Connectivity and natal sources of Greenland halibut in the gulf of St. Lawrence inferred from otolith chemistry

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Abstract

Recent genomic study on Greenland halibut *Reinhardtius hippoglossoides* throughout the Northwest Atlantic revealed genetic differentiation between the Gulf of St. Lawrence and the remainder of the Northwest Atlantic. Knowledge of migration and thus connectivity among fish populations is key to understanding the stock dynamics of commercial species and establishing effective management strategies. In this study, we use otolith microchemistry to assess the population structure and define more clearly the potential connectivity of Greenland halibut among Saguenay Fjord, the Estuary, and the Gulf of St. Lawrence (EGSL) and outside this system. Otolith margins and cores were analyzed to determine the elemental fingerprints. The margin elemental fingerprints (Mg, Sr, and Ba) were used to infer spatial differentiation, and quadratic discriminant analysis resulted in an overall reclassification success of fish to their capture site of 59%. The core elemental fingerprints indicated three chemically distinct juvenile sources, suggesting high connectivity in the EGSL supporting the St. Lawrence Estuary as a main nursery. Our results also suggest that one of these three sources has a smaller contribution relative to the other sites.

Key words: *Reinhardtius hippoglossoides*, otolith microchemistry, elemental fingerprint, natal sources, stock contribution

1. Introduction

Coastal communities depend on the exploitation of fishery resources to support their economies. Sustainable fishing practices must be used by the fishing industry to limit the risk of resource overexploitation (Walters and Martell 2002), and extensive studies on the connectivity and stock structure of commercial species as well as their population dynamics are required. While knowledge of connectivity among populations is a prerequisite for studying population dynamics and managing fish stocks (Thorrold et al. 2007; Reiss et al. 2009), this information is generally limited by the difficulties of conducting such studies on marine organisms, especially for deepwater fishes.

Greenland halibut (*Reinhardtius hippoglossoides*) is a circumpolar flatfish of the northern hemisphere; it is a long-lived and slow-growing species with a high commercial value, and it supports several fisheries throughout the Arctic and North Atlantic oceans as well as the Estuary and Gulf of Saint Lawrence (EGSL), Canada (Bowering and Brodie 1995; Bowering and Nedreaas 2000; Treble et al. 2008; Delaney et al. 2012). Since the mid-1990s, Greenland halibut has supported one of the main groundfish fisheries in the EGSL with annual landings of ~3200 t (DFO 2019). Despite its high socio-economic importance, key questions remain about stock structure and connectivity of Greenland halibut in the Northwest Atlantic. Both the high larval dispersal and migratory capacity of the species make it difficult to define stock limits and to assess connectivity and population dynamics (Jørgensen 1997; Boje 2002; Stenberg et al. 2016; Barkley et al. 2018). Moreover, nursery areas are not well known, and only four have been confirmed in the North Atlantic and the St. Lawrence system. In the North Atlantic two nurseries have been identified, the first in Disko Bay (Greenland) (Stenberg et al. 2016) and the second around the Svalbard archipelago (Albert and Vollen 2015). In the St. Lawrence system, there are two known nurseries described by Ait Youcef et al. (2013), the main nursery being located in the estuary and the secondary in the northeast of Anticosti Island.

Several studies have aimed at documenting the genetic structure of Greenland halibut in the Northwest Atlantic, and most of them failed to demonstrate any clear structure. Based on microsatellite markers, Pomilla et al. (2008) identified two genetically distinguishable stocks with geographic intermingling and possibly admixing, suggesting that only a single unit for management through the Northwest Atlantic would be reasonable until more information is available. Based on the same type of molecular marker, Roy et al. (2014) concluded that the species comprises a single panmictic

population in the Northwest Atlantic. A relative distinctiveness of the EGSL population when comparing with the rest of the Atlantic was detected by Vis et al. (1997) based on mitochondrial haplotypes. Genotyping by sequencing has confirmed the significant distinctiveness of the EGSL while revealing a fluctuating contribution from the NL area to the EGSL stock through the years (Carrier et al. 2020). Furthermore, a very recent study based on whole-genome sequencing revealed that except from EGSL, Greenland halibut appear to be panmictic throughout the Northwest Atlantic and that environmental association is mainly driving the distinctiveness of the EGSL (Ferchaud et al. 2022).

In the absence of clear stock or population structure in 1978, the Northwest Atlantic Fisheries Organization (NAFO) and International Council for the Exploration of the Sea divided the Northwest Atlantic into four separate management units on the basis of limited empirical evidence: (1) Baffin Island—West Greenland, (2) West Greenland inshore, (3) Labrador—eastern Newfoundland, and (4) the Gulf of St. Lawrence (Bowering and Chumakov 1989; Bowering and Brodie 1995). The population in the EGSL has long been considered to be isolated from the rest of the Northwest Atlantic based on prevalence of blood parasites (Arthur and Albert 1993; DFO 2006).

Conventional tagging methods are logistically difficult to use to characterize fish movements throughout the life cycle, especially in deep-sea and long-lived species. An alternative method for assessing connectivity in fish populations relies on elemental concentrations in otoliths (Campana 1999; Elsdon et al. 2008; Reis-Santos et al. 2013). All teleost fish possess metabolically inert otoliths that grow by the accretion of successive annuli throughout the life on individual. During otolith growth, some minor and trace elements (e.g., lithium, magnesium, strontium, and barium) are incorporated on otolith's structure. The predominant source of elements and isotopes is the water surrounding the fish, and their incorporation rate into otoliths varies according to various biotic and abiotic factors (Kerr and Campana 2014). Elemental concentration is currently used as a polyvalent and powerful tool for different applications, such as reconstructing movement or migration patterns (Hamer et al. 2006; Kerr and Secor 2012), stock identification (Campana et al. 2000; Tanner et al. 2016), estimating connectivity (Fairclough et al. 2011), and determining natal sources and evaluating their contribution to adult populations (Thorrold et al. 2001; Gillanders 2002; Tanner et al. 2012; Reis-Santos et al. 2013; Lazartigues et al. 2016; Wright et al. 2018).

