Preliminary results for the investigation of natal origin in capelin (*Mallotus villosus)* from the Gulf of St. Lawrence using otolith chemistry

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Coussau, L., Van Beveren, E., Boudreau, M., and Jac, R. 2026. Preliminary results for the investigation of natal origin in capelin (*Mallotus villosus)* from the Gulf of St. Lawrence using otolith chemistry. Can. Tech. Rep. Fish. Aquat. Sci. 3762: vii + 40 p. https://doi.org/10.60825/97zm-6s40

This study investigates the use of otolith elemental fingerprints to infer natal origin and habitat connectivity of capelin (*Mallotus villosus*) in the Gulf of St. Lawrence (GSL). Otolith elemental fingerprints were extracted from capelin larvae, juveniles and adults collected throughout most of the GSL in 2022 and 2023. Larval otolith elemental fingerprints differed significantly from the larval otolith portion (core) of juveniles and adults. Elevated elemental concentrations in the larval otolith fingerprints, beyond values observed in juvenile and adult cores, currently impedes the assignment of post-recruit individuals of unknown origin to a specific location. Despite this challenge, our results confirm that geographic variability in environmental conditions in the GSL produced distinct elemental fingerprints, reinforcing the reliability of otolith chemistry for tracking capelin movements. Random Forest clustering of elemental fingerprints from juvenile and adult core otoliths identified two putative natal origins in the population. The contributions of these two natal sources indicated some geographic structuring of juveniles, especially in the southern GSL (sGSL). Adult contributions of each source were similar in the Estuary but dominated by one natal source in the sGSL. We discuss implications for future otolith chemistry analyses and provide recommendations for upcoming sampling and data analysis.

RÉSUMÉ

Coussau, L., Van Beveren, E., Boudreau, M., and Jac, R. 2026. Preliminary results for the investigation of natal origin in capelin (*Mallotus villosus)* from the Gulf of St. Lawrence using otolith chemistry. Can. Tech. Rep. Fish. Aquat. Sci. 3762: vii + 40 p. https://doi.org/10.60825/97zm-6s40

Cette étude examine l'utilisation des empreintes élémentaires des otolithes pour déduire l'origine natale et la connectivité de l'habitat du capelan (*Mallotus villosus*) dans le golfe du Saint-Laurent (GSL). L'empreinte élémentaire des otolithes a été extraite chez des larves de capelan, des juvéniles et des adultes prélevés dans la majeure partie du golfe du Saint-Laurent en 2022 et 2023. Les empreintes élémentaires des otolithes larvaires différaient significativement de la portion larvaire des otolithes (noyau) des juvéniles et des adultes. Les concentrations élémentaires élevées dans les otolithes larvaires, dépassant les valeurs observées dans les noyaux des juvéniles et des adultes, empêchent actuellement l'attribution d'individus post-établis d'origine inconnue à un lieu spécifique. Malgré ce défi, nos résultats confirment que la variabilité géographique des conditions environnementales dans le GSL se traduit par des empreintes élémentaires distinctes, ce qui renforce la fiabilité de la chimie des otolithes pour le suivi des mouvements du capelan. L'analyse de regroupement par Random Forest des empreintes élémentaires du cœur des otolithes de juvéniles et adultes a permis d'identifier deux origines natales potentielles dans la population. Leur contribution aux habitats des juvéniles indique un certain degré de structuration géographique de la population, en particulier dans la partie sud du GSL (sGSL). Chez les adultes, la contribution des deux sources était plus égale dans l'estuaire, mais toujours dominée par une seule source dans le sGSL. Nous discutons des implications de ces résultats pour les futures analyses de chimie des otolithes et formulons des recommandations pour les prochains efforts d'échantillonnage et d'analyse des données.

1. INTRODUCTION

Estimating the spatial scale of fish population connectivity is a fundamental ecological question that remains largely unresolved for many species. Most marine fish populations have a high potential for dispersal and demographic exchange due to their pelagic planktonic larval stage (Kingsford et al., 2002; Cowen et al., 2007; Cowen & Sponagle, 2009). Demographic exchanges can also result from the active migratory behaviour of individuals, which fulfills ecological functions such as accessing preferred breeding sites, optimizing foraging opportunities and avoiding unfavourable conditions (Nathan et al., 2008; Secor, 2015). Understanding the degree of demographic exchange and spatio-temporal isolation among fish populations is important for assessing their structure and dynamics. This is key in the management of exploited marine populations, as management strategies should be tailored to protect well-defined populations and designed at the appropriate spatial scale (Fogarty & Bostford, 2007; Kerr et al., 2017). However, tracking lifetime movements is complex, with constraints such as species longevity and the capturability of specific ontogenic stages, leaving our understanding of these dynamics incomplete.

Fish otoliths, known for their ability to incorporate chemical elements from surrounding water (Campana and Neilson, 1985), serve as lifetime records of individual environmental histories and movement patterns (Campana, 1999; Reis-Santos et al., 2012). Minor and trace elements are transferred from the water into the bloodstream through intestinal or branchial uptake, ultimately becoming accreted onto the growing calcified structure (Campana, 1999). Chemical elements incorporate either by substituting for calcium (Ca) within the crystal lattice, bonding with the organic matric constituents, or by filling interstitial space of the crystalline structure (Kalish, 1989; Mugiya & Yoshida, 1995; Thomas & Swearer, 2019; Hüssy et al., 2020). The resulting otolith elemental composition is influenced by a combination of environmental factors (e.g., ambient elemental concentrations, temperature, salinity, pH) and intrinsic physiological processes (e.g., growth, reproduction, metabolic processes) (Barnes and Gillanders, 2013; Walther et al., 2010; Miller, 2011; Reis-Santos et al., 2012; Sturrock et al., 2014). This complex process makes the elemental fingerprint of otoliths a valuable natural tracer of a fish's lifetime exposure to water bodies with varying physico-chemical properties.

Tracking the natal origin of post-recruit individuals using otolith elemental fingerprints offers valuable insights into the connectivity and movement of fish populations (Cowen et al., 2007). Analysis of the elemental fingerprints of otolith cores, which contain the chemical information of a fish's early life stage, has allowed the estimation of dispersal distances or natal homing behaviour (e.g., Gillanders, 2002a; Thorrold et al., 2001; Standish et al., 2008, Rogers et al., 2019). Two principal methodologies can be used to investigate the natal origins of post-recruit (i.e., post-larval stage) individuals based on otoliths core fingerprint. The first method aims to assign a precise location of origin by comparing the fingerprints of post-recruit otolith cores of unknown origin with a pre-established reference collection of elemental fingerprints from larvae sampled at known locations (Gillanders, 2002b; Thresher, 1999; Wright et al., 2010). This approach requires that assignments be cohort-specific and that larvae have been adequately

sampled from all possible natal origins. Without these two conditions being fulfilled, a second more parsimonious method can be applied. The diversity of natal sources from a sample of post-recruit individuals can be estimated through cluster analysis of the elemental fingerprints of otolith cores (e.g., Artetxe- Arrate et al., 2019; Gibb et al., 2017; Régnier et al., 2017; Coussau et al., 2023).

Capelin (*Mallosus villosus*) is a short-lived pelagic forage fish with a circumpolar distribution, inhabiting cold water masses of the coasts and continental shelves of the North Atlantic and Arctic waters (Carscadden et al., 2013; McQuinn et al. 2012). In the Northwest Atlantic, three different haplogroups have been identified on the Newfoundland and Labrador Shelf and in the Estuary and Gulf of St. Lawrence (GSL; Cayuela et al., 2020). The GSL, which includes Northwest Atlantic Fisheries Organization (NAFO) divisions 4RST, is considered a single stock and is managed separately from the southeast and eastern coasts of Newfoundland (NAFO Divisions 2J3KL + 3Ps; southeastern shoal: NAFO Divisions 3NO) (DFO, 2022). In the GSL, capelin might engage in both beach and demersal spawning (as observed for the Newfoundland capelin stock, NAFO Div. 2J3KL+3Ps; Penton et al., 2012; Crook et al., 2017b) from April to August, with beach spawning occurring earliest in the Estuary and latest on the West coast of Newfoundland (Boudreau et al., 2023).

A deeper knowledge of GSL capelin stock structure and dynamics is highly desirable. Over the past decade, Fisheries and Oceans Canada (DFO) assessments have revealed geographic differences in abundance indices for capelin (Chamberland et al., 2022). Specifically, the northern GSL (nGSL), where larger capelin were found, may be experiencing a decline in abundance, while the southern GSL (sGSL), where smaller individuals are found, may have experienced a relative increase in recent years (DFO, 2022; Lehoux et al., 2022). The current lack of information on the GSL capelin population structure and spatial dynamics hampers our ability to interpret these trends. In addition, the GSL has also experienced rising water temperatures, reduced sea ice cover and warming of the cold intermediate layer (CIL) in recent decades (Galbraith et al., 2022). These changes, driven by global warming, may prompt capelin to shift their distribution (Carscadden et al., 2013). Given the high migratory capacity of the species (Huse & Ellingsen, 2008; Carscadden et al., 2013), addressing these knowledge gaps is important for effective management and conservation of capelin stocks.

The presence of pronounced gradients in environmental conditions in the Gulf of St. Lawrence (GSL) (Galbraith et al., 2022) has enabled the successful application of otolith chemistry to investigate stock structure and migratory movements in several commercially important species, including the redfish complex (*Sebastes* spp.) (Campana et al., 2007; Coussau et al., 2023), herring (*Clupea harengus*) (Couillard et al., 2022), Greenland halibut (*Reinhardtius hippoglossoides*) (Bassi et al., 2023) and Atlantic halibut (Gauthier et al., 2024). In capelin, otolith elemental fingerprints have been successfully used as natural tracers of natal origin in the Saguenay–St. Lawrence Marine Park (Lazartigues et al., 2016) and in eastern Newfoundland coastal waters (Tripp et al., 2020).

