Connectivity of Newfoundland Forage Fish using Otolith Chemistry

by

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Thesis Abstract

Connectivity refers to the movement and exchange of individuals of the same species among regions, linking individuals across different regions of their geographic range. Forage fish species, such as capelin (*Mallotus villosus*) and sand lance (*Ammodytes dubius*), serve as critical prey for numerous commercially and ecologically important marine predators, yet their dispersal patterns, natal origins, and connectivity in the Northwest Atlantic (NWA) remain poorly understood and studied. Identifying the natal sources of forage fish and understanding the scales at which their early life stages disperse are essential for assessing population structure and for effective management. However, the larval phase, which is the dominant phase of dispersal, remains notoriously difficult to study. Otolith chemistry can be used as a tool to infer movement and natal origins, but its effectiveness varies across species. This thesis investigated the potential of otolith chemical signatures to uncover natal origins and connectivity patterns of capelin and sand lance on the Newfoundland Shelf in eastern Canada. We quantified age/stage-specific otolith concentrations of a variety of trace elements (strontium, barium, manganese, magnesium) using Laser Ablation Inductively Coupled Plasma-Mass Spectrometry for larval capelin and adult sand lance (aged 1) to determine whether otolith chemical signatures can be distinguished across regions. For larval capelin (Chapter 2), chemical signatures in the otolith pre-hatch region varied among sites within bays, and this fine spatial scale variation decreased the classification success at broader-scales, evidenced by low-moderate classification success of chemical signatures among Newfoundland bays (2023: 31.3-73.3%; 2024: 5.9-61.1%). These patterns demonstrated that site-specific environmental conditions experienced during early life history stages of capelin may limit the ability to distinguish natal bays of adult capelin. For sand lance (Chapter 3), near-core chemical signatures showed minimal distinctions between inshore and offshore regions, consistent with larval mixing or shared water masses during larval dispersal. By contrast, post-settlement otolith chemistry exhibited more clear regional distinctions, supporting strong site-fidelity after settlement. Together, these results demonstrate the merits and challenges of using otolith chemistry to determine natal origin and connectivity of marine fish, emphasizing the need for careful interpretations when examining the pre-hatch region of the otolith when attempting to assign adults to their natal origins.

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Table of Contents

List of Tables

List of Figures

Thesis Format

This thesis is written in a grouped manuscript format. Chapter 1 offers a broad introduction and outlines the key concepts that frame the research presented in Chapters 2 and 3. Each chapter functions as an independent manuscript, with its own abstract, introduction, methods, results, discussion, conclusion, and references, along with their respective tables, figures, and supplementary materials. The final chapter (Chapter 4) brings together the main findings from Chapters 2 and 3, highlighting their implications for management and future research directions.

Contribution of Authors

Sierra Schlief conducted all field work (in Chapter 2), data analysis, and wrote this thesis. Samples in Chapter 3 were supplied by Fisheries and Oceans Canada. Gail Davoren (thesis advisor) obtained the funding necessary to complete projects within Chapters 2 and 3. Sierra Schlief and Gail Davoren both determined the appropriate data analyses, manuscript structure, editing, and revising the manuscript. Dr. Ashley Tripp assisted in project development, field sampling and manuscript editing (Chapter 2), along with sand lance age estimations (Chapter 3). Dr. Hannah Murphy and Dr. Gary Anderson (thesis committee) provided input.

Chapter One: Introduction

Marine food webs are under ‘wasp-waist’ control, where the lower (e.g., phytoplankton, zooplankton) and upper (e.g., seabirds, marine mammals) trophic levels have higher species diversity than the intermediate trophic level (e.g., forage fish; Cury et al., 2000). Therefore, the intermediate trophic level is critical to energy flow in many marine systems, where the energy is funnelled through a few forage fish species (Cury et al., 2000; Pikitch et al., 2012). Forage fish species are short-lived, small, schooling fish and their populations are characterized by ‘boom-bust’ cycles, where population size substantially and rapidly changes due to natural environmental conditions and food availability (Pikitch et al., 2012; Schwartzlose & Alheit, 1999). As forage fish account for up to one-third of global marine fisheries landings, and are essential for energy flow in marine systems, there is an imminent need for precautionary fisheries management for these species (Cury et al., 2000; Pikitch et al., 2012; Smith et al., 2011).