In this study, we use otolith microchemistry to assess the population structure and potential connectivity of Greenland halibut in the EGSL. Elemental fingerprints in both the margins and cores of otoliths were analyzed to test the hypothesis that Greenland halibut sampled in different sites have different origins inside the EGSL. Specifically, elemental fingerprint margins were used to assess the presence of spatial chemical variations among capture sites in the EGSL and the Saguenay Fjord. Furthermore, elemental fingerprint cores were analyzed using an unsupervised random forest procedure to identify potential origin sources, such as a nursery, from sampled Greenland halibut. The presence of differ-

ential spatial elemental fingerprints is used to infer potential source locations of Greenland halibut juveniles through the comparison between otoliths margins and core. Thus, connectivity among Saguenay Fjord, the Estuary, and Gulf of Saint-Lawrence and outside this system could be evaluated. Increasing knowledge regarding the structure of Greenland halibut stocks would provide useful elements on the biology of this species and help the development of sustainable management strategies for this resource.

2. Materials & methods

2.1. Sample collection

All Greenland halibut were collected in the EGSL during summer 2016 (June–August) during bottom-trawl surveys conducted by Fisheries and Oceans, Canada (DFO 2018), from seven sampling sites: Saguenay (SAG), Estuary (EST), Sept-Îles (SIL), Gaspésie (GAS), South Anticosti (S.ANT), North Anticosti (N.ANT), and Esquiman (ESQ) (Fig. 1). The fishing gear used was an Alfredo III bottom otter trawl (140 mm mesh, 30 mm mesh liner in the cod end). A total of 175 adult (47.66 ± 5.78 cm, mean ± SD) Greenland halibut were sampled for otoliths; thus, for each site, 25 otoliths were analyzed for elemental fingerprint. Maturity of individuals was verified by gonads inspection directly on board during the survey, and only confirmed adults were used for the present study.

2.2. Otolith extraction and sample preparation

Otoliths were extracted in a metal-free environment laboratory. To avoid any contamination by Teflon, polyethylene, or polypropylene, tools were cleaned with nitric acid (10%), and Nitech gloves were changed every two hours. After otolith extraction, blood and organic tissues were removed, and otoliths were triple cleaned using Super Q water, dried with Kimwipes, and stored in acid-washed polypropylene vials until analysis. Otoliths were set in epoxy resin (Miapoxy 95, Miapoxy 100; Avon, OH, USA) for laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis. Each otolith was cut in a transverse plane using a diamond saw (Isomet low speed, Buehler; Lake Bluff, IL, USA) to obtain a slice containing the core and growth annuli on the cross-section. Slices were sanded on both sides with different grades of aluminum oxide lapping film (3M Wetordry™ Polishing Paper #400, #1200, 5M, and 1M). Otolith slices were fixed in random order of fish on a petrographic slide with Crystalbond thermoplastic glue (AREMCO Inc., Valley Cottage, NY, USA) to avoid artificial bias by instrument drift. Each petrographic slide was sonicated in ultrapure water for 5 min, triple rinsed in Super Q water, and then dried in a class 100 laminar flow hood for 24 h (Lazartigues et al. 2016).

2.3. LA-ICP-MS method

The LA-ICP-MS analysis was performed using an Agilent 7900 ICP-MS (Agilent, Santa Clara, CA, USA) coupled to a Resolution (ASI) 193 nm Excimer laser system-equipped cell with S-155 ablation (Australian Scientific instruments, Fyshwick, Australia). Greenland halibut otoliths were ablated in two places—at the margin and at the core location—to obtain the