The objectives of this study were to evaluate whether natal origins and connectivity patterns within the GSL capelin population could be resolved using otolith chemistry. First, we assessed whether elemental fingerprints from larval otoliths could discriminate

among geographic regions where capelin larvae were collected in the GSL. Larval sampling conducted over two consecutive years allowed us to account for potential interannual variability in chemical signatures driven by environmental change. Second, using larval chemical fingerprints preserved in juvenile and adult otolith cores, we estimated the number of chemically distinct natal origins and quantified the relative contribution of each putative origin to the post-recruit population. Finally, our results provide guidance for future otolith sampling strategies and establish a baseline for future statistical analyses.

2. METHODS

2.1 Study area

The GSL is a semi-enclosed sea characterized by diverse bathymetric features and heterogeneous oceanographic conditions (Galbraith et al., 2022). The southern GSL consists of a broad shallow plateau with homogenous depths averaging 80 m, while the northern GSL is more complex, featuring deep channels and slopes, with an average depth of 240 m, reaching 500 m at its deepest (Koutitonsky and Bugden, 1991). The GSL's summer thermal structure includes a warm surface layer, a cold intermediate layer (between 50 and 120m), and a deeper and warmer layer originating from the Arctic (Galbraith et al., 2022). For the purpose of this study, the GSL capelin habitat was divided in four geographic regions based on the physico-chemical characteristics of water masses (Galbraith et al., 2022): the St. Lawrence Estuary (NAFO subdivisions: 4Tp, 4Tq, 4Sz, 4To), the southern Gulf (NAFO subdivisions: 4Tm, 4Tn, 4Tk, 4Tj, 4Tg), the northern Gulf (NAFO subdivisions: 4Sy, 4Sx, 4Sw) and western Newfoundland (NAFO subdivisions: 4Ra, 4Rb, 4Rc, 4Rd) (Figure 1).

2.2 Sampling

Capelin larvae were sampled across nine NAFO subdivisions in the GSL in 2022, and seven subdivisions in 2023 (Table 1; Figure 1). These larvae were collected from multiple sources, including the DFO mackerel egg survey in the sGSL (June; Lehoux et al., 2024) and a new complementary mackerel egg survey conducted in western and southern Newfoundland (July; Van Beveren, pers. comm.). Both surveys used a 0.6 m diameter bongo sampler with 333 µm mesh nets, towed in a saw-tooth profile between the surface and a maximum depth of 50 m, or to within 5 m of the bottom at shallower stations. Additional larvae were obtained through sporadic ichthyoplankton bottom-to-surface sampling with ring nets (200 µm mesh) as part of the Atlantic Zone Monitoring Program (AZMP). Larvae from the Estuary were caught caught with a small pelagic trawl (500-µm mesh) equipped with a removable cod end during the juvenile rainbow smelt (*Osmerus mordax*) monitoring program conducted by the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (MFFP). All samples were preserved in ethanol.

Juvenile and adult capelin were sampled in 2022 from two sources: during wharf sampling as part of the DFO commercial port sampling program between May and July (see description of the sampling in Boudreau et al., 2023), and during the DFO multi-species bottom trawl surveys in the nGSL in August and sGSL in September (Chamberland et al., 2022). Individuals targeted by commercial fisheries are typically

larger capelin spawners, while the scientific survey captures a broader range of fish lengths. Individuals were measured (total length, nearest mm), and their sex and maturity status were determined (see Supplementary Figures 3 and 4 for the spatial distribution of maturity status and sex ratio). Individuals were separated into juveniles or adults based on their maturity status (i.e., immature, maturing, pre-spawning, spawning, or recovering). Individuals who had not yet reached maturity (i.e., immature) were classified as juveniles. All other maturity statuses were considered adults.

2.3 Otolith preparation

Manipulation tools and storage containers for capelin otoliths were decontaminated in 10% nitric acid for 24h for glass and 10 minutes for plastic materials and were then rinsed with ultrapure water and dried under a laminar flow flume hood.

Capelin larval otoliths were extracted under a stereomicroscope (63×) using transmitted light and a dark background. Each larva was positioned in a small drop of water, and the right sagittal otolith was carefully removed with fine entomological needles by opening the otic capsule. The otolith was then transferred onto a petrographic slide coated with double-sided 3M™ adhesive tape, using a moistened needle to avoid direct contact with the otolith surface.

For juvenile and adult capelin, right sagittal otoliths were extracted, cleaned of organic tissues, triple-rinsed in ultrapure water and stored in polyethylene vials before being dried under a laminar flow flume hood for 24 h. Otoliths were mounted on petrographic slides with thermoplastic glue (Crystalbond™ 509; Aremco™ products, NY, USA), and oriented consistently. Each otolith was progressively polished using 1200 µm, 5 µm, and 1 µm lapping films until the core became clearly visible under microscope. They were finally transferred onto a petrographic slide and photographed to identify core location prior to laser ablation.

2.4 LA-ICP-MS analysis

Otolith trace elemental concentrations were obtained from laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) using a Resonetic Excimer 193 nm ArF laser coupled to an Agilent 7900x qICP-MS. Laser ablation was performed along a transect for both larval, juvenile, adult otoliths. In larvae, the transect spanned the entire otolith, while in juveniles and adults the ablations targeted the core portion (approximately 20 µm) containing the larval fingerprint.

LA-ICP-MS analysis was carried out at Earth's Material Laboratory (LabMaTer) from the University of Quebec at Chicoutimi. Laser spot energy density was set to 3 J/cm^2 with 15 Hz frequency, 15 µm beam diameter, and 2.5 µm/s ablation rate for larval otoliths and 5 J/cm^2 with 25 Hz frequency, 44 µm beam diameter, and 5.5 µm/s ablation rate for juvenile and adult otoliths. The ablated material from the otoliths was carried into the ICP-MS by an Argon–Helium gas mix at a rate of 0.8^{-1} L min^{-1} for Argon and 350 mL min^{-1} for Helium, and 2 mL min^{-1} for Nitrogen also added to the mixture. A 20 s gas blank acquisition preceded each ablation. Four reference materials (SRM-610 and SRM-612 obtained from NIST, MD, USA; GSE-1, GP4-A and MACS-3 obtained from USGS, CO, USA, see Lazartigues et al., 2014 for details on the reference materials) were analyzed at the beginning and the end of each LA-ICP-MS session, and

approximately every 30 minutes during analysis. A total of 38 elements and isotopes were measured: ^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{29}Si, ^{31}P, ^{34}S, ^{39}K, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{56}Fe, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{61}Ni, ^{63}Cu, ^{64}Zn, ^{65}Cu, ^{66}Zn, ^{69}Ga, ^{85}Rb, ^{86}Sr, ^{87}Sr, ^{88}Sr, ^{111}Cd, ^{114}Cd, ^{118}Sn, ^{119}Sn, ^{120}Sn, ^{136}Ba, ^{137}Ba, ^{138}Ba, ^{202}Hg, and ^{208}Pb. Data reduction was carried out using the Iolite package for Igor Pro software from Wavemetrics Incorporated (Paton et al., 2011). Calcium (^{44}Ca) was used as an internal standard and assumed to compose 38.02% of the otolith mass (Campana, 1999). Calibration was performed using NIST SRM-610 reference material (Chen et al., 2011). ^{43}Ca profiles were checked for the presence of irregularities in the otolith matrix and only samples considered stable in their calcium concentrations were retained for further analysis.

2.5 Statistical analysis

2.5.1 Data preparation

Trace elements measured as ppm concentrations were expressed as molar ratios relative to calcium. Statistical analyses were performed on ln (x + 1) transformed elemental concentration data to meet the assumptions of normality and homoscedasticity of residuals. Outliers exceeding 5 times the standard deviation from the mean were removed from the dataset (Kerr and Campana, 2014). Twelve larvae and fourteen post-recruits were considered outliers.

2.5.2 Larval otoliths versus post-recruit otolith cores

Accurately assigning capelin of unknown origin to specific natal locations requires a comparison of the elemental concentrations of larval otoliths with those of post-recruit otolith cores. The objective is to ensure that the reference collection of larval elemental fingerprints accurately reflects the potential natal origins of the post-recruited population. This comparison was conducted using both univariate (ANOVA and boxplot, Supplementary Figure 1) and multivariate (PCA, Figure 2) analyses.

2.5.3 Larval otoliths: Spatial and temporal variation in elemental fingerprint

The variability in elemental fingerprints was further explored to 1) confirm that environmental conditions in the GSL are sufficiently heterogeneous to support otolith microchemistry analyses, 2) reveal how regional environmental conditions might affect elemental concentrations and 3) assess inter-annual variability.

First, we tested the ability of larval otolith elemental fingerprint to reassign capelin larvae to their respective habitat (capture location). To determine the optimal number and combination of trace elements that maximized habitat assignment accuracy for the St. Lawrence Estuary, Southern GSL, and Western Newfoundland, we applied the statistical approach outlined by Mercier et al. (2011). We compared classification success among two common multivariate ordination models and one machine learning method: linear and quadratic discriminant analysis (LDA and QDA) and random forest classification (RF). Machine learning has an advantage over ordination analysis as it does not require distributional assumptions of the data set and avoids overfitting (Breiman, 2003). We used a cross-validation method where 75% of individuals were randomly selected to train the classifiers, and the remaining 25% were used to assess classification accuracy. For each method tested, the number of iterations was fixed to 100 and the number of trees to 500.

Principal component analysis (PCA) was performed to visualize geographical differences in larval fingerprints and identify the chemical elements driving the observed spatial variations. MANOVA and ANOVA were used to test for significant variability in elemental fingerprints, both spatially (between the three regions of capture) and temporally (between the years of capture within each region).

Habitat-specific differences in otolith elemental composition might be confounded with variations in larval lengths between locations. Indeed, larvae caught in the southern GSL were larger than other capture sites in the estuary and on the Newfoundland coast (Supplementary Figure 2). Therefore, before all analyses, we evaluated the relationship between larval lengths and the selected trace elements through an analysis of covariance (ANCOVA; see Kerr and Campana, 2014). When a significant effect of length (L) was detected for a given element (e, including Na, P, Sr and Ba), elemental concentrations (C) were adjusted (C^{adj}) for each individual (i) by subtracting the predicted effect size of length ($effect$; $C_{e,i}^{adj} = C_{e,i} - effect_{e,i} * L_i$, see ANCOVA estimated effect sizes in Supplementary Table 1).

