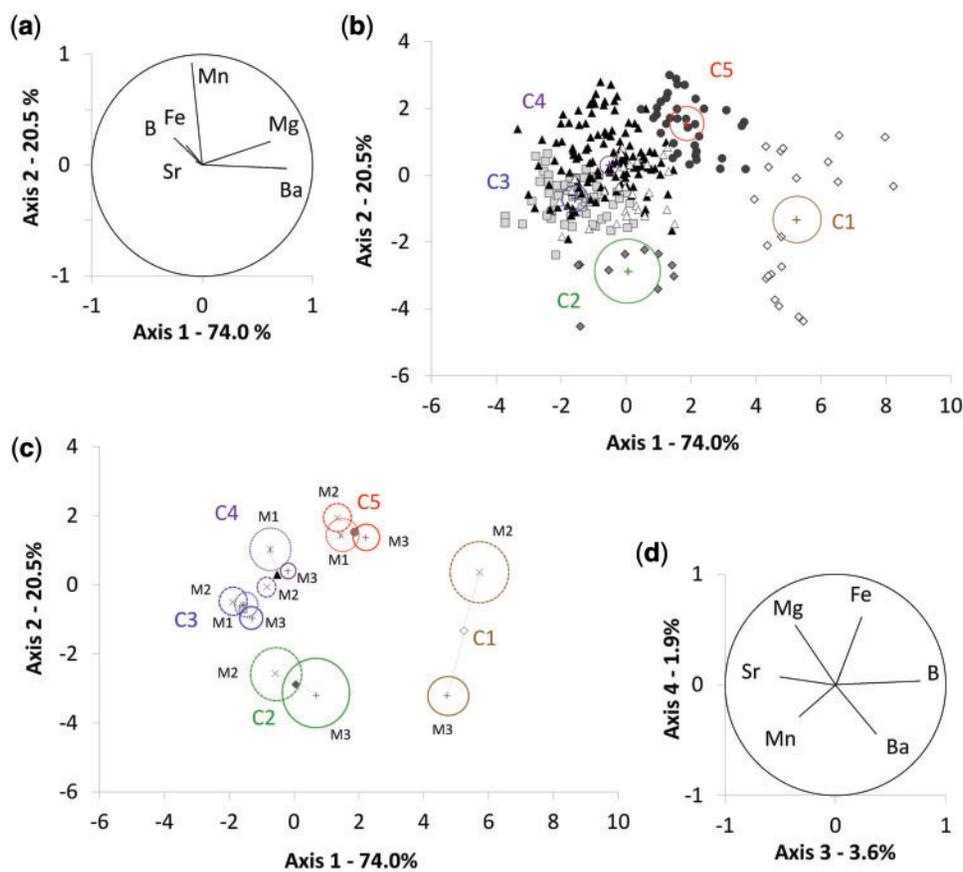
Figure 3. Linear discriminant function analysis (geographical groups). (a, d) Description of the axes. (b) Plot of the 280 capelin larvae collected from the five geographical groups (C1–C5). (c) Variations in otolith signature among the missions (M1–M3). B, boron (^{11}B); Ba, barium (^{138}Ba); Fe, iron (^{56}Fe); Mg, magnesium (^{24}Mg); Mn, manganese (^{55}Mn); Sr, strontium (^{88}Sr). Groups C1–C5 are the same as those noted in Figure 1 (C1: SSLMP—fjord upstream, white diamonds; C2: SSLMP—fjord middle, grey diamonds; C3: SSLMP—fjord mouth and estuary, grey squares; C4: remaining estuary, triangles, including C4a, black triangles and C4b, white triangles; C5: north-western gulf, grey circles). Circles enclosing centroids represent 95% confidence limits (graphs b and c). Three sampling campaigns were completed: M1: mission 1, dotted line circles; M2: mission 2, dashed line circles; M3: mission 3, full line circles (graph c).

Table 1. Reclassification of capelin larvae into geographical groups from otolith elemental signatures.