Fig. 1. Locations of sampling sites and sectors (groups of sites sharing the same symbol) defined by otolith margin elemental fingerprints for Greenland halibut, *Reinhardtius hippoglossoides*, in the Gulf of St. Lawrence. The 200 m isobaths are represented by the solid line. Black dashed line delimits the "Maritime Estuary" section of the EGSL, which includes EST site, and the "Northwest Gulf" section, which includes SIL and GAS sites. Red dashed line represents the St. Lawrence estuary defined by Ait Youcef et al. (2015), where juvenile Greenland halibut settle and stay sedentary for at least their first two years of life (map projection: WGS84).
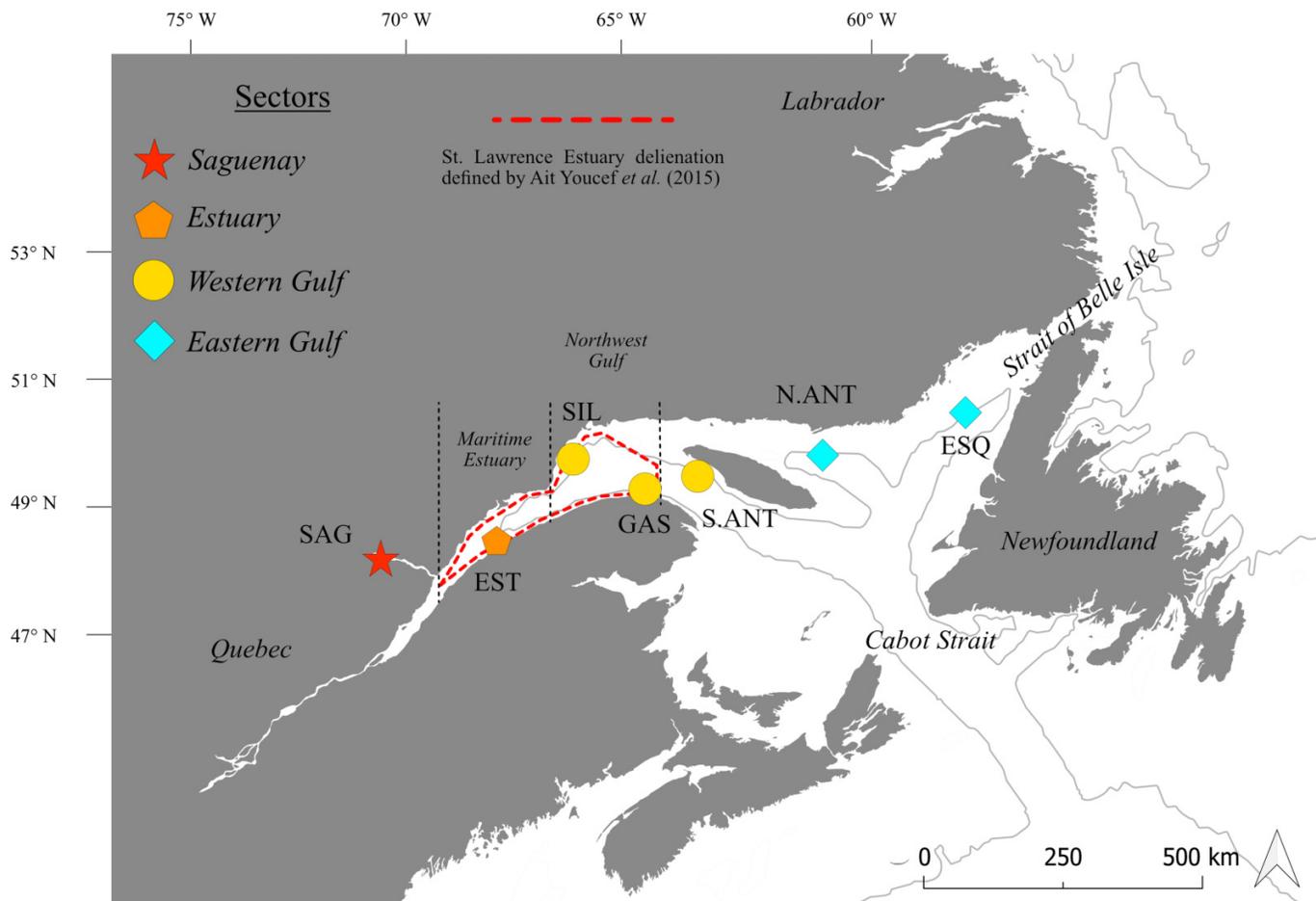
elemental fingerprint from the capture site and from the juvenile source. The laser ablation of otoliths was conducted using the following parameters: 5 J·cm^2 fluency, 20 µm diameter, 15 Hz frequency, 5 µm·s speed, and 0.233 s dwell time at LabMaTer (Université du Québec à Chicoutimi, Québec, Canada). Generally, time ablation was 94 s (∼470 µm) for margin and 140 s (∼700 µm) for core sections. The target elements were ^{7}Li, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{34}S, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{64}Zn, ^{65}Cu, ^{66}Zn, ^{69}Ga, ^{85}Rb, ^{86}Sr, ^{87}Sr, ^{88}Sr, ^{136}Ba, ^{137}Ba, ^{138}Ba, and ^{208}Pb. Calcium content was assumed to be 38.02% in each otolith (Campana 1999) and was used as the internal standard for calibration. Standard materials were ablated every hour (every ∼12 samples) to correct for temporal drift in the sensitivity of the mass spectrometer. Some elements were below the ICP-MS's limit of detection (LOD), i.e., ^{7}Li, ^{55}Mn, ^{64}Zn, ^{69}Ga, ^{85}Rb, ^{136}Ba, and ^{208}Pb; these data were not analyzed. The LOD was determined as three times the standard deviation of the gas blank (SD$_{blank}$) divided by the sensitivity of the signal (Lazartigues et al. 2014). Aluminum was used to verify the absence of contamination during manipulation

of otoliths. Calibration was performed using NIST SRM 610 reference material (Chen et al. 2011) with reference working values (±uncertainties) obtained from the Geological and Environmental Reference Materials database (http://georem.m pch-mainz.gwdg.de/). The US Geological Survey Denver provided the MACS-3 working values (±uncertainties) for quality control (Ca, 38.02%; http://crustal.usgs.gov/geochemical _reference_standards/microanalytical_RM.html). ICP-MS signal treatments were performed using the Iolite function (Paton et al. 2011) of the Igor Pro software (Wavemetrics Inc., Portland, OR, USA). Integration was performed using the *trace elements IS* procedure in Iolite on a 20 s stable signal (100 µm).

2.4. Statistical sector definition

Otolith margin data were used to assess the elemental variation among sampling sites. Each element was analyzed by univariate analysis of variance (ANOVA) performed using R software (R Core Team 2020, version 4.0.3), with the

Table 1. Summary of otolith margin elemental fingerprint concentrations by sector.

Sector	n	Mg	Sr	Ba
Saguenay	23	*20.24 ± 6.05*	*1933.13 ± 199.91*	*0.99 ± 0.26*
Estuary	25	22.17 ± 5.46	2376.16 ± 437.3	1.1 ± 0.36
Western Gulf	71	**30.31 ± 10.16**	**2687.18 ± 505.51**	1.48 ± 0.58
Eastern Gulf	49	23.22 ± 6.92	2682.43 ± 585.5	**1.71 ± 0.62**

Note: Elemental fingerprint mean concentrations and standard deviations are indicated ($\mu g \cdot g^{-1}$). The highest values are in bold and the lowest in italic for each element.