2.5.4 Post-recruit otolith cores: Natal origin investigation

The precise location of natal origin of post-recruits could not be identified based on the reference collection of larval otolith fingerprints, due to significant differences in elemental concentrations between the two datasets (see Results). We therefore assessed the diversity of natal sources in juvenile and adult capelin through a cluster analysis of the elemental fingerprints of their otolith cores, which reflected the conditions experienced during the larval stage. Specifically, we used the unsupervised random forest clustering analysis which is well suited in cases where no reference collection is available (e.g., Gibb et al., 2017; Régnier et al., 2017; Artetxe-Arrate et al., 2019; Coussau et al., 2023). The analysis focused on the set of elements that we identified as the most effective for discriminating larval habitats in the study area.

The unsupervised RF approach, developed by Shi & Horvath (2006), consisted of a two-step procedure. Briefly, RF predictors were used to distinguish between the observed dataset and a synthetic one that was generated from a reference distribution (Breiman and Cutler, 2003). The dissimilarity measure between the two datasets then served as input for a partitioning around medoids (PAM) clustering algorithm (Kaufman & Rousseeuw,1990) that allowed the detection of groups or underlying structures in the observed dataset. The optimal number of clusters was determined using the clValid R package (Brock et al., 2008) which allowed multiple combinations of validation measures to be tested while varying the number of clusters. Differences in elemental concentrations between the identified clusters were compared using MANOVA for multi-elemental fingerprints and ANOVA for single element analysis. The statistical significance of the MANOVA was tested with the Pillai trace statistic as it is the most robust to deviations from multivariate normality (Quinn and Keough, 2002). P-values from ANOVA were corrected with Bonferroni corrections for statistical significance. The contribution of the natal origins to capture sites was examined as a function of the individual life stage (juvenile: immature, or adult: individuals that have already reproduced). Analyses and visualizations were performed in R (version 4.3.2; R Core Team, 2023). Key packages included ggplot2 (Wickham, 2016), dplyr (Wickham et al.,

2022), tidyr (Wickham et al., 2022), FactoMineR (Lê et al., 2008), factoextra (Kassambara & Mundt, 2020), randomForest (Liaw & Wiener, 2002), caret (Kuhn, 2008), MASS (Venables & Ripley, 2002), and NbClust (Charrad et al., 2014).

3. RESULTS

3.1 Larval otoliths: Comparison with post-recruit otolith cores

A total of 349 capelin larvae were sampled, with 220 larvae in 2022 and 129 in 2023. Larvae ranged in length from 4.61 to 17 mm in 2022 (mean ± SD: 8.06 ± 2.93 mm; median: 7.04 mm; SE: 0.20 mm) and from 4.94 to 19 mm in 2023 (mean ± SD: 9.63 ± 3.40 mm; median: 9.46 mm; SE: 0.30 mm). Juvenile capelin (n = 144) ranged from 75 to 144 mm in length (mean ± SD: 117.03 ± 12.65 mm; median: 118 mm; SE: 1.05 mm), whereas adult capelin (n = 242) ranged from 115 to 183 mm (mean ± SD: 145.40 ± 14.89 mm; median: 145 mm; SE: 0.96 mm).

Univariate and multivariate analysis revealed a clear distinction between the two sets of multi-elemental fingerprints (Figure 2). Elemental concentrations were significantly higher in larval otoliths, exceeding those found in juvenile and adult otolith cores by a factor of two for Sr, Na and P, up to ten for Ba, and up to 163 for Zn (Supplementary Figure 1).

3.2 Larval otoliths: Spatial and temporal variation in elemental fingerprint

The highest classification success rate for assigning 2022 capelin larvae to their respective capture regions in the GSL was obtained with the RF classification method, based on the following set of elements: ^{23}Na, ^{24}Mg, ^{31}P, ^{66}Zn, ^{88}Sr, ^{138}Ba (Figure 3; Table 2). RF analysis with 1000 iterations and 100 trees achieved an overall accuracy of 80%. Classification accuracy for each region was respectively 62% for the Estuary (N=50), 80% for the sGSL (N=80) and 92% for western Newfoundland (N=81) (Table 3).

The plot of the first two principal components of the PCA, explaining 28.2% and 24.4%, respectively, of the variation in the elemental fingerprints, allowed a visual assessment of the spatial discrimination of capelin larval capture sites (Figure 4). Newfoundland showed the highest classification success rate and was mostly discriminated through elevated concentrations in Zn and Ba. The Estuary and Southern GSL sites presented higher concentrations of P, Mg, and Na and were more difficult to distinguish visually based on the first two principal components.

Univariate analyses of variance revealed significant spatial differences in Na, Mg, P and Zn concentrations measured in larval otoliths from 2022 (Table 4). For each region, we observed significant differences in the concentrations of Na, P, Zn, and Ba when contrasting 2022 with 2023 (Table 5; Figure 5). The interannual variations in element concentrations sometimes exceeded regional variations.

3.3 Post-recruit otolith core: Natal origins investigation

Two significantly different natal fingerprints were identified from RF clustering on Na, Mg, P, Zn, Sr, and Ba concentrations measured in the core of post-recruit otoliths (MANOVA, Pillai's trace statistic = 0.59, F = 92.73, df = 1, $p < 0.0001$). The natal

fingerprint of cluster 1 was characterized by significantly lower concentrations of Na, P, Mg, Zn and Sr. No significant differences were found for Ba (Table 6; Figure 6). Both clusters contributed to all four regions, but with different proportions between regions and between capelin juvenile and adult populations (Figure 7; Table 7). In the nGSL, cluster 1 was dominant, accounting for 67%, 75%, or 88% of the individuals, depending on the sampling method and the life stage. In the Estuary, cluster 1 was dominant in juveniles (69% versus 31%), while adults showed nearly equal contributions from both clusters, regardless of whether they were sampled at the wharf (48% versus 52%) or during research efforts (44% versus 56%). In the southern GSL, both juveniles and adults were predominantly from cluster 2 (accounting for 76% and 100%, respectively), although cluster 1 was dominant among adults from the wharf sampling (61% versus 39%). In western Newfoundland, cluster 2 was dominant among juveniles (63% versus 37%) and adults from wharf sampling (71% versus 29%), but the adult population from research surveys showed a more balanced distribution between the two clusters (46% versus 54%).

4. DISCUSSION

4.1 Larval otoliths: comparison with post-recruit otolith cores

Theoretically, we can determine the natal origin of post-recruit individuals by reassigning the elemental fingerprints of their otolith cores, representing the larval stage, to a reference collection of elemental signatures of larval otoliths of known origin. We were, however, unable to do so because the elemental concentrations of larval otoliths greatly exceeded those of the larval portion of juvenile and adult otoliths. Several hypotheses are proposed to explain this observed mismatch between larval otolith fingerprints and the core of the pre-recruits, and enrichment of larval fingerprints.

A first hypothesis involves methodological factors. The larval portion of juvenile and adult otoliths may not have been accurately targeted during LA-ICP-MS analysis. Larval otoliths are only a few dozen micrometers in diameter whereas the larval portion in post-recruit otoliths is embedded under subsequent calcite layers which can dilute the signal during LA-ICP-MS analysis. Our ability to target the three-dimensional larval portion of the otolith is therefore highly dependent on the accuracy of the transverse cut through the otolith core. The process of otolith polishing may also have removed or altered the portion of material that contains the signature of the larval stage.

A second hypothesis, as suggested by Brophy et al. (2003) for herring, is that post-mortem contamination by residual endolymph or tissue was a possible source of elemental enrichment in larval otoliths compared to post-recruits. Although a rigorous cleaning protocol for capelin larvae otoliths limits contamination during extraction (see section 2.3), we cannot rule out potential effects of larval storage before otolith extraction. In contrast, potential contamination from the 3M™ tape used to mount larval otoliths during laser ablation was dismissed. Despite the tape containing high concentrations of several elements (Na, P, S, and K), baseline correction during data reduction in Iolite software confirmed that the 3M™ tape did not significantly affect otolith elemental concentrations.

A third hypothesis that resorption or remobilization of chemical elements during capelin ontogeny could explain the lower concentrations observed in post-recruits was deemed unlikely. Otoliths are metabolically inert structures (Campana, 1999), and resorption has only been documented under extreme stress conditions (Mugiya & Uchimura, 1989).

Assuming that the otolith core was accurately targeted in post-recruits, the lack of overlap in chemical signatures with the larval reference collection suggests that juveniles and adults may originate from unsampled larval sources. Exhaustive sampling of potential larval sources is essential, since incomplete coverage can lead to erroneous origin assignments (Gillanders, 2005; Crook et al., 2017a). In our study, the absence of larval samples from NAFO division 4S represents a possible gap in the reference collection. However, this is unlikely to explain the important mismatch between larval and post-recruit fingerprints. Environmental conditions in 4S are not drastically different from those in adjacent sampled regions (Galbraith et al., 2022), making it highly unlikely that post-recruits originated exclusively from this single unsampled habitat.

Although we could not differentiate if the capelin larvae in this study originated from either beach or demersal spawning habitats, it seems unlikely that this spawning habitat difference would be reflected in the larval fingerprint. Captured larvae averaged approximately 8 mm in length and were likely several weeks old; therefore, the pre-hatch chemical signature associated with the spawning habitat likely represents only a small fraction of the larval otolith and may be overshadowed by the post-hatch signature. It is more likely that the elemental differences in the larval fingerprint reflect habitat conditions from several weeks after hatching.

An additional hypothesis for the observed mismatch between larvae and post-recruited individuals is interannual variability in otolith elemental concentrations (i.e., temporal variability). An important premise in natal origin investigations is that reassignments should be cohort-specific in order to prevent interannual variability in environmental conditions from being a confounding factor in spatial origin assignments (Campana et al., 2000; Gillanders, 2002b; Gillanders, 2005; Elsdon et al., 2008). In this study, since larvae and post-recruits belong to different year-classes, their otolith fingerprints may reflect not only spatial variation (habitat), but also temporal variation (year of birth). The physico-chemical conditions in the GSL (e.g., temperature, salinity, nutrient inputs, circulation) are known to vary from year to year (Galbraith et al., 2022; Blais et al., 2024), potentially influencing elemental incorporation into otoliths. However, previous studies examining temporal variability in capelin larval fingerprints have reported limited interannual differences compared to spatial contrasts (e.g., Tripp et al., 2020). Such temporal variability has not been shown to produce the order-of-magnitude differences observed between larvae and post-recruited individuals in the present study. We therefore conclude that temporal variability alone is unlikely to account for the patterns reported here.