Geographic source		Correspondence to the source groups based on the elemental signature in larval otoliths[a]						
		Bootstrap-assigned group (deduced from elements)						Success of reclassification
Area name	Group	C1	C2	C3	C4	C5	n	in geographic groups (%)
SSLMP								
Fjord upstream	C1	21	0	0	0	0	21	100
Fjord middle	C2	0	10	0	0	0	10	100
Fjord mouth and estuary	C3	0	0	59	4	0	63	94
ESTUARY[b]	C4	0	3	34	106	7	150	71
NW GULF	C5	0	0	0	0	36	36	100

B, boron (^{11}B); Ba, barium (^{138}Ba); Fe, iron (^{56}Fe); Mg, magnesium (^{24}Mg); Mn, manganese (^{55}Mn); Sr, strontium (^{88}Sr); NW, north-western.
[a]The characterization of elemental signature in each geographical group was based on concentrations of B, Ba, Fe, Mg, Mn, and Sr in larval otoliths.
[b]Excluding the estuary portion of the SSLMP.

2013), both of which can affect the boundaries of the area thereby affecting the otolith signature. However, temporal variability did not hamper spatial discrimination. Single-mission discriminations were tested against another mission and lead to high reclassification success rates (Supplementary Data S4). In the same way, reclassification success rates of the pooled temporal data were high (Table 1). In consequence, we considered that source signatures were representative of geographic groups over the entire spawning period.

This study did not identify why the geographic groups were well discriminated by otolith composition-driven differences in Ba, Mg, and Mn. According to the literature, microchemistry is influenced by the environment (e.g. salinity and temperature), physiology (i.e. active transfer of elements from water to egg,
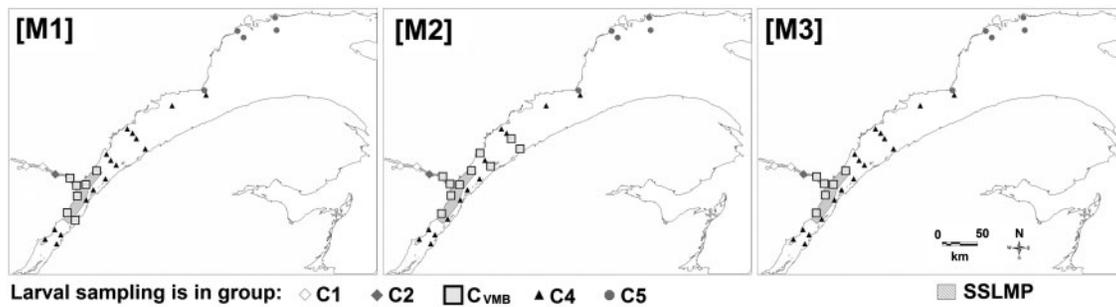
Figure 4. Influence of spatio-temporal variations in larval otolith signatures on the performance of the general geography-based model.

Table 2. Source of capelin juveniles as determined the HISEA procedure (Millar, 1987, 1990).

Geographic source		HISEA attribution of natal source for juveniles[a] Source contribution of juveniles collected in the SSLMP (%; mean ± SD)
Area name	Group	
SSLMP		
Fjord upstream	C1	NA
Fjord middle	C2	9 ± 7
Fjord mouth and estuary	C3	1 ± 4
ESTUARY[b]	C4	**74 ± 12**
NW GULF	C5	16 ± 8

B, boron (^{11}B); Ba, barium (^{138}Ba); Fe, iron (^{56}Fe); Mg, magnesium (^{24}Mg); Mn, manganese (^{55}Mn); Sr, strontium (^{88}Sr); NW, north-western; NA, origin was not attributed during the simulation.
[a]The characterization of elemental signature in each geographical area was based on concentrations of B, Ba, Fe, Mg, Mn, and Sr in larval otoliths (resampled baseline based on data from 180 larvae) and in the core of juvenile otoliths (mixed stocks, $n = 35$). Source contribution values are mean ± SD. Value in bold (source contribution >20%) presented low bias in HISEA assignation.
[b]Excluding the estuary portion of the SSLMP.