Demographic connectivity (hereafter referred to as ‘connectivity’) involves the exchange and movement of individuals among regions of high abundance (‘local populations’) within a species’ geographic range (Cowen et al., 2006; Taylor et al., 1993; Turgeon et al., 2010) and the degree of connectivity is determined by whether individuals can successfully disperse to other regions (Cowen et al., 2006; Cowen & Sponaugle, 2009; Palumbi, 2003). Understanding connectivity of marine fish is critical for defining management units, or stocks, which is crucial for designing effective management regulations as well as establishing effective Marine Protected Areas (MPAs) (Sinclair & Smith, 2002; Stephenson, 2002).

For marine fish, the larval phase is the dominant life phase during which dispersal, or movement away from natal origins, occurs (Cowen et al., 2006; Cowen & Sponaugle, 2009). As such, it is essential to focus on the larval stage to understand connectivity within a marine fish species (Cowen & Sponaugle, 2009). Determining dispersal away from natal sites, however, poses challenges because larvae are often too small for external tagging. Consequently, alternative tools are required to determine natal origins within these species (Cowen & Sponaugle, 2009).

Otolith microchemistry is one tool that can be used to discriminate between different contingents of a population that may have minimal mixing, thereby providing key information to define stocks for fisheries management (Edmonds et al., 1991). Otoliths are inner ear stones that allow fish to hear, orientate, and balance (Campana & Neilson, 1985). Otoliths grow continuously throughout the fish's life and are metabolically inert, meaning all deposited trace elements become permanent within the otoliths. This chronological chemical record provides valuable insights into the environmental conditions the fish experienced throughout its lifespan, thereby providing information regarding an individual’s natal origins as well as dispersal and movement patterns (Campana & Neilson, 1985; Fowler et al., 1995). When otolith chemical signatures are compared across local populations, their differences or similarities can shed light on the degree of connectivity among these local populations.

Environmental factors, including salinity, temperature, and ambient water chemistry, influence otolith trace element concentrations (Campana, 1999; Elsdon & Gillanders, 2002). As these factors vary across regions and years within the same region, they influence the rate at which certain elements are incorporated into the otolith, resulting in distinct otolith chemical signatures (Campana, 1999; Rogers et al., 2019). Non-essential elements (e.g. strontium, barium) are not under physiological control during incorporation into the otolith (Campana, 1999) and, thus, their concentrations primarily vary with ambient water chemistry, being influenced by factors such as bedrock and sediment composition, salinity, temperature, pH, and dissolved oxygen levels (Bath et al., 2000; Campana, 1999; Chowdhury & Blust, 2001; Hegg et al., 2013). By contrast, essential elements (e.g., magnesium, manganese) are under physiological control (Loewen et al., 2016) and typically vary less with ambient water chemistry. However, because temperature influences growth, otolith concentrations of non-essential elements may increase under warmer conditions due to a higher rate of incorporation into the body and, thus, the otoliths (Sturrock et al., 2014; Woodcock et al., 2012).

Study area and study species

The Newfoundland and Labrador Shelf ecosystem is comprised of several forage fish species, Atlantic herring (*Clupea harengus)*, Atlantic mackerel (*Scomber scrombus*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes dubius* and *A. americanus*). These forage fish, except for sand lance, are commercially fished and, thus, undergo a bi-annual stock assessment by Fisheries and Oceans Canada.

Larval capelin drift passively through surface waters to offshore nursery habitat near the shelf break, where they remain until sexually mature (2-3 years old) after which they migrate to coastal subtidal (15-40 m) or intertidal spawning sites (Carscadden et al., 2013; Crook et al., 2017). The Newfoundland capelin stock collapsed in the early 1990's and persists in a low productivity phase after 30 years (Buren et al., 2019). Capelin on the eastern Newfoundland and

Labrador Shelf (Northwest Atlantic Fisheries Organization, NAFO, divisions 2J3KL) is managed as a single stock (DFO, 2015) as tagging evidence suggests intermixing among post-spawning capelin from differing regions within this larger area (Nakashima, 1992). In support, age-specific otolith chemistry did not differ between spawning adults collected in two bays (Trinity Bay, subtidal; Notre Dame Bay, intertidal; Davoren & Halden, 2014) and adults from different regions also could not be distinguished based on age-specific morphological and meristic characteristics (Carscadden & Misra, 1980; Misra & Carscadden, 1987; Nakashima, 1992; Sharp et al., 1978). These studies, however, were based on limited data, possibly limiting their ability to indicate separate stocks. More recently, larval capelin otolith chemistry was shown to vary across bays (Tripp et al., 2020), suggesting that chemical signatures in the pre-hatch otolith region might be a good tool to determine the natal origins of adult fish within this stock. It is unclear, however, whether otolith chemistry in the pre-hatch regions is influenced more by site-specific than bay-specific environmental conditions, as only larval capelin from one site per bay were sampled in this previous study (Tripp et al., 2020).