Table 2. Results of univariate ANOVA comparing the elemental concentrations of otolith margins between sample sites and sectors.

	df	MS	F	P
Mg				
Sites	6	0.702	8.449	<0.0001
Residuals	161	0.083		
Sectors	3	1.272	15.14	<0.0001
Residuals	164	0.084		
Sr				
Sites	6	0.373	11.78	<0.0001
Residuals	161	0.032		
Sectors	3	0.671	20.64	<0.0001
Residuals	164	0.032		
Ba				
Sites	6	1.001	9.473	<0.0001
Residuals	161	0.106		
Sectors	3	1.927	18.34	<0.0001
Residuals	164	0.105		

seven sample sites as tested factors. ANOVA was run following the screening of residual data to validate normality and homoscedasticity using the expected normal probability plot. Data were ln-transformed when necessary to achieve normality (Queen et al. 2002). Tukey–Kramer honestly significant difference (HSD) multiple comparison tests ($p < 0.05$) were used to determine differences between means (*multcomp* R package 1.4–17; Hothorn et al. 2008). Multivariate ANOVA (MANOVA, Pillai's trace) was used to test the overall among-site differences in elemental fingerprint composition using elements showing spatial variability. The isotopic masses used for each element were set to the most abundant natural isotope and assumed to be representative of the total concentration of that element in the otolith. For simplification, no mass numbers will be presented in the text (e.g., $Mg = {}^{24}Mg$). When adjacent sites showed no significant difference in their elemental fingerprints, they were grouped to form a "sector". The ability of the otolith's margin elemental fingerprint to discriminate the sector was assessed using quadratic discriminant analysis (QDA). The QDA (MASS R package 7.3–54; Venables and Ripley 2002) was performed on the sectors (Table 1). To avoid over-representing a specific sector due to the unequal representation of sampling sites within each sector, the prior probabilities of data were specified equally among each group.

2.5. Statistical source definition

Clustering analysis, which does not require any reference baseline, was performed to explore the number of putative juvenile sources and the degree of connectivity between locations. In this study, the term "juvenile source" refers to potential nursery area. The clustering method developed by Shi and Horvath (2006) was applied, using a supervised random forest procedure in an unsupervised way (*randomForest* R package 4.6–14, Liaw and Wiener 2002; *UnsupRF* R package 1.0, Ngufor 2021). The random forest unsupervised test was applied to otolith core data, and the class variable was ignored. Instead, an artificial dataset was produced by random sampling in the product of empirical marginal distributions of the different elements (Shi and Horvath 2006). This method produced a similarity matrix, defined by the frequency at which two individuals end up in the same terminal node of the trees (Breiman 2001). In the second step, the similarity matrix was transformed into a dissimilarity matrix that was used to partition the medoid clustering algorithm (Kaufman and Rousseeuw 2009). The appropriate number of clusters was determined by the Dunn index (Halkidi et al. 2001) using the *clValid* R package 0.7 (Brock et al. 2008).

3. Results
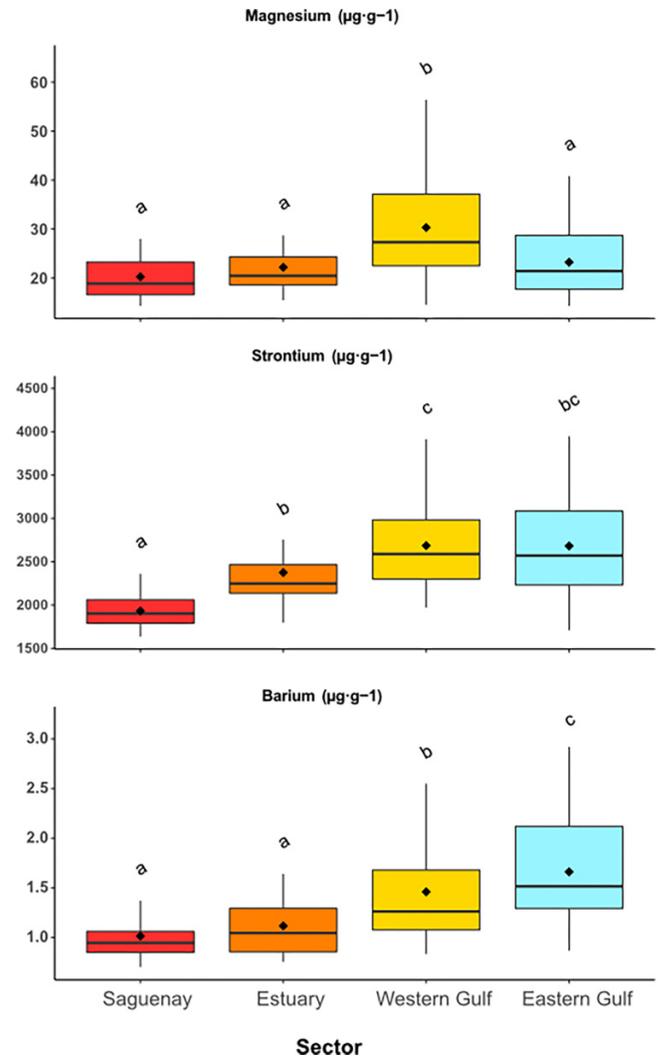
3.1. Spatial variation in elemental fingerprint

The elemental composition of otoliths varied among sites (Pillai's trace value: 0.728, F = 8.6, $p < 0.0001$). Stepwise forward QDA showed that Mg, Sr, and Ba contributed the most to site differences; therefore, these were used in further analyses. The elemental fingerprints of otolith margins, based on these three elements, showed significant variations among sites (ANOVA and HSD Tukey–Kramer, $p < 0.05$; Table 2, Fig. 2). Several adjacent sites, such as SIL–GAS–S.ANT and N.ANT–ESQ, showed no difference in elemental concentration (Fig. 2), so we were able to group them into two sectors called western Gulf (WG) and eastern Gulf (EG), respectively; the SAG and EST sites were distinct. Spatial variation in elemental fingerprints was investigated at the sector scale ($n = 4$). Each of the three elements showed significant differences among sectors (ANOVA and HSD Tukey–Kramer, $p < 0.05$; Table 2, Fig. 3). Mg was highest in WG ($30.31 \pm 10.16\ \mu g \cdot g^{-1}$) and lowest in SAG ($20.24 \pm 6.05\ \mu g \cdot g^{-1}$); Sr was highest in WG ($2687.18 \pm 505.51\ \mu g \cdot g^{-1}$) and lowest in SAG ($1933.13 \pm 199.91\ \mu g \cdot g^{-1}$); and Ba was highest in EG ($1.71 \pm 0.62\ \mu g \cdot g^{-1}$) and lowest in SAG ($0.99 \pm 0.26\ \mu g \cdot g^{-1}$).