maternal transfer, etc.), and/or it may be dependent on the carbonate structure (Michibata and Hori, 1979; Volk et al., 2000; Brophy et al., 2004; Melancon et al., 2008; Walther et al., 2010; Miller, 2011; Reis-Santos et al., 2013; Sturrock et al., 2014). Furthermore, the interactions between environmental and physiological factors are likely important, especially as the physiology of eggs and larvae is known to be influenced by the ambient temperature (Rombough, 1988; Kamler, 1992). In our study, physiologically influenced elements such as K (intergroup pattern similar to Mn) or Na (intergroup pattern similar to Mg) sometimes presented more important differences between groups than environment-influenced elements such as Sr (Figure 2). This suggests that physiological processes may be important for discriminating capelin larval sources during a protracted spawning period. In a separate study, Ba, Mn, and Mg (as well as B and Fe) were recorded at relatively high concentrations in the prehatching sector (related to the egg stage) and at relatively low concentrations in the post-hatching rings of the capelin larval otoliths (Lazartigues et al., 2014). Maternal contribution may have contributed to differences in larval otolith composition. Capelin female are known to remain near spawning site for several weeks prior to spawning (Jangaard, 1974). During this period, maternal uptake of elements from water and diet might contribute to a geographically structured signature in larval otolith.

Advances in knowledge on capelin

In this study, otolith signatures of larvae caught in 2009 were used as a baseline to classify 1+ individuals captured in 2010 in the SSLMP to estimate their natal origin. Capelin sources were located in the fjord (~10%), in the estuary (~74%), and in the gulf (~16%), meaning that spawning sites located both inside and outside the SSLMP contributed to juvenile recruitment in the conservation area. The connectivity between SSLMP, estuary, and gulf has been demonstrated for copepods *Calanus* spp. (Perrin et al. 2014) in 2009–2010. The contribution gradient inferred from our study was estuary (C4a + C4b + C3) ≫ gulf (C5) > central portion of the fjord (C2) ≫ fjord upstream portion (C1) (Table 2). These results agree with previous studies based on larval density (Jacquaz et al., 1977; Ouellet et al., 2013). The estuary portion that surrounded the SSLMP (C4a) was the most important source of capelin in this study. And historically, the highest densities of newly hatched capelin larvae were observed in a region equivalent to C4a. In this area, numerous sampled stations presented high densities comparing to other sectors (such as C4b area or gulf region) where densities are lower and individuals are concentrated in a few number of stations (Jacquaz et al., 1977, Ouellet et al., 2013). Thus, in C4a sector, densities of newly hatched larvae were up to two orders of magnitude greater than in a region equivalent to C4b (Jacquaz et al., 1977, Ouellet et al., 2013). The density in the north-western Gulf of St. Lawrence was lower than in the estuary (by more than two orders of magnitude—Jacquaz et al., 1977, Ouellet et al., 2013), although the former region significantly contributed to juvenile production in the SSLMP (16%, this study).

Results from Jacquaz et al. (1977) showed low density of capelin larvae in the Saguenay Fjord in the 1970s. However, more recent studies have shown high densities of larvae in this ecosystem (Sirois et al., 2009; Ouellet et al., 2013). In our study, the upstream portion of the fjord was not an important source of juveniles that were caught close to the mouth of the fjord even though larval density was relatively high along the entire fjord in June and July during the same year (Ouellet et al., 2013). Sirois et al. (2009) hypothesized that capelin in the upstream portion of the fjord recruit locally. This idea is supported by the presence of several late-stage larvae, measuring >10–15 mm, in the upstream portion of the fjord in late summer (Sirois et al., 2009; Ouellet et al., 2013). It is also reinforced by genetic research suggesting that subpopulations from the Saguenay Fjord and the St. Lawrence Estuary may be at the initial stage of isolation (Colbeck et al., 2011).