Connectivity along with the general ecology of sand lance (*A. dubius*) in the Northwest Atlantic (NWA) remain poorly understood (Staudinger et al., 2020). NWA *Ammodytes* appear to begin spawning in late fall and winter, producing demersal, adhesive eggs that attach to sandy substrate and develop over approximately 1-3 months, depending on temperature (2-10°C) (Smigielski et al., 1984; Winters, 1983). Once hatched, larvae transition to a planktonic phase, remaining in the water column for several months (Norcross et al., 1961; Scott, 1968). Evidence from other *Ammodytes* species, such as A. *marinus* and A. *tobianus* in the Northeast Atlantic and A. *hexapteru*s in the Pacific, indicates that juveniles then settle into demersal habitats with suitable habitat characteristics, where they remain resident for their lifespan, rarely moving beyond 15 km (Gauld, 1990; Kunzlik et al., 1986; Robinson et al., 2013). This reduced dispersal as adults appears to result in minimal mixing and connectivity among regions, as illustrated by different age-specific otolith trace element concentrations among fish sampled in different areas (Gibb et al., 2017; Laugier et al., 2015; Wright et al., 2018). These restricted movement/dispersal patterns may result from their lack of a swim bladder and associated habitat preferences for coarse sand with minimal silt (<10%), in which they bury when they are not feeding to minimize energy expenditure and avoid predation (Engelhard et al., 2008; Robinson et al., 2013; van Der Kooij et al., 2008; Wright et al., 2000).

Objectives

Overall, the objective of my thesis was to investigate whether otolith chemistry can be used as a tool to understand the natal origins and connectivity patterns of Newfoundland forage fish. In my second chapter, I aimed to determine if trace element concentrations from the pre-hatch (embryonic) otolith region of larval capelin can be distinguished across different Newfoundland bays, sites within bays, and years. To accomplish this, late-stage eggs and recently hatched larvae were collected at two to three intertidal sites per bay across six bays throughout the southern and eastern coastal regions of Newfoundland during July-August, 2022, 2023, and 2024 within NAFO Divisions 3KL and 3Ps. I hypothesized that capelin eggs raised in different bays could be distinguished based on trace element concentrations in the otolith pre-hatch region due to variable bay-specific environmental factors (e.g, water chemistry, temperature, salinity). I predicted that pre-hatch otolith trace element concentrations differ more among bays than among sites within a bay with little differentiation at sites across sampled years.

The objectives of my third chapter were to examine connectivity of *Ammodytes dubius* across different regions on the northeastern (inshore) and southeastern (offshore) Newfoundland Shelf using otolith chemistry. To accomplish this, I used sand lance otoliths collected from these two regions within NAFO Division 3L during 2019 and 2020. I hypothesized that sand lance larvae mix over a large geographic region but remain where they settle as juveniles throughout their lifespan, similar to *Ammodytes* species in other regions (Gibb et al., 2017). I predicted that adult sand lance collected from inshore and offshore regions will show less distinct otolith chemistry signatures in the otolith region representing larval dispersal, but otolith chemistry will be more distinct post-settlement, demonstrating limited connectivity as adults.