Fig. 2. Box and whisker plots of the mean concentrations ($\mu g \cdot g^{-1}$) of three elements (Mg, Sr, and Ba) in otolith margins by sample site. Sites were grouped into sectors according to the similarity of their elemental otolith fingerprints: Saguenay (red), Estuary (orange), Western Gulf (yellow), and Eastern Gulf (turquoise). For each element, mean concentrations (mean $\pm$ SD) having the same letter are not significantly different (ANOVA, Tukey–Kramer HSD performed on the natural-log transformed variable); diamond: mean value; horizontal line: median value.

Fig. 3. Box and whisker plots of the mean concentrations ($\mu g \cdot g^{-1}$) of three elements in otolith margins (Mg, Sr, and Ba) by sector. For each element, mean concentrations (mean $\pm$ SD) having a common letter are not significantly different (ANOVA, Tukey–Kramer HSD performed on the natural-log transformed variable); diamond: mean value; horizontal line: median value.
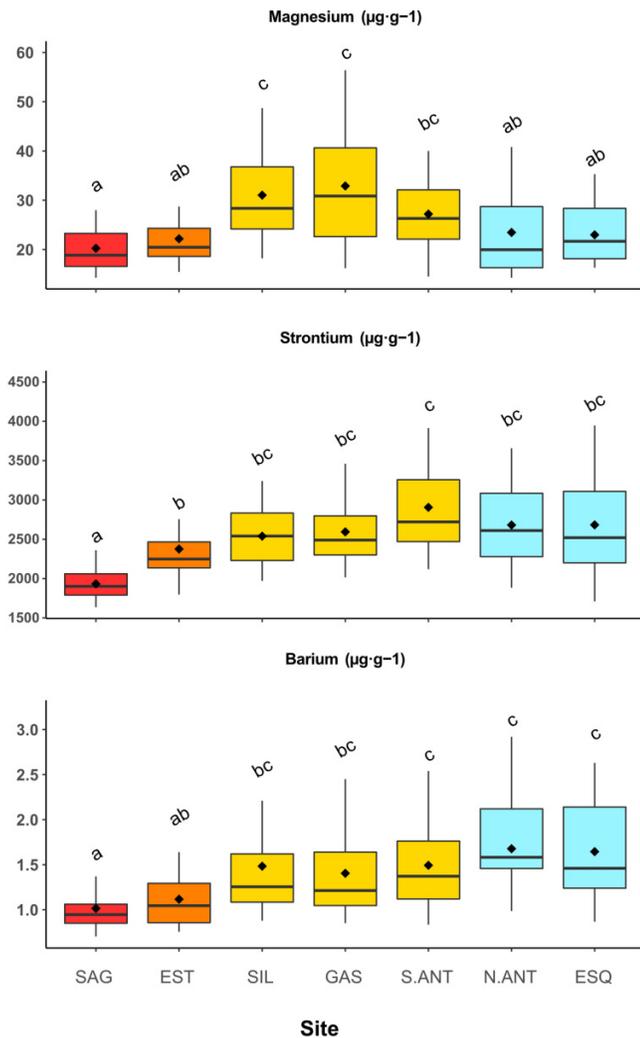
SAG showed the lowest elemental concentrations for all three elements (Table 1; Fig. A1). Mg was stable among sectors (20–23 $\mu g \cdot g^{-1}$) and did not differ among sites, except for WG. The concentrations of Sr and Ba increased along an upstream–downstream gradient, with low concentrations in the western sectors and high concentrations in the eastern sectors (Fig. 3). Sr concentrations allowed us to clearly differentiate SAG from other EGSL sectors, and Ba revealed the east–west gradient concentration (Figs. 3 and A1). An MANOVA of the combined Mg, Sr, and Ba values confirmed the degree of discrimination between the four sectors (Pillai's trace value: 0.66, F = 15.409, $p < 0.0001$).

Discriminant analyses were consistently successful in reclassifying fish to their sector of origin on the basis of their elemental fingerprints. Quadratic discriminant analysis achieved an overall classification success of ~59%, and most classification errors were observed among adjacent sectors (Table 3). The success in classification by sector ranged from 53% (WG) to 78% (SAG) (Table 3); 60% of Greenland halibut captured in EST were correctly reclassified as were 57% of Greenland halibut captured in EG. Therefore, SAG had the most distinct elemental fingerprint compared to other EGSL sectors.

3.2. Sources of Greenland halibut
The random forest clustering approach applied to Mg, Sr, and Ba identified three potential sources (A, B, and C) having

Table 3. Success of matching sampled Greenland halibut to their predicted elemental fingerprint sector using quadratic discriminant analysis.

		QDA % success reassignment of Greenland halibut			
	n	Saguenay	Estuary	Western Gulf	Eastern Gulf
Saguenay	23	**78.3**	13	0	8.7
Estuary	25	16	**60**	16	8
Western Gulf	71	2.8	26.8	**53.5**	16.9
Eastern Gulf	49	12.2	18.4	12.2	**57.1**

Note: Reclassification success rate for each sector is indicated in bold characters.

distinct elemental fingerprints. Source A was a minor contributor for SAG sector, comparable to the contribution of Source C for sectors WG and EG and slightly higher than Source C in EST sector (Fig. 4), and was characterized by the highest Sr ($1761.19 \pm 456.19\,\mu g \cdot g^{-1}$) and Ba ($4.04 \pm 2.38\,\mu g \cdot g^{-1}$) levels (Table 4; Fig. A2). Source B was the main contributor to SAG, EST, and EG, with values of 48%, 64%, and 55%, respectively (Fig. 4), and it had the lowest Mg ($23.03 \pm 9.14\,\mu g \cdot g^{-1}$), Sr ($1335.09 \pm 137\,\mu g \cdot g^{-1}$), and Ba ($1.31 \pm 0.71\,\mu g \cdot g^{-1}$) concentrations (Table 4; Fig. A2). Source C was the main contributor to WG (51%) and a minor contributor to EST (16%); it had the highest Mg concentration ($35.03 \pm 13.64\,\mu g \cdot g^{-1}$) (Table 4; Fig. A2).