Overall, the mismatch between larval and pre-recruit core signatures currently limits the reliability of larval elemental fingerprints for determining the natal origin of juvenile and adult capelin in the GSL. Successful reassignments using larval reference collections remain rare (e.g., Lazartigues et al., 2016), likely due to the methodological and ecological limitations discussed above. Consequently, connectivity studies either rely on later life stages for reassignments, or restrict natal origin investigations to larvae only

(e.g., Tripp et al., 2020). Future work should therefore focus on better understanding the factors contributing to the discussed mismatch, to determine whether larval fingerprints can be used with confidence as reference collections for natal origin assignments.

4.2 Larval otoliths: Spatial and temporal variation in elemental fingerprint

The potential of using otoliths as natural tracers of natal origin depends on the ability of their elemental fingerprint to discriminate spatially discrete habitats in the studied system. Spatial variation in elemental fingerprints can result from both variations in ambient elemental concentrations (Bath et al., 2000; Hamer and Jenkins, 2007) and physico-chemical parameters (Elsdon & Gillanders, 2004; DiMaria et al., 2010; Izzo et al., 2018; Hüssy et al., 2020). The variation in physico-chemical parameters within the GSL has already been shown to result in distinct fingerprints in capelin otoliths (Lazartigues et al., 2016). A similar observation was made in coastal southern and northeastern Newfoundland (Tripp et al., 2020).

In our study, the elemental fingerprints of larval otoliths collected from three distinct regions could be clearly distinguished. Larvae could be re-assigned to their capture region with a high overall accuracy of 80%, indicating that their otolith chemistry reflects exposure to regionally distinct water masses during early development. Western Newfoundland showed particularly clear differentiation, consistent with the unique physico-chemical properties of these water masses influenced by the Labrador Current El-Sabh,1976; Galbraith et al., 2022). In contrast, the Estuary and sGSL were more difficult to discriminate, as shown by overlap in the multi-elemental fingerprints in the PCA biplot and the moderate classification accuracy. This reduced discriminability may reflect both the similarity of water mass properties in these adjacent regions and the effects of larval transport following the summer surface water flow from the estuary to the sGSL (Ouellet et al., 2013). While capture location does not necessarily indicate natal origin, the high classification success suggests that larval otolith chemistry retains sufficient regional signal to distinguish broad geographic areas.

The chemical elements important for the geographic discrimination of larvae in the GSL are consistent with those used in previous capelin connectivity studies in the North Atlantic (Lazartigues et al., 2016; Tripp et al., 2020; Davoren & Halden, 2014). Laboratory experiments have also identified Sr, Ba, and Mg as key elements for distinguishing capelin natal environments (Davoren et al., 2015; Loeppky et al., 2018; Loeppky and Davoren, 2018). The main drivers determining their concentrations in larval otoliths were ambient concentrations (Tripp et al., 2022), and water temperature and salinity (Loeppky et al., 2018; Loeppky and Davoren, 2018). Unexpectedly, we found that Sr and Ba were not the primary elements responsible for spatial discrimination in the GSL. Instead, Mg, Zn, P, and Na, elements that are typically considered physiological rather than environmental markers (Hüssy et al., 2020), played a more significant role. This discrepancy highlights the need for further research to investigate whether physiological processes significantly influence spatial discrimination in the GSL.

Assessing temporal variability in elemental fingerprints is important to avoid confounding individual movement from interannual changes in environmental conditions

(Gillanders, 2002a; Crook et al., 2017a). The scale of the observed interannual variability in larval elemental fingerprint between 2022 and 2023 is consistent with findings from other capelin habitats, such as eastern Newfoundland coastal waters (Davoren et al., 2015; Loeppky et al., 2018; Tripp et al., 2022). Capelin larvae develop in the upper layers of the water column (DFO, 2022), which are highly sensitive to temporal variability in ambient elemental concentrations and environmental factors. These fluctuations can be driven by river discharge, rainfall events, tidal cycles, or interannual variations in upwelling intensity (Elsdon & Gillanders, 2006; Reis-Santos et al., 2012; Morissette et al., 2021). Our results reinforce the importance of ensuring that temporal variability in the elemental fingerprint is smaller than spatial variability in the studied system, as this balance is key to evaluate population connectivity using otolith chemistry.

4.3 Post-recruit otolith core: Natal origin investigation

As discussed earlier, several limitations lead us to prefer an unsupervised approach for natal origin investigations in capelin. We thus relied on a cluster analysis of the core region of otoliths from post-recruited fish to assess the diversity of larval sources that contribute to the population (e.g., Artetxe-Arrate et al., 2019; Gibb et al., 2017; Régnier et al., 2017; Coussau et al., 2023).

In our study, the marked spatial variability of larval otolith fingerprints confirms the prediction that the two identified clusters likely reflect geographically distinct natal origins. Unsupervised analysis does not provide a location to be attributed to either of the two putative origins. However, hypotheses can be derived from 1) the elemental concentrations characterizing both sources and 2) the spatial patterns in the contribution of the sources to capture regions.

Source 2 was significantly more concentrated in P, Sr, Mg, Zn and Na, listed here in order of importance. P, Zn and Na are rarely reported in connectivity studies, as they typically are considered physiological markers rather than environmental tracers (Hüssy et al., 2020). This suggests that physiological processes may play an important role in the discrimination of capelin natal origins (see also Lazartigues et al., 2016). Among recognized environmental tracers, observations from recent laboratory studies have shown that higher Sr concentrations in capelin otoliths are positively correlated with environmental concentrations (Tripp et al., 2022), and with water temperature (Loeppky & Davoren, 2018). Similarly, Mg incorporation into capelin otoliths was also shown to be positively influenced by temperature (Loeppky & Davoren, 2018). On this basis, the higher Sr and Mg concentrations observed in individuals from source 2 would be characteristic of warmer water masses, corresponding best to the shallow habitats of the southern GSL (Galbraith et al., 2022). The predominant contribution of source 2 in the southern Gulf further supports the hypothesis that individuals captured in this region are more likely to originate from a spatially restricted location.

In contrast, source 1, characterized by lower Sr and Mg concentrations, likely reflects colder waters, although the exact location of this natal source remains unknown. One possibility is that it corresponds to an estuarine origin identified by Lazartigues et al. (2016) as a major contributor to the Saguenay Fjord, St. Lawrence Estuary, and the northwestern GSL.

Spatial patterns in the contribution of the two potential sources varied between ontogenic stages (juveniles versus adults). For juvenile capelin, certain regions were clearly dominated by a single cluster. Cluster 1 was predominantly associated with the Estuary and northern GSL, while cluster 2 clearly dominated in the southern GSL and Newfoundland. This observation suggests that there might be distinct natal sources in these regions and some degree of population structure. The adult population, on the other hand, was rather homogenous in the Estuary, indicating that a portion of the capelin population might migrate across the GSL during its lifetime. For the southern GSL, the dominance of source 2 was still very strong in the adult population suggesting the sGSL functions as a more isolated region, with less demographic exchange with capelin from other areas.

Since adults were sampled between May and September, which includes capelin's spawning season, capture locations may correspond to the area of spawning, in which case spawning area fidelity should be important in the sGSL and more limited in the Estuary. Further work is, however, needed to validate this interpretation and to fully understand the factors influencing this apparent structure in GSL capelin population.

5. CONCLUSION

Spatial variability found in otolith larval fingerprints confirmed the potential of using otolith chemistry to investigate natal origins and habitat connectivity of capelin in the GSL.

The unsupervised RF clustering analysis appeared to be a parsimonious approach that provided a first overview of the connectivity between capelin pre- and post-recruit habitats, revealing some degree of population structure especially between sGSL and the Estuary.

Further investigations of the possible factors explaining the observed mismatch between larval fingerprint and core fingerprint of post-recruit individuals is required to confidently use the larval fingerprint as reference collection for natal origin assignment. As a result, the addition of larvae samples from 2024 to the dataset is not recommended at this time.

Cohort tracking over time will be essential given the demonstrated temporal variability in larval fingerprints. To maximize the chances of targeting the cohorts sampled in 2022 and 2023, the focus should be on collecting adult samples in 2024.

Future research should be directed towards the analysis of the elemental fingerprint along the entire otolith profile, from core to edge. This approach provides chemical information across the entire life history of the fish, offering a more complete understanding of capelin's lifetime movement patterns and habitat use in the GSL.

An important area for future research will be estimating the contribution of nursery habitats to the adult capelin population. This will allow to test the hypothesis that the southern GSL serves as a nursery for capelin populations in other regions of the GSL.

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We are grateful to the two reviewers for their careful reading and insightful suggestions which significantly enhanced this work.

TABLES

Table 1: Number of capelin captured per life stage, NAFO division and subdivision, for 2022 and 2023.

		Larvae		Juvenile/Adults
Division	**Subdivision**	**2022**	**2023**	**2022**
	4Ra	0	0	28
4R	4Rb	28	0	39
	4Rc	28	9	0
	4Rd	28	30	32
		84	**39**	**99**
	4Tk	0	0	10
4T	4Tj	0	0	10
	4Tgh	35	45	10
	4Tl	4	1	0
	4Tm	6	33	9
	4Tn	40	1	62
	4To	16	0	23
	4Tp	35	10	51
	4Tq	0	0	26
		136	**90**	**201**
4S	4Sx	0	0	8
	4Sy	0	0	18
	4Sz	0	0	23
		0	0	51
				100
TOTAL		**220**	**129**	**400**

Table 2: Maximum, mean and minimum accuracy (proportions) (95% CI) for assigning capelin larvae to their respective capture region, using random forest (RF), linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) and the best combination of elements.