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Chapter Two: Fine-scale variation in chemistry in the pre-hatch otolith region of capelin reduces classification success into bays in coastal Newfoundland, Canada

Abstract

Identifying natal origins of fish is key to understanding movement of individuals among regions and relative contributions of different regions to recruitment dynamics. Forage fish species, such as capelin (*Mallotus villosus*), play a vital role in pelagic marine food webs, serving as prey for commercially and ecologically important species. Despite their importance, natal origins and ecological connectivity of forage fish remain largely unknown. We aimed to investigate whether the natal bay of capelin in coastal Newfoundland could be identified based on otolith chemical signatures. As capelin disperse immediately away from natal sites upon hatching, capelin eggs and recently hatched larvae were sampled from 2-3 intertidal sites per bay across six bays on the south and east coasts of Newfoundland during the summers of 2022, 2023, and 2024. Trace element concentrations (strontium, barium, magnesium, and manganese) in the otolith pre-hatch region were quantified using Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). Although otolith chemical signatures differed among sites within bays and years within sites, signatures differed among bays each year, but classification was generally low (2023: 31.3-73.3%; 2024: 5.9-61.1%). Site-specific inter-annual variation in otolith chemical signatures suggests that bay-specific otolith chemical signatures may differ across years. This research shows that fine spatial scale variation decreased the accuracy to assign larvae to broader scales, suggesting that bay-specific (i.e., multiple sites within a bay pooled) otolith chemistry from the natal year may be necessary to assign adults to their natal bay. This research suggests caution when using otolith chemistry as a tool to

determine natal origins for adult capelin.

Introduction

Identifying natal origins of fish is key to understanding dispersal, distinctiveness of local populations, metapopulation structure and relative contributions of different locations to recruitment dynamics (Cowen & Sponaugle, 2009; Taylor et al., 1993). Forage fish species play a vital role in pelagic marine food webs, serving as prey for commercially and ecologically important species. Despite their importance, dispersal patterns from natal sites remain largely unknown. Understanding this demographic connectivity (hereafter referred to as ‘connectivity’), or the movement of individuals among local populations (Cowen et al., 2006; Taylor et al., 1993; Turgeon et al., 2010), is critical for defining fisheries management units and, thus, designing effective management plans for fish species as well as establishing Marine Protected Areas (MPA) (Sinclair & Smith, 2002; Stephenson, 2002).

Determining natal origins of adult marine fishes pose challenges because dispersal away from the natal site predominantly occurs in the larval phase (Cowen et al., 2006; Cowen & Sponaugle, 2009) and larvae are often too small for external tagging. Consequently, alternative tools are required to determine natal origins within these species, such as otolith chemistry (Cowen & Sponaugle, 2009). For species that disperse immediately upon hatching, the pre-hatch region of the otolith provides the most direct record of natal origin as it reflects the environmental conditions experienced at the spawning site. Therefore, otolith chemistry has served as a tool to infer natal origins (e.g., Lazartigues et al., 2016; Rachel et al., 2008; Rogers et al., 2019; Tripp et al., 2020) and, by extension, improve our understanding of connectivity in marine fish.

Capelin (*Mallotus villosus*) is the dominant forage fish species during the summer in coastal Newfoundland. In this region, larval capelin drift passively in surface waters away from natal sites immediately upon hatching to offshore nursery habitat near the shelf break. In this region, they mix with capelin larvae from other bays (Dalley, 2002; deYoung et al., 1994; Frank & Leggett, 1981) and remain until sexually mature (2-3 years old) after which they migrate to coastal subtidal (15-40 m) or intertidal sites to spawn (Carscadden et al., 2013; Crook et al., 2017). Capelin on the eastern Newfoundland and Labrador Shelf is managed as a single stock (Northwest Atlantic Fisheries Organization (NAFO) divisions 2J3KL; DFO, 2015). This stock collapsed in the early 1990's and persists in a low productivity phase after 30 years (Buren et al., 2019). Evidence supporting a single stock comes from tagging studies showing movement of mature capelin among multiple bays during spawning (Nakashima, 1992) and an inability to distinguish among adults from different regions based on age-specific morphological and meristic characteristics (Carscadden & Misra, 1980; Misra & Carscadden, 1987; Sharp et al., 1978). These studies, however, were based on limited data, possibly limiting their ability to indicate separate stocks. More recently, chemistry in the pre-hatch otolith region of larval capelin was shown to vary across bays (Tripp et al., 2020), suggesting that these chemical signatures may be a good tool to further investigate dispersal patterns of larvae and connectivity among bays within this stock. It is unclear, however, whether pre-hatch otolith chemistry is influenced more by site-specific than bay-specific environmental conditions (Tripp & Davoren, 2025), as only larval capelin from one site per bay were sampled in the aforementioned study (Tripp et al., 2020). Indeed, although this and another study in coastal

Newfoundland have shown variation in otolith chemistry among bays for juvenile and larval fish of two species (capelin: Tripp et al., 2020; Atlantic cod *Gadus morhua*: Stanley et al., 2016), this variation may arise from a combination of factors, including broad-scale variation in water chemistry (e.g., underlying bedrock; Catto, 2012) and/or oceanography (e.g., influence of Labrador Current; Cyr, 2022), along with fine-scale variation in these environmental factors.