4. Discussion

The three identified natal sources contributed to Greenland halibut population throughout the sampled sectors, with a major contribution of juveniles from source B (SAG, EST, and EG) and source C (WG). That suggests a pronounced connectivity level of Greenland halibut throughout the EGSL, including the Saguenay Fjord, preventing an isolated stock or population in that area. The elemental composition characterizing sources B and C were comparable to the elemental fingerprints from otolith margins of samples from the Estuary and Western Gulf sectors, suggesting that the estuarine area is the main nursery within the EGSL. Among the three identified sources of elemental fingerprint, none seemed to correspond to the area north of Anticosti Island (Eastern Gulf sector), the site of a long-presumed nursery area (DFO 2019). The origin of the third source, represented by cluster A, remains unknown, as no correspondence was associated with elemental fingerprints from otolith margins. However, the high concentration of Ba observed in source A could indicate a juvenile source external to the EGSL, likely located along the Labrador coast. This result agrees with a recent study that demonstrated the existence of an external source contributing to the EGSL population based on genomics (Carrier et al. 2020).

4.1. Spatial variability of elemental fingerprints in EGSL

Our model based on the three most discriminating elements—Mg, Sr, and Ba—revealed spatial variability in elemental fingerprints from otolith margins in the EGSL and Saguenay regions. Variation in elemental fingerprints

among sites allowed us to identify four distinct sectors: three inside the EGSL (EST, WG, and EG) and another representing the Saguenay Fjord (SAG). This is the first evidence of spatial variation in Greenland halibut elemental fingerprints within the EGSL and one of its tributaries. Patterns of elemental fingerprint variations differed depending on the element. For example, Mg was higher in the WG, whereas Sr and especially Ba were characterized by an upstream–downstream gradient. However, Sr and Ba gradients revealed different discrimination patterns: SAG and EST were statistically separated by Sr, whereas Ba allowed the discrimination of WG and EG. For elemental fingerprint margins, we assumed that any temporal and ontogenetic variations would be negligible since we sampled adult Greenland halibut (>40 cm) during one single summer period (Table 5). In 2016 in the EGSL, 50% of females were mature at ~42 cm, whereas size at 50% maturity for males was ~32 cm (Gauthier et al. 2020). We thus assumed that differences observed in the concentration of elemental fingerprint margins reflected spatial variability. These three elements are routinely used in otolith microchemistry studies, but causes of their variations can be multiple and complex and are often species specific (Hüssy et al. 2020).

Strontium is the most frequently used elemental marker for tracking fish movement because it allows discrimination of salinity gradients (Limburg 1995; Secor et al. 1995), with increasing concentrations as salinity increases (Walther and Limburg 2012). This provides a useful tool to track fish movements between fresh and brackish waters (Kraus and Secor 2004) or to detect anadromy (Coutant and Chen 1993). In the present study, otolith margin concentrations of Sr and Ba increased progressively from the upstream sector (Saguenay) to the downstream sector (Eastern Gulf). Greenland halibut caught in SAG and EST probably grew in waters with an estuarine influence, characterized by lower concentrations of Sr and Ba. This could explain the low Sr and Ba levels found in elemental fingerprints associated with these regions (Bath et al. 2000; Miller 2011; Reis-Santos et al. 2013). Like Sr, Ba variations are often related to local geochemistry or reflects known coastal/offshore movements across salinity gradients.

Individuals caught in EG were distributed deeper on the bottom, close to the Strait of Belle Isle, where waters from the Labrador current enter in the EGSL. In another study on Greenland halibut caught in 2016 in the Northern Atlantic, the elemental fingerprint for the Labrador coast was similar for the same three elements, with relatively high Ba

Fig. 4. Pie charts representing the relative contributions of the three juvenile sources (A, B, and C), identified through random forest clustering, to sectors of the EGSL and Saguenay (map projection: WGS84).

Table 4. Summary of otolith core elemental fingerprint concentrations by source.

Source	n	Mg	Sr	Ba
A	37	27.11 ± 10.06	**1761.19 ± 456.19**	**4.04 ± 2.38**
B	72	*23.03 ± 9.14*	*1335.09 ± 137.00*	*1.31 ± 0.71*
C	52	**35.03 ± 13.64**	1506.93 ± 126.45	1.76 ± 0.58

Note: Mean concentrations and standard deviations of elemental fingerprints are indicated ($\mu g \cdot g^{-1}$). The highest values are in bold and the lowest in italic for each element.

$(2.47 \pm 0.81 \, \mu g \cdot g^{-1})$ and Sr $(2908.63 \pm 447.54 \, \mu g \cdot g^{-1})$ concentrations (Bassi 2022). Hence, the strong input of Labrador waters through the Strait of Belle Isle (Galbraith et al. 2017) likely contributes to enriching the Eastern Gulf sector in Sr and especially Ba, as was found in EG otolith margins. While Mg concentrations showed similar trends in otolith margins across our study areas, we found slightly higher concentrations of this element in otolith cores. Some studies have shown that Mg exhibits a pronounced seasonality (Grammer et al. 2017) in many fish species, where the minimum value corresponds to winter, but the amplitude in Mg differences between summer and winter decreases with age (Hüssy et al.

2016; Limburg et al. 2018). In the present study, EGSL sites were sampled during summer 2016, which refutes the hypothesis that the variation are due to temporal or seasonal effects. Thus, the mechanism that could explain the high level observed in WG is poorly understood, though it is likely due to an environmental effect.