Classification method	Combination of elements	Maximum accuracy	Mean accuracy	Minimum accuracy
RF	^{23}Na, ^{24}Mg, ^{31}P, ^{66}Zn, ^{88}Sr, ^{138}Ba	0.80 (0.05)	0.72 (0.07)	0.51 (0.06)
LDA	^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, ^{66}Zn	0.73 (0.06)	0.61 (0.08)	0.38 (0.05)
QDA	^{23}Na, ^{31}P, ^{39}K, ^{57}Fe, ^{66}Zn	0.75 (0.05)	0.57 (0.09)	0.37 (0.06)

Table 3: Classification accuracy (%) of capelin larvae to their capture region from RF based on the best combination of elements. Results are obtained from RF with 1000 iterations and 100 trees

Combination of elements	N	Capture region	Classification accuracy	Overal accuracy
^{23}Na, ^{24}Mg, ^{31}P, ^{66}Zn, ^{88}Sr, ^{138}Ba	50	St. Lawrence Estuary	62	80
	76	Southern Gulf	80	
	74	Western Newfoundland	92	

Table 4: Results of one-way a) MANOVA and b) ANOVA) examining spatial variation in elemental concentrations of capelin larvae captured in three regions of the GSL in 2022. Significant differences among regions (p-value <0.05) are indicated in bold.

a) MANOVA

Pillai Trace	df	F	p-value	
0.70	2	17.97	**<.0001**	***

b) ANOVA

	df	F	p-value	
Na	2	23.37	**<.0001**	**
Mg	2	6.28	**0.0022**	**
P	2	21.07	**<.0001**	***
Zn	2	83.59	**<.0001**	***
Sr	2	0.34	0.7137	ns
Ba	2	2.67	0.0721	ns

Table 5: Results of one-way a) MANOVA and b) ANOVA) examining temporal variation in elemental concentrations of capelin larvae captured in three regions of the GSL in 2022 and 2023. Significant differences between years (p-value <0.05) are indicated in bold.

a) MANOVA

Pillai Trace	df	F	p-value	
0.24	1	17.13	**<.0001**	***

b) ANOVA

	df	F	p-value	
Na	1	17.35	**<.0001**	**
Mg	1	1.28	0.2891	ns
P	1	27.68	**<.0001**	***
Zn	1	16.48	**<.0001**	***
Sr	1	3.33	0.644	ns
Ba	1	12.70	**<.0001**	***

Table 6: Results of one-way a) MANOVA and b) ANOVA) examining variation in elemental concentrations between the two identified clusters from unsupervised RF clustering analysis. Significant differences between clusters (p-value <0.05) are indicated in bold.

a) MANOVA

Pillai Trace	df	F	p-value	
0.59	1	92.73	**<.0001**	***

b) ANOVA

	df	F	p-value	
Na	1	33.37	**<.0001**	***
Mg	1	81.31	**<.0001**	***
P	1	322.96	**<.0001**	***
Zn	1	66.37	**<.0001**	***
Sr	1	110.19	**<.0001**	***
Ba	1	1.10	0.2976	

Table 7: Percentage contribution of the two natal sources identified from RF clustering analysis by capture region and life stage.

Life Stage	Sampling	Capture Region	Cluster 1 (%)	Cluster 2 (%)
Adult	*Research*	Estuary	44	56
		Northern GSL	67	33
		Southern GSL	0	100
		Newfoundland	46	54
Adult	*Wharf*	Estuary	48	52
		Northern GSL	75	25
		Southern GSL	61	39
		Newfoundland	29	71
Juvenile	*Research*	Estuary	69	31
		Northern GSL	88	12
		Southern GSL	24	76
		Newfoundland	37	63

FIGURES

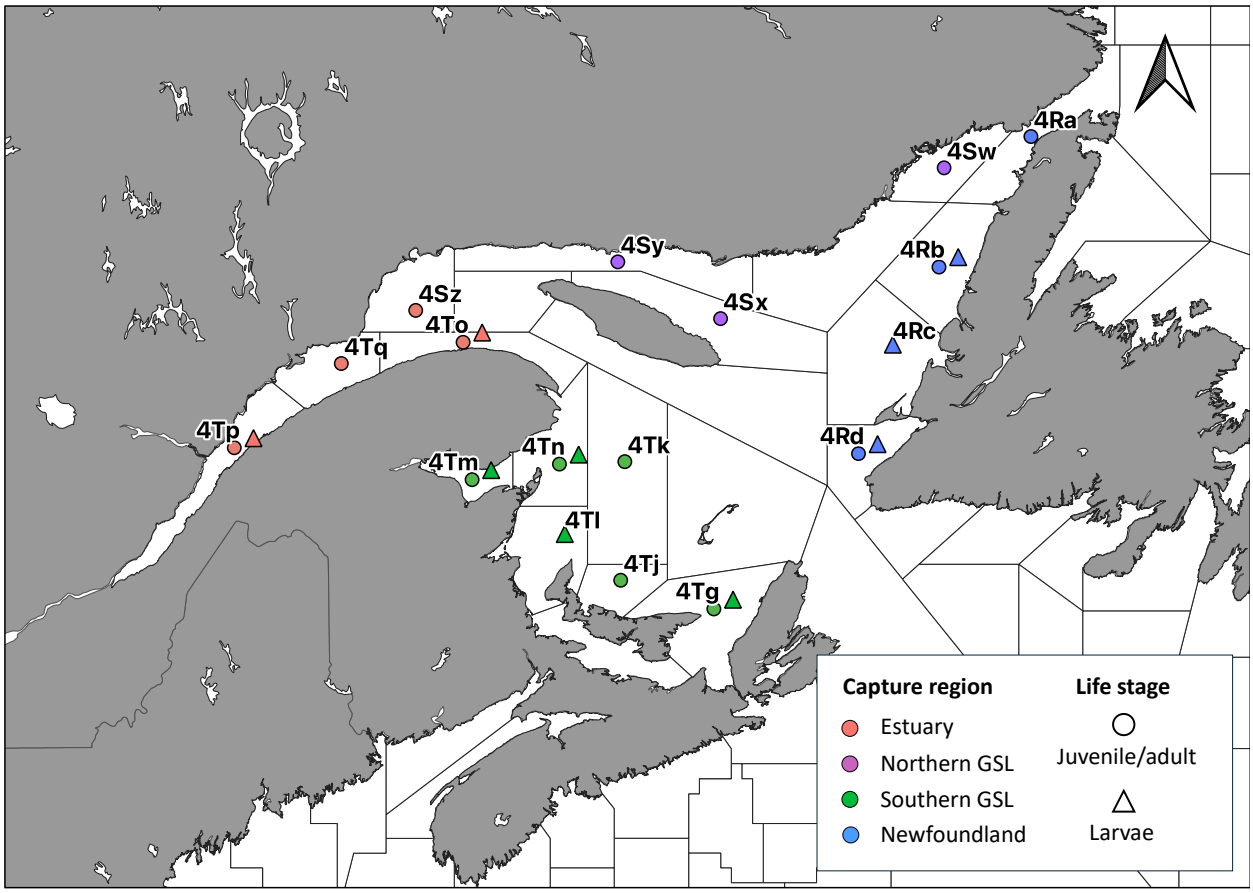

Figure 1: Sampling locations of capelin larvae and post-recruits (juveniles and adults) across NAFO subdivisions in 2022 and 2023.

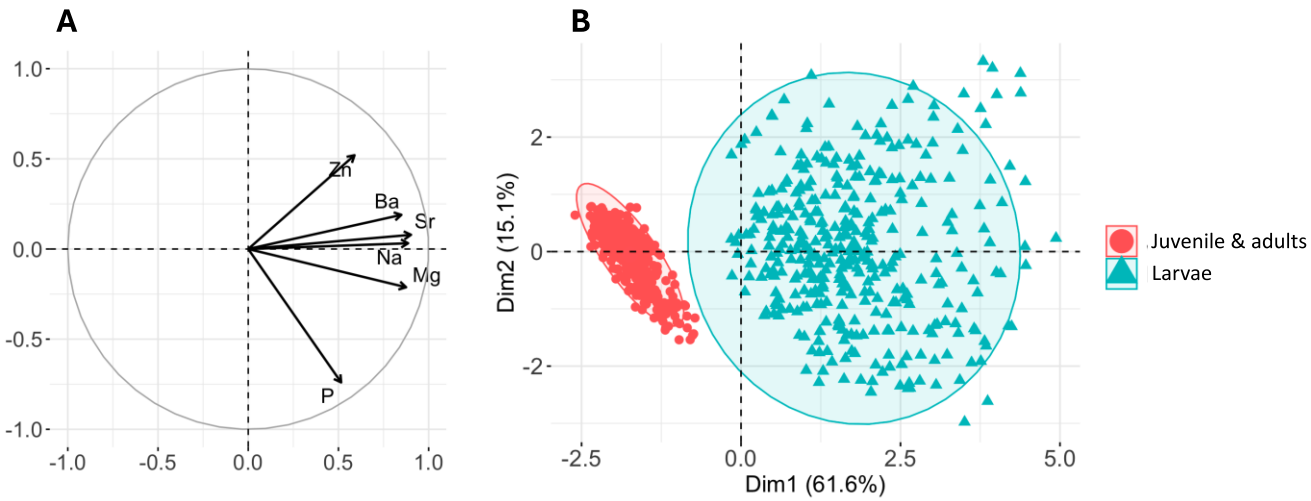

Figure 2: Principal component analysis. A) Biplot and B) correlation circle of elemental concentrations from otolith cores of juvenile and adult capelin (red) compared to larval otoliths (blue).