An understanding of the sources of recruits of key prey species (e.g. 1+ capelin in this study) is critical for marine park

management. In the SSLMP, a challenge for the managers is that they cannot protect most of the spawning sites which are located outside of the current park boundaries although they should protect a sizeable proportion of capelin recruits. Ultimately, our results raise new questions. First, our findings suggest that capelin may migrate significant distances during their first year as we observed 1+ capelin caught close to the mouth of the fjord that originated from natal sources as far as the gulf region, up to 300 km away. As a consequence, further investigations of young-of-the-year capelin in terms of their dispersal and movements are required to assess the importance of SSLMP occupation during their first year of life. Second, our results support the view that most capelins hatched in the upper portion of the Saguenay Fjord (i.e. C1) do not contribute to the 1+ capelin stock sampled from the lower portion of the fjord and in the estuary. This upstream portion was not sampled for juveniles during this study and investigation on these upstream recruits is merited. Third, in 2009, the 10% contribution from habitats within the park boundaries may be sufficient to maintain recruitment of this cohort within the SSLMP. However, capelin is known to present a high interannual variability in habitat use and recruitment (Carscadden *et al.*, 2013). Moreover, several studies showed that natal source contributions can vary in supporting contingents and subpopulations from 1 year to another (Kraus and Secor, 2005; Kerr *et al.*, 2010). In consequence, additional studies should focus on temporal variability in habitat use by capelin within the park boundaries.

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Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

References

Able, K. W. 1978. Ichthyoplankton of the St. Lawrence Estuary: composition, distribution, and abundance. Journal of the Fisheries Research Board of Canada, 35: 1518–1531.

Anadón, J. D., Mancha-Cisneros, M. D. M., Best, B. D., and Gerber, L. R. 2013. Habitat-specific larval dispersal and marine connectivity: implications for spatial conservation planning. Ecosphere, 4: 82.

Bailey, R. F. J., Able, K. W., and Leggett, W. C. 1977. Seasonal and vertical distribution and growth of juvenile and adult capelin (*Mallotus villosus*) in the St. Lawrence Estuary and western Gulf of St. Lawrence. Journal of the Fisheries Research Board of Canada, 34: 2030–2040.

Brophy, D., Jeffries, T. E., and Danilowicz, B. S. 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. Marine Biology, 144: 779–786.

Bui, A. O. V., Castonguay, M., and Ouellet, P. 2012. Distribution and abundance of larval and juvenile fish in the Lower St. Lawrence Estuary: connectivity among the Gulf, the Estuary, and the Saguenay Fjord. Canadian Technical Report of Fisheries and Aquatic Sciences, 2981: 1–57.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263–297.

Carscadden, J. E., Gjøsæter, H., and Vilhjálmsson, H. 2013. Recruitment in the Barents Sea, Icelandic, and eastern Newfoundland/Labrador capelin (*Mallotus villosus*) stocks. Progress in Oceanography, 114: 84–96.

Colbeck, G. J., Turgeon, J., Sirois, P., and Dodson, J. J. 2011. Historical introgression and the role of selective *vs.* neutral processes in structuring nuclear genetic variation (AFLP) in a circumpolar marine fish, the capelin (*Mallotus villosus*). Molecular Ecology, 20: 1976–1987.

Cook, G. S. 2011. Changes in otolith microchemistry over a protracted spawning season influence origin of natal origin. Marine Ecology Progress Series, 423: 197–209.

Cook, G. S., Parnell, P. E., and Levin, L. A. 2014. Population connectivity shifts at high frequency within an open-coast marine protected area network. PLoS One, 9: e103654.

Costello, M. J. 2014. Long live marine reserves: a review of experiences and benefits. Biological Conservation, 176: 289–296.

Davoren, G. K., May, C., Penton, P., Reinfort, B., Buren, A., Burke, C., Andrews, D., Montevecchi, W. A., et al. 2007. An ecosystem-based research program for capelin (*Mallotus villosus*) in the northwest Atlantic: overview and results. Journal of Northwest Atlantic. Fishery Science, 39: 35–48.

Davoren, G. K., Woloschiniwsky, C. S. A., Halden, N. M., and Wang, F. 2015. Does otolith chemistry indicate the natal habitat of Newfoundland capelin *Mallotus villosus*? Journal of Experimental Marine Biology and Ecology, 464: 88–95.

Dodson, J. J., Tremblay, S., Colombani, F., Carscadden, J. E., and Lecomte, F. 2007. Trans-Arctic dispersals and the evolution of a circumpolar marine fish species complex, the capelin (*Mallotus villosus*). Molecular Ecology, 16: 5030–5043.