For chemical signatures in the pre-hatch region of the otolith to reflect the natal site, trace element incorporation rates must be influenced by environmental factors and these factors must vary across regions, resulting in distinct, region-specific otolith chemical signatures (Campana, 1999; Rogers et al., 2019). The key environmental factors that influence otolith trace element concentrations include salinity, temperature, and ambient water chemistry (Campana, 1999; Elsdon & Gillanders, 2002). Otolith trace elements widely studied include strontium, barium, manganese, and magnesium because their concentrations occur at detectable concentrations and reliably track environmental variation (Campana, 1999, 2005; Rogers et al., 2019). In particular, strontium and barium are non-essential elements for fish and, as such, do not have physiological mechanisms for uptake and incorporation into the otolith (Campana, 1999). Therefore, their concentrations primarily vary with ambient water chemistry, which is influenced by factors such as bedrock and sediment composition, salinity, temperature, pH, and dissolved oxygen levels (Bath et al., 2000; Campana, 1999; Chowdhury & Blust, 2001; Hegg et al., 2013). By contrast, essential elements (e.g., magnesium, manganese) have physiological mechanisms for uptake (Loewen et al., 2016) and typically vary less with ambient water chemistry. As temperature influences growth, however, otolith magnesium and manganese concentrations may increase under warmer conditions due to a higher rate of incorporation into the body and, thus, the otolith (Sturrock et al., 2014; Woodcock et al., 2012). Although studies have shown that trace element concentrations in the pre-hatch otolith region may also be influenced by the maternal environment during gonadal development (Limburg et al., 2001; Volk et al., 2000; Zimmerman & Reeves, 2002), trace element concentrations in the pre-hatch region can be altered when eggs are raised in varying water chemistry for capelin (Loeppky & Davoren, 2018; Tripp et al., 2022), along with other species (e.g., Atlantic salmon, Warren-Myers et al., 2015).

Objectives

In this manuscript, I investigated whether capelin eggs raised in different bays and sites within bays and across years can be distinguished using trace element concentrations in the pre-hatch (embryonic) otolith region. To do this, I designed a coast-wide sampling program to collect capelin eggs in late developmental stages and recently hatched larvae in the sediment at intertidal spawning sites across multiple bays and years, from which trace element concentrations were quantified in the otolith pre-hatch region (Campana & Neilson, 1985; Tripp et al., 2020). I hypothesized that eggs raised in different bays could be distinguished based on trace element concentrations in the otolith pre-hatch region due to variable bay-specific environmental factors (e.g, water chemistry, temperature, salinity). I predicted that pre-hatch otolith trace element concentrations differ more among bays than among sites within a bay with little differentiation at sites across sampled years. Overall, this research will inform the spatial scale that otolith chemistry can effectively differentiate capelin larvae raised at intertidal spawning sites from two stock areas in NAFO Divisions 3KL and 3Ps. Although 3Ps is a separate stock, it is managed under the 2J3KL stock assessment. If bay-specific otolith chemical concentrations in the pre-hatch region can be distinguished, researchers could potentially determine the natal origins of adult spawning fish to investigate connectivity among bays and stock areas (i.e. whether capelin return to their natal bay to spawn) as well as the relative productivity of different bays.

Methods

Fertilized capelin eggs (hereafter referred to as “eggs”) in late developmental stages and recently hatched larvae (3-6 mm, yolk sac present) were collected from two to three intertidal sites per bay across six bays throughout the southern and eastern coastal regions of Newfoundland during July-August, 2022, 2023, and 2024 (Table 2.1, Figure 2.1). As capelin only spawn at a few beaches within each bay, as preferred spawning conditions vary across beaches, sampled sites are likely representative of spawning sites within each bay during a given year. The bays included in this study are Notre Dame Bay (NAFO Division 3K), Witless Bay/Southern Avalon (3L), Tor Bay (3L), Trinity Bay (3L), Conception Bay (3L), and Placentia Bay (3Ps) (Table 2.1, Figure 2.1) These bays have diverse environmental characteristics, including distinct coastal features (i.e., freshwater input) and oceanographic conditions, allowing for a comprehensive study of larval capelin otolith trace element concentrations throughout NAFO Divisions 3KLPs (Figure 2.1).