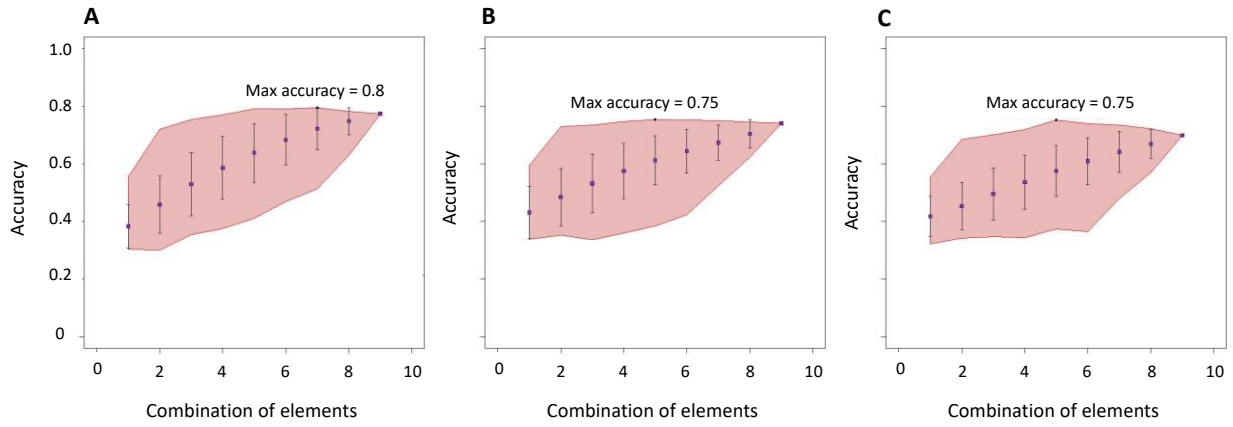

Figure 3: Accuracy for assigning capelin larvae to their respective region of capture based on model performance using three algorithms: A) Random Forest (RF), B) Linear Discriminant Analysis (LDA), and C) Quadratic Discriminant Analysis (QDA), each applied to different combinations of elements. The optimal element set for RF was ^{23}Na, ^{24}Mg, ^{31}P, ^{66}Zn, ^{88}Sr, and ^{138}Ba; for LDA it was ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, and ^{66}Zn; and for QDA it was ^{23}Na, ^{31}P, ^{39}K, ^{57}Fe, and ^{66}Zn. The shaded red area indicates the range between maximum and minimum accuracies, with points and bars representing the mean values and their 95% confidence intervals.

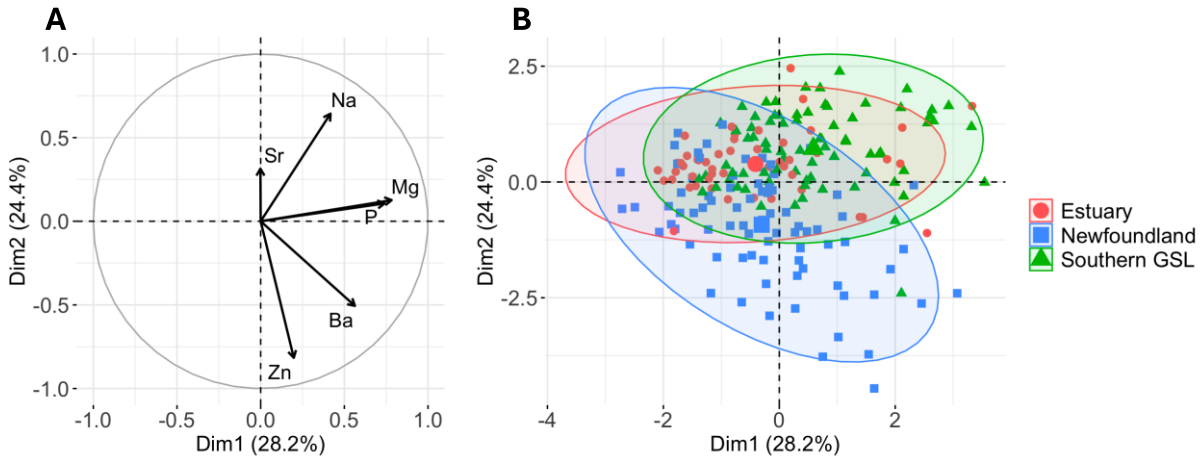

Figure 4: Principal component analysis A) Biplot and B) correlation circle of elemental concentrations measured in otoliths from capelin larvae captured in 2022 from the St. Lawrence Estuary (red), western Newfoundland (blue) and southern GSL (green).

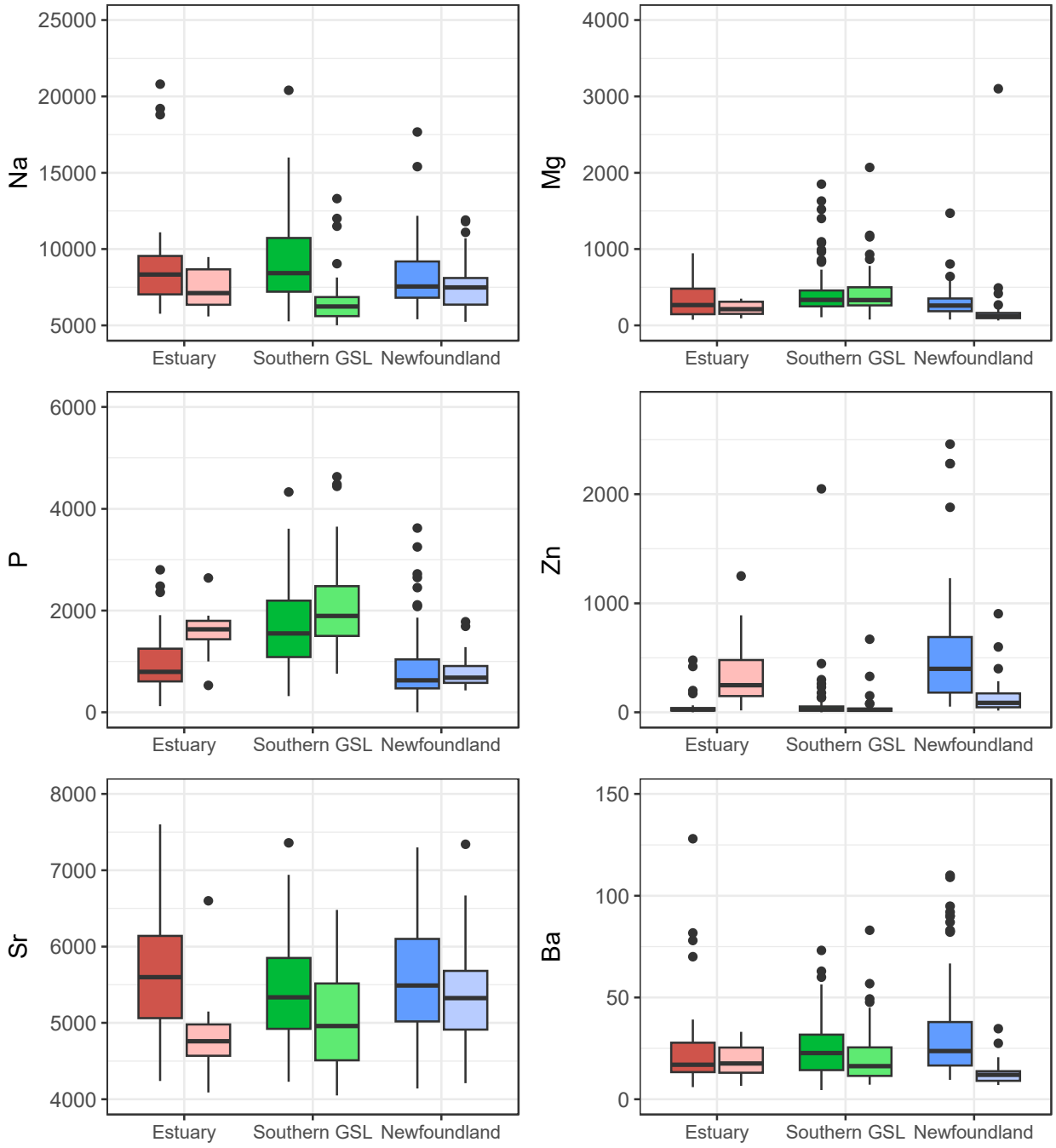

Figure 5: Boxplot of Na, Mg, P, Zn, Sr and Ba concentrations (ppm) measured in capelin larval otoliths, by capture region (colors) and sampling year (2022 = dark, 2023 = light).