Engstedt, O., Engkvist, R., and Larsson, P. 2014. Elemental fingerprinting in otoliths reveals natal homing of anadromous Baltic Sea pike (*Esox lucius L.*). Ecology of Freshwater Fish, 23: 313–321.

Fortier, L., and Leggett, W. C. 1982. Fickian transport and the dispersal of fish larvae in estuaries. Canadian Journal of Fisheries and Aquatic Sciences, 39: 1150–1163.

Gillanders, B. M. 2002. Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. Canadian Journal of Fisheries and Aquatic Sciences, 59: 669–679.

Hamer, P. A., Jenkins, G. P., and Gillanders, B. M. 2003. Otolith chemistry of juvenile snapper *Pagrus auratus* in Victorian waters: natural chemical tags and their temporal variation. Marine Ecology Progress Series, 263: 261–273.

Hedges, K. J., Ludsin, S. A., and Fryer, B. J. 2004. Effects of ethanol preservation on otolith microchemistry. Journal of Fish Biology, 64: 923–937.

Jacquaz, B., Able, K. W., and Leggett, W. C. 1977. Seasonal distribution, abundance, and growth of larval capelin (*Mallotus villosus*) in the St. Lawrence Estuary and northwestern Gulf of St. Lawrence. Journal of the Fisheries Research Board of Canada, 34: 2015–2029.

Jangaard, P. M. 1974. Le capelan (*Mallotus villosus*): biologie, distribution, exploitation, utilisation et composition. Bulletins of the Fisheries Research Board of Canada, 186F: 1–73.

Kamler, E. 1992. Early life history of fish: an energetics approach. *In* Fish and Fisheries Series 4, pp. 267. Ed. by E. Kamler. Chapman & Hall, London.

Kerr, L. A., Cadrin, S. X., and Secor, D. H. 2010. The role of spatial dynamics in the stability, resilience, and productivity of an estuarine fish population. Ecological Applications, 20: 497–507.

Kraus, R. T., and Secor, D. H. 2005. Application of the nursery-role hypothesis to an estuarine fish. Marine Ecology Progress Series, 291: 301–305.

Lazartigues, A. V., Sirois, P., and Savard, D. 2014. LA-ICP-MS analysis of small samples: carbonate reference materials and larval fish otoliths. Geostandards and Geoanalytical Research, 38: 225–240.

Lovvorn, J. R., Anderson, E. M., Rocha, A. R., Larned, W. W., Grebmeier, J. M., Cooper, L. W., Kolts, J. M., et al. 2014. Variable wind, pack ice, and prey dispersion affect the long-term adequacy of protected areas for an Arctic sea duck. Ecological Applications, 24: 396–412.

Lubchenco, J., Palumbi, S. R., Gaines, S. D., and Andelman, S. 2003. Plugging a hole in the ocean: the emerging science of marine reserves. Ecological Applications, 13 (Suppl. 1): S3–S7.

Magris, R. A., Pressey, R. L., Weeks, R., and Ban, N. C. 2014. Integrating connectivity and climate change into marine conservation planning. Biological Conservation, 170: 207–221.

Melancon, S., Fryer, B. J., Gagnon, J. E., and Ludsin, S. A. 2008. Mineralogical approaches to the study of biomineralization in fish otoliths. Mineralogical Magazine, 72: 627–637.

Michibata, H., and Hori, R. 1979. The accumulation of manganese from the environmental medium by the egg of *Oryzias latipes.* Journal of Cellular Physiology, 98: 241–243.

Millar, R. B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Canadian Journal of Fisheries and Aquatic Sciences, 44: 583–590.

Millar, R. B. 1990. Comparison of methods for estimating mixed stock fishery composition. Canadian Journal of Fisheries and Aquatic Sciences, 47: 2235–2241.

Miller, J. A. 2011. Effects of water temperature and barium concentration on otolith composition along a salinity gradient: implications for migratory reconstructions. Journal of Experimental Marine Biology and Ecology, 405: 42–52.