4.2. Adult origins

The random forest clustering approach performed on otolith cores identified three potential areas of juvenile origin, two of which dominated (B and C) in most sectors. To confirm the chemical distinction of these three sources, we performed a QDA as we did previously for the sectors (see Section 2.4). The three sources had distinct elemental fingerprints that led to an 84% success in reclassification by a reassignment procedure, suggesting that they represent different areas of origin. The three sources were detected in otoliths from all sample sites. WG fish primarily originated from source C, whereas source B dominated in the SAG, EST, and EG sectors. Source A contributed to each sector, but always as the second (EST) or third (SAG, WG, and EG) contributor. According to current knowledge, the St. Lawrence Estuary comprises the main nursery area for Greenland halibut in the EGSL, hosting high numbers of one- and two-year-old fish

Table 5. Summary of sample site information: sampling area, site name, Northwest Atlantic Fisheries Organization (NAFO) division, sample site abbreviation, sector, sampling depth (m; below ocean surface), number of sampled fish, fish size (mean ± SD), number of otoliths analyzed, and sample site longitude and latitude.

Sampling area	Site	NAFO division	Site abbreviation	Sector	Depth (m)	N (Fish)	Fish size (cm ± SD)	n (Otoliths)	Longitude	Latitude
Saguenay River	Saguenay	–	SAG	*Saguenay*	76	55	48.94 ± 3.91	23	70°51′0.36″W	48°24′50.04″N
EGSL	Rimouski	4T	EST	*Estuary*	236	50	46.72 ± 4.59	25	68°28′36.48″W	48°39′33.12″N
	Sept-Îles	4S	SIL		249	50	49.4 ± 3.43	22	66°46′19.92″W	49°46′59.88″N
	Gaspésie	4T–4S	GAS	*Western Gulf*	339	50	49.2 ± 3.23	24	65°16′58.80″W	49°23′42.00″N
	South Anticosti	4S	S.ANT		242	50	50.22 ± 3.37	25	64°8′35.88″W	49°33′0.00″N
	North Anticosti	4S	N.ANT	*Eastern Gulf*	240	50	43.31 ± 7.32	25	62°16′1.56″W	49°46′45.48″N
	Esquiman	4S–4R	ESQ		254	50	47.95 ± 9.48	24	59°1′36.48″W	50°1′19.92″N

(Ait Youcef et al. 2013). The area north of Anticosti Island is a second nursery location in the EGSL, with high concentrations of juvenile Greenland halibut also being found in this area (DFO 2018). While no other juvenile source or nursery is currently known within the EGSL, our results indicate a third potential juvenile source somewhere inside or outside of the EGSL.

The unsupervised random forest approach does not directly provide the natal location, so we investigated the composition of each elemental fingerprint obtained from otolith cores to try to identify their potential location through comparison with the margin signatures. These comparisons must be interpreted with caution: since there is a gap of several years between margin and core elemental fingerprints, some ontogenetic effects could occur during the larval/juvenile stage that could affect elemental incorporation (de Pontual et al. 2003). Thus, Greenland halibut exhibit ontogenetic migration by gradually moving to deeper and(or) saltier water as they grow (Morgan et al. 2003). That behavior could explain the difference of concentration between margins and cores observed for Sr. Due to these differences, Sr was useful to discriminate sectors by margin elemental fingerprints but unsuitable for inferring nursery locations from core elemental fingerprints. However, Mg and Ba seem to be less affected by ontogenetic modifications because the values observed among the elemental fingerprints are comparable between cores and margins and thus may be more useful for identifying nursery areas.

The main contributor to the Saguenay and Estuary sectors was source B, which was characterized by the lowest concentrations of all three elements. It is currently thought that the Saguenay Fjord is a sink for Greenland halibut, due to passive transport of eggs and larvae or juvenile migration from the estuary (Sirois et al. 2009). So, if the Saguenay is not a source—which remains to be clearly demonstrated—the elemental fingerprint of source B could be the chemical signature of the Estuary sector due to the similar concentrations of Mg and Ba. Source C was characterized by high Mg, which was specific to WG. Mg concentrations were fairly constant among our sampling sectors except for WG. Mg appears to be the element characterizing this sector, with a slightly higher concentration for the otolith core (35 µg·g^{-1}) than for the margin (30 µg·g^{-1}). This difference could be caused by physi-

ological effects during the juvenile stage, since Mg seems to be higher in the core section for Greenland halibut (Limburg et al. 2018) and(or) due to some variation of water chemistry occurring throughout the entire lifespan of adult individuals. Greenland halibut caught in the western Gulf were adults, with a mean size of 49.6 cm and an estimated age of more than 4 years (Ait Youcef et al. 2015), which could explain the slight Mg variations between the margin and core in addition to physiological effects. If our assumption was right that the respective locations of sources B and C were the EST and WG sectors, these elemental fingerprints distinguished the St. Lawrence Greenland halibut nursery. Ait Youcef et al. (2015) defined the Greenland halibut nursery as the "St. Lawrence Estuary," a large single area overlapping the EST and WG sectors (Fig. 1). We rather identified two distinct elemental fingerprints in the St. Lawrence Estuary: one upstream near the maritime estuary (EST sector) and the other further downstream, located in the northwest gulf (WG sector; Fig. 1). This evidence corroborates the notion that the St. Lawrence Estuary is the main Greenland halibut nursery in the EGSL, with the cumulative percentage of sources B and C indicating that around 78% of our sampled Greenland halibut originated from these sources.

Source A shows an atypical signature, with above-average Ba levels—up to four times higher than those observed in Saguenay elemental fingerprints. Mg concentrations were higher than all sectors except for WG and source C, and the Sr concentration was highest among all the elemental fingerprint sources. The chemical signature of source A could indicate a new juvenile source inside the EGSL, possibly around Cabot Strait and(or) the southwest coast of Newfoundland, as these areas were missing in our sample. Currently, there is no information or mention in the literature of a third nursery inside the EGSL. Another possibility is that the elemental fingerprint of source A reveals an unknown nursery located outside of the EGSL that would highlight an immigration of individuals into the EGSL.