Larvae that had hatched but had not emerged from the beach sediments (‘pre-emergent larvae’), were opportunistically sampled by scooping ~ 1 L of sediment with adherent capelin eggs in late developmental stages into 4 L plastic bags. Bags were filled with seawater, gently inverted to release pre-emergent larvae from sediment, and kept in a cooler for up to 12 h during transport prior to processing. At each site, a water sample was collected on the same day, or within a week, of sediment collection. Water samples were collected at waist height by filling a 60-mL disposable syringe, which was then pressed through a 0.45-µm filter, collected in a plastic sample bottle and preserved by adding 3 mL of 1:3 ultrapure nitric acid and deionized water.

The seawater from each sediment-egg-larvae bag was poured over a 0.27-mm mesh sieve to collect larvae and eggs in later developmental stages (i.e., stages V–VII; Fridgeirsson, 1976). When present, all larvae and late-staged eggs (typically 10-100) in good condition were immediately preserved in 95% ethanol. Within 6 months, the right sagittal otolith was extracted with tungsten needles (Roboz) from 10 larvae per intertidal site under a dissecting microscope (Olympus SZX7) equipped with a polarizing lens (Olympus SZX-PO) and placed on a glass slide with double sided tape (Scotch^{TM}), with each slide containing up to 60 otoliths (as described in Loeppky & Davoren, 2018).

Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS; New Wave UP-213 Laser Ablation System attached to an Agilent 8900 triplequad ICP-MS) was conducted at the University of Manitoba in the Department of Geological Sciences. We ablated 8-10 otoliths per site based on availability. Each otolith was ablated with a beam size of 40 µm using a spot technique to ablate the whole otolith (following Loeppky & Davoren, 2018; Tripp et al., 2020). This involved ablating the otolith surface repeatedly until all otolith material was ablated. As each larval otolith is ~ 30 µm, spot tests were performed on the tape to assess if there were background chemical signatures. Laser parameters included a repetition rate of 5 Hz for samples and 10 Hz for standards, with fluence of ~6 J/cm^2. Each analysis included a 30 s gas blank, and 30 s ablation time. After each laser ablation, a 60-s washout period was applied to remove any remaining residual ablated particles before the next otolith was ablated.

Data reduction was performed using Iolite 4 (Paton et al., 2011). First, otolith trace element concentrations with spikes >3 SD above the mean for that otolith were removed, as these spikes likely indicate surface-level contamination. Calcium (^{43}Ca) counts per second (CPS) was used as an internal standard, allowing otolith trace element concentrations to be standardized based on the amount of ablated material. Standardization assumed a fixed CaO content of 40.04 wt.%, which was applied individually to each element measured. For every trace element, the mean concentration and SE were calculated across the ablation period (i.e. repeatedly ablating the otolith surface), which was defined by the period ^{43}Ca (CPS) was consistently high and stable (i.e. otolith material was ablated). Overall, our dataset consisted of the concentrations of trace elements (strontium, barium, manganese, magnesium, zinc, lithium, boron, sodium, potassium, iron, rubidium, molybdenum, uranium, copper, and lead) in parts per million (ppm or µg/g) from each site, following Tripp et al. (2020). These trace elements were selected as they were previously important for distinguishing capelin spawning bays and habitats (intertidal, subtidal) in the embryonic region of capelin otoliths (Davoren et al., 2015; Lazartigues et al., 2016; Loeppky & Davoren, 2018; Tripp et al., 2020) and to represent the trace elements found in the water chemistry samples across sites.

Elements with concentrations frequently below or near the limit of detection were excluded from the analysis (i.e., uranium, molybdenum, copper, lead, rubidium, iron, aluminum, boron), leaving strontium, barium, magnesium, and manganese. Limits of detection

(LODs) for the otolith samples were defined as three times the standard deviation of the blank signal, which were averaged across all blank analyses for strontium (Sr), barium (Ba), magnesium (Mg), and manganese (Mn). The external precision estimates (%RSD) and LODs were as follows for the studied elements: ^{88