Ouellet, P., Bui, A. O. V., Lavoie, D., Chassé, J., Lambert, N., Ménard, N., and Sirois, P. 2013. Seasonal distribution, abundance, and growth of larval capelin (*Mallotus villosus*) and the role of the Lower Estuary (Gulf of St. Lawrence, Canada) as a nursery area. Canadian Journal of Fisheries and Aquatic Sciences, 70: 1508–1530.

Palumbi, S. R., Sandifer, P. A., Allan, J. D., Beck, M. W., Fautin, D. G., Fogarty, M. J., Halpera, B. S., et al. 2009. Managing for ocean biodiversity to sustain marine ecosystem services. Frontiers in Ecology and the Environment, 7: 204–211.

Pater, C., and Oxley, I. 2014. Developing marine historic environment management policy: the English Heritage experience. Marine Policy, 45: 342–348.

Paton, C., Hellstrom, J., Paul, B., Woodhead, J., and Hergt, J. 2011. Iolite: freeware for the visualisation and processing of mass spectrometric data. Journal of Analytical Atomic Spectrometry, 26: 2508–2518.

Perrin, G., Plourde, S., DiBacco, C., Winkler, G., and Sirois, P. 2014. Tracing the origins of Calanus sp. in the Saguenay-St. Lawrence Marine Park (Québec, Canada) using δ13C as a marker. Marine Ecology Progress Series, 499: 89–102.

Rombough, P.J. 1988. Respiratory gas exchange, aerobic metabolism and effects of hypoxia during early life. *In* Fish Physiology, Vol. 11A, pp. 59–161. Ed. by W.S. Hoar and D.J. Randall. Academic Press, San Diego. 545 pp.

Reis-Santos, P., Gillanders, B. M., Tanner, S. E., Vasconcelos, R. P., Elsdon, T. S., and Cabral, H. N. 2012. Temporal variability in estuarine fish otolith elemental fingerprints: implications for connectivity assessments. Estuarine, Coastal and Shelf Science, 112: 216–224.

Reis-Santos, P., Tanner, S. E., Elsdon, T. S., Cabral, H. N., and Gillanders, B. M. 2013. Effects of temperature, salinity and water composition on otolith elemental incorporation of *Dicentrarchus labrax.* Journal of Experimental Marine Biology and Ecology, 446: 245–252.

Simard, Y. 2009. Le Parc Marin Saguenay-Saint-Laurent: processus océanographiques à la base de ce site unique d'alimentation des baleines du nord-ouest Atlantique. Journal of Water Science, 22: 177–197.

Sirois, P., Diab, G., Fortin, A. L., Plourde, S., Gagné, J. A., and Ménard, N. 2009. Recrutement des poissons dans le Fjord du Saguenay. Journal of Water Science, 22: 341–352.

Standish, J. D., White, J. W., and Warner, R. R. 2011. Spatial pattern of natal signatures in the otoliths of juvenile kelp rockfish along the Californian coast. Marine Ecology Progress Series, 437: 279–290.

Sturrock, A. M., Trueman, C. N., Milton, J. A., Waring, C. P., Cooper, M. J., and Hunter, E. 2014. Physiological influences can outweigh environmental signals in otolith microchemistry research. Marine Ecology Progress Series, 500: 245–264.

Tanner, S. E., Reis-Santos, P., Vasconcelos, R. P., França, S., Thorrold, S. R., and Cabral, H. N. 2012. Otolith geochemistry discriminates among estuarine nursery areas of *Solea solea* and *S. senegalensis* over time. Marine Ecology Progress Series, 452: 193–203.

Trenkel, V. M., Huse, G., MacKenzie, B. R., Alvarez, P., Arrizabalaga, H., Castonguay, M., Goñi, N., et al. 2014. Comparative ecology of widely distributed pelagic fish species in the North Atlantic: implications for modelling climate and fisheries impacts. Progress in Oceanography, 129: 219–243.

Volk, E. C., Blakley, A., Schroder, S. L., and Kuehner, S. M. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: using otolith core chemistry to distinguish maternal associations with. Sea and Freshwaters. Fisheries Research, 46: 251–266.

Walther, B. D., Kingsford, M. J., O'Callaghan, M. D., and McCulloch, M. T. 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environmental Biology of Fishes, 89: 441–451.

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