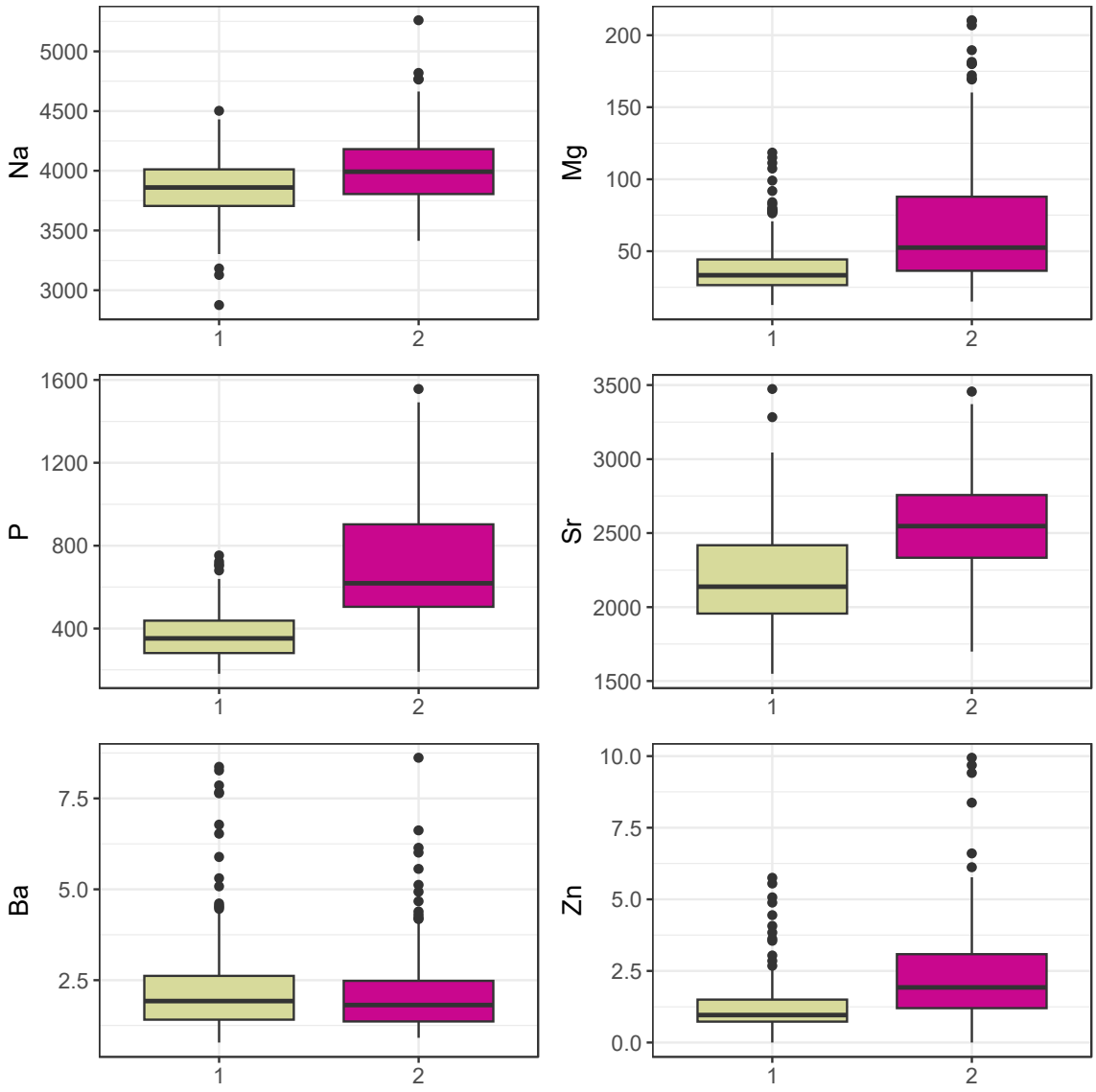

Figure 6: Boxplot of Na, Mg, P, Zn, Sr and Ba concentrations (ppm) for the two clusters identified by the unsupervised random forest clustering analysis.

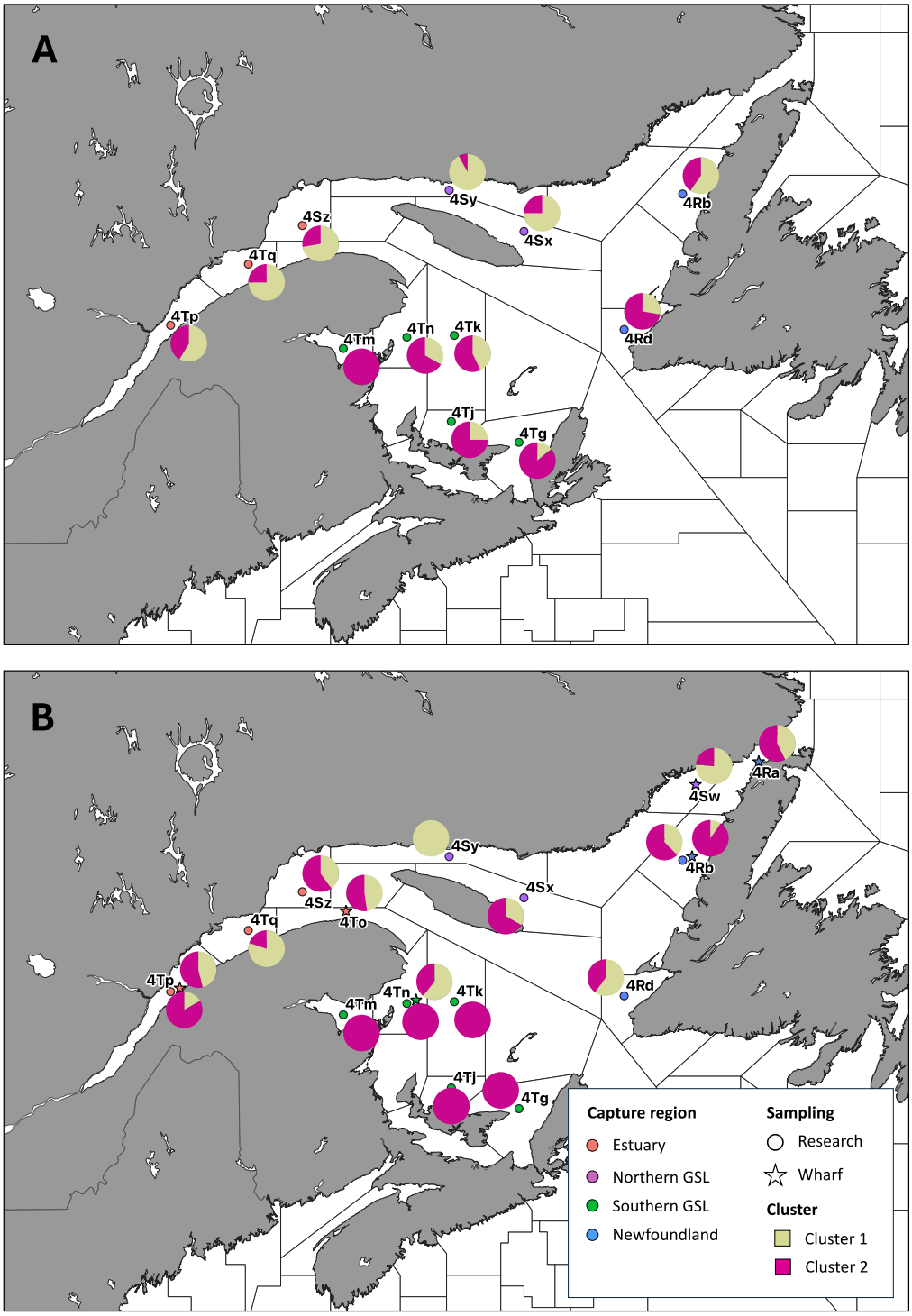

Figure 7: Pie charts representing the contribution per capture region (%) and sampling origin of the two putative natal sources identified by unsupervised RF clustering analysis for A) juvenile and B) adults.

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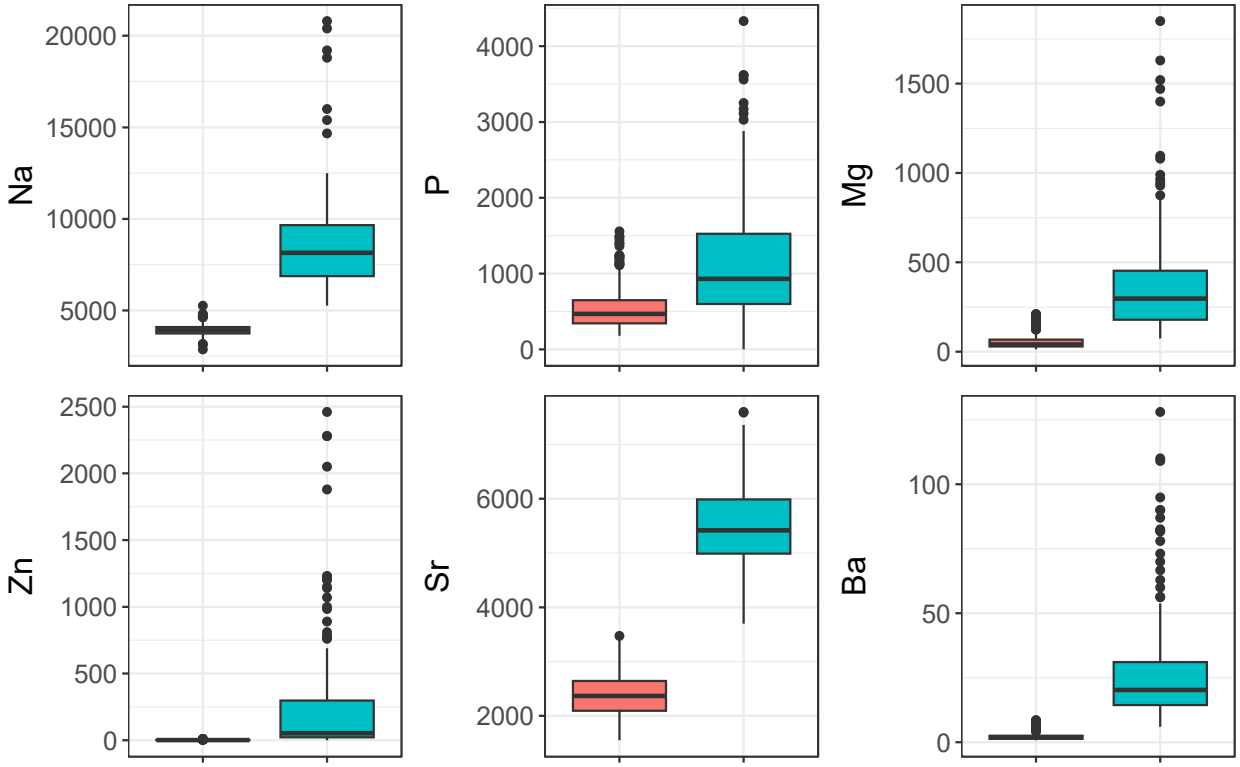
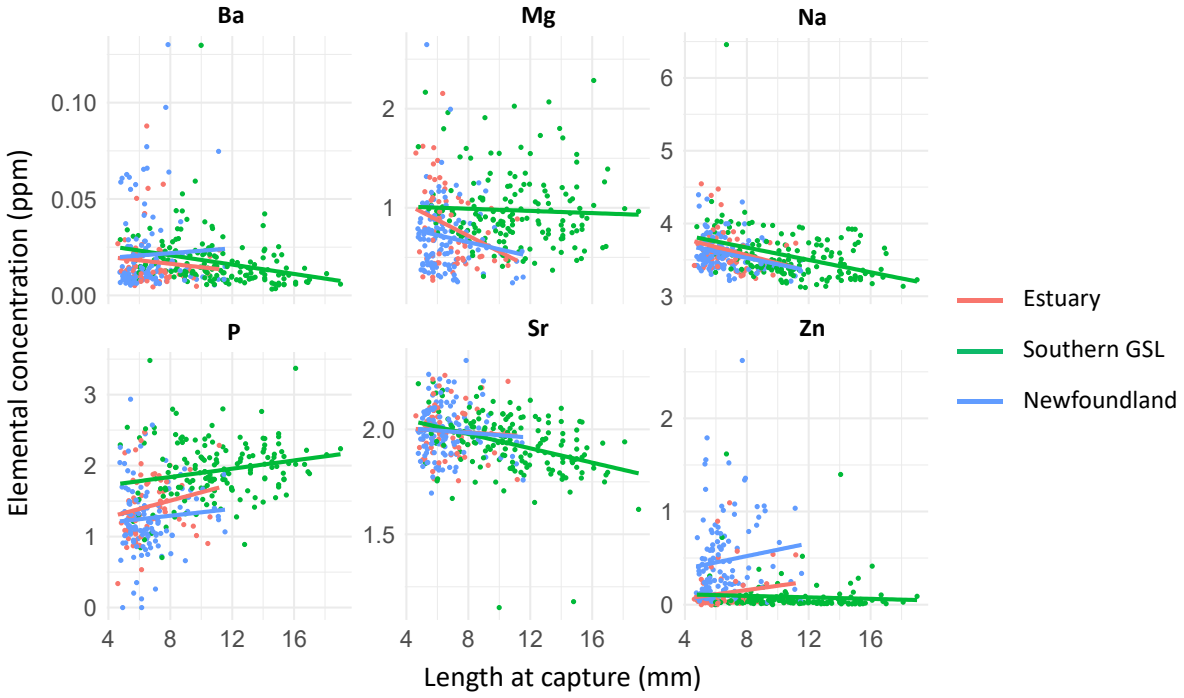
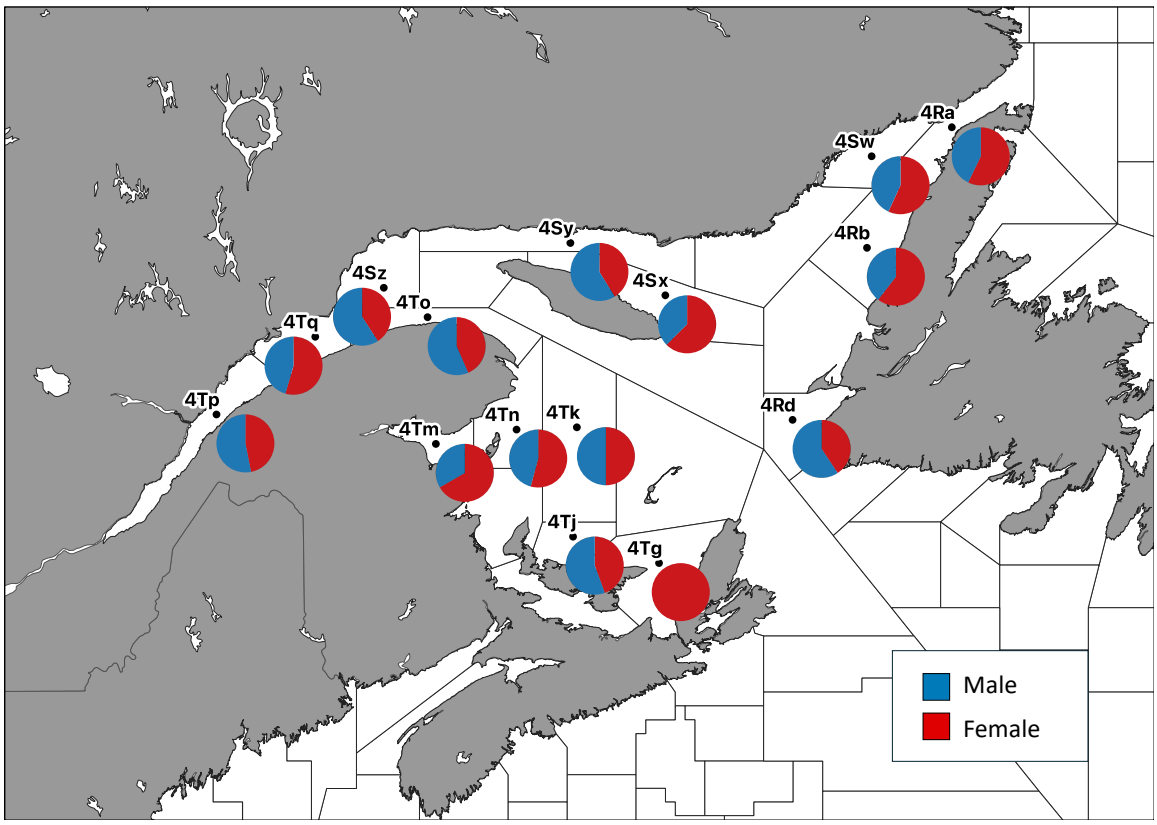
SUPPLEMENTARY MATERIAL