There are several points that may support the hypothesis of an external juvenile source, starting with elemental concentrations. Ba was much higher than all other elemental fingerprints in both margins and cores. However, previous work showed that Greenland halibut caught in 2016 along the Labrador coast ($n = 61$) had the highest Ba value

(2.47 ± 0.81 µg·g⁻¹) (Bassi 2022). This study also showed that the high Ba levels were observed only in Canadian waters along the NU and Labrador coasts, with Labrador coast sampling showing the highest Ba values. In the absence of such a high level of Ba inside the EGSL, the Ba concentration in cluster A could correspond to the elemental signature of the Northeast coast of NL. In addition, cluster A contributes only ~22% ($n = 37$) of sampled Greenland halibut origins, which makes it the tertiary source of overall juveniles in the EGSL. It is conceivable that such a small proportion of individuals comes from an external source. Recent work based on genomics has suggested that the Greenland halibut stock off the Northeast coast of Newfoundland contributes up to one third of the EGSL stock depending on the year (Carrier et al. 2020). These authors proposed that the contributions of the Newfoundland stock to the eastern north Anticosti and the estuary nurseries were respectively 30% and 14%. In the present study, we found source A contributions of 21% and 20% for EG and EST, respectively. Specifically, for the north Anticosti site, contribution of source A was estimated at 29%. The similarity of contributions from a third source estimated using genomics and otolith chemistry for north Anticosti and the Estuary provides support to the hypothesis that source A originates from outside the EGSL.

Greenland halibut have a large egg and larva dispersal capacity: these early life stages have been shown to drift in water masses for more than half a year between spawning in Davis Strait and post-metamorphosis settlement along the Labrador coast (Stenberg et al. 2016). Eggs and larvae from outside the EGSL could potentially drift during their prolonged pelagic phase, or small juveniles (<15 cm) could enter the Strait of Belle Isle via incoming currents near the coast of Labrador (Han et al. 1999; Wu et al. 2012; Galbraith et al. 2019). The Strait of Belle Isle is an important migratory route for several species, including Atlantic redfish (*Sebastes mentalla*; Benestan et al. 2021), snow crab (*Chionoecetes opilio*; Puebla et al. 2008), and capelin (*Mallotus villosus*; Kenchington et al. 2015; Cayuela et al. 2019). Moreover, Bowering (1982) previously postulated that there was significant gene flow through migrations between the Northeast coast of Newfoundland and the EGSL through the Strait of Belle Isle based on stock dynamics in the 1970s. The question however remains whether those migrations correspond to the passive larval drift or the active movements of young juveniles and(or) larger individuals from Labrador to the GSL.

4.3. The contributions of otolith chemistry for Greenland halibut stocks

Since 1990, the Greenland halibut population of the EGSL has been considered an isolated population (Arthur and Albert 1993) and exploited as such because attempts from genetic studies failed to reach a consensus about population structure. Otolith chemistry allowed us to identify three distinct sources of juveniles that contributed to the EGSL population, indicating a high degree of mixing inside the EGSL. The two main sources identified likely correspond to the two known St. Lawrence nurseries; source B refers to the maritime Estuary (EST) and source C to the northwest gulf (WG) of St. Lawrence system, which is consistent with past evidence of nursery location (Ait Youcef et al. 2013, 2015), whereas the third source would either correspond to a currently unknow source within the EGSL or a source located outside of the EGSL. The St. Lawrence estuary is an essential habitat for Greenland halibut, and their preservation requires more biological information, like those obtained in this study to support sustainable exploitation. Currently, important environmental changes occurs in the EGSL system, especially in the estuary part, by incursion of warmer, saltier, and less oxygenated water from the Gulf Stream through the Laurentian Channel (Galbraith et al. 2019). Rising temperature and oxygen depletion could negatively impact the Greenland halibut nursery in the estuary, halting population replacement and leading to long-term declines. As we identified the potential of unknown source of juveniles outside the EGSL system, identification of this source seems essential for stock management.

Further work is required to identify the precise location of juvenile sources of Greenland halibut revealed by otolith chemistry and in particular whether an external source contributes to the EGSL population. The random forest clustering approach applied here could be further developed by the inclusion of targeted larval or juvenile samples from known nurseries, which would help in identifying the geographic location of juvenile sources.

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Appendix A

Fig. A1. Elemental fingerprints of the four sectors represented as radar charts on scaled values.
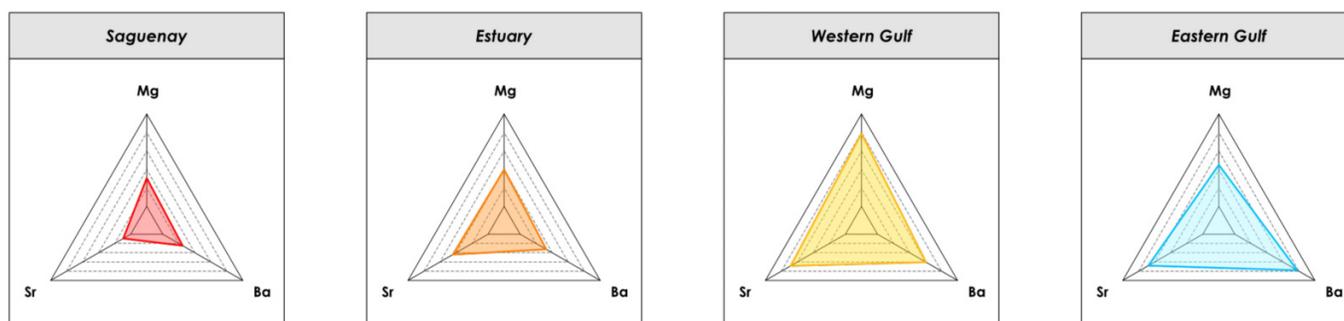

Fig. A2. Elemental fingerprints of three juvenile sources represented as radar charts on scaled values.