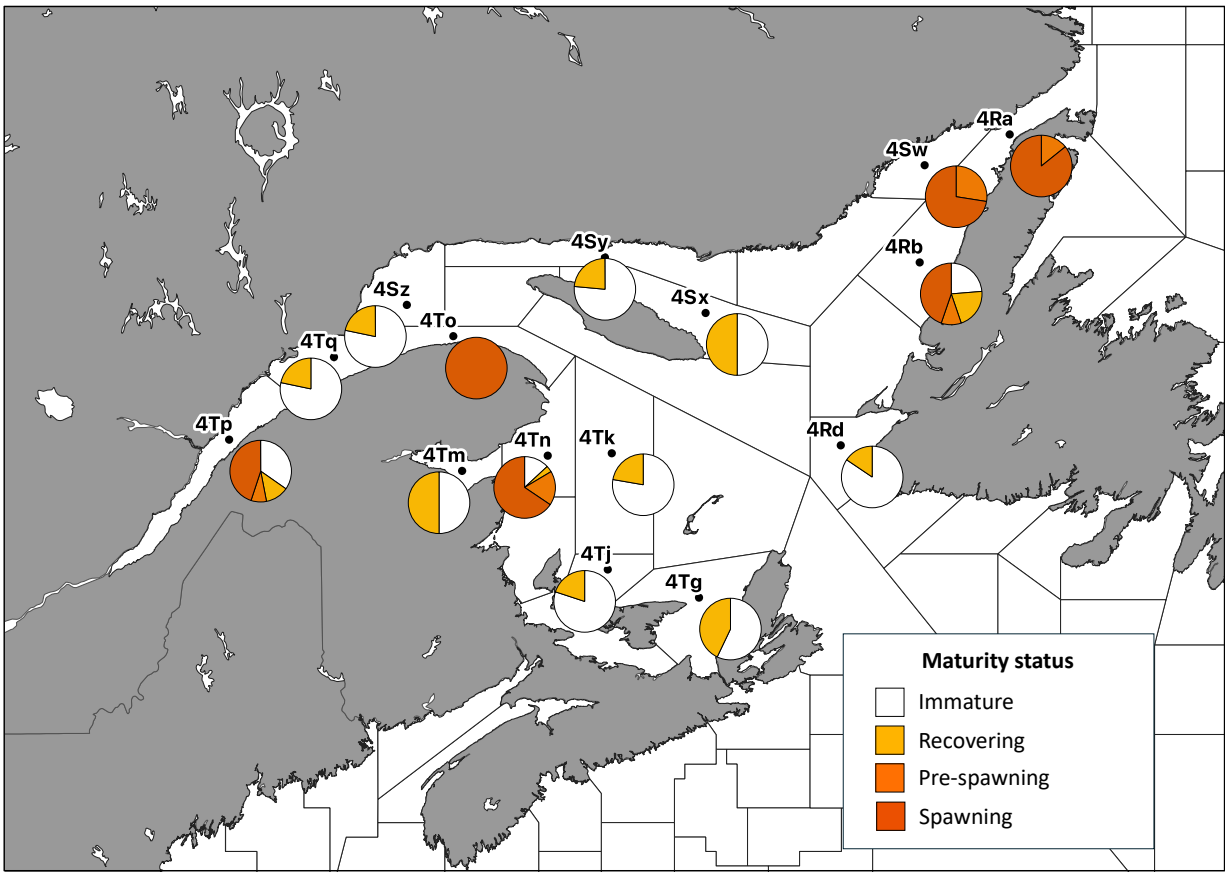
Supplementary Figure 1: Boxplots of elemental concentrations from otolith cores of juvenile and adult capelin (red) compared to larval otoliths (blue). ANOVA indicated that all comparisons were statistically significant ($p < 0.05$).



Supplementary Figure 2: Relationship between elemental concentrations and capelin larvae length at capture, grouped by region (Estuary, southern Gulf of St. Lawrence and western Newfoundland).



Supplementary Figure 3: Spatial distribution of capelin sex ratios.



Supplementary Figure 4: Spatial distribution of capelin maturity status.

Supplementary Table 1: Results from ANCOVA analysis of the relation between larval fish length and region on otolith elemental concentrations. Significant parameters (p-value <0.05) are indicated in bold.

Element		Estimate	Std Error	t value	p-value
Na	(Intercept)	3.929	0.056	70.40	**<.0001**
	length	-0.044	0.006	-6.87	**<.0001**
	Site (Southern GSL)	0.092	0.049	1.87	0.0628
	Site (Newfoundland)	-0.053	0.044	-1.20	0.2296
Mg	(Intercept)	0.917	0.074	12.45	**<.0001**
	length	-0.015	0.008	-1.80	0.0732
	Site (Southern GSL)	0.222	0.065	3.39	**0.0008**
	Site (Newfoundland)	-0.114	0.058	-1.99	**0.0478**
P	(Intercept)	1.229	0.089	13.76	**<.0001**
	length	0.030	0.010	3.01	**0.0028**
	Site (Southern GSL)	0.363	0.079	4.59	**<.0001**
	Site (Newfoundland)	-0.170	0.070	-2.43	0.0156
Zn	(Intercept)	0.107	0.060	1.76	0.0788
	length	0.003	0.007	0.48	0.6318
	Site (Southern GSL)	-0.060	0.054	-1.12	0.2652
	Site (Newfoundland)	0.344	0.047	7.28	**<.0001**
Sr	(Intercept)	2.090	0.026	81.16	**<.0001**
	length	-0.015	0.003	-4.98	**<.0001**
	Site (Southern GSL)	-0.003	0.023	-0.12	0.9022
	Site (Newfoundland)	-0.003	0.020	-0.14	0.8872
Ba	(Intercept)	0.024	0.003	7.31	**<.0001**
	length	-0.001	0.000	-2.51	**0.0125**
	Site (Southern GSL)	0.004	0.003	1.26	0.2097
	Site (Newfoundland)	0.003		1.31	0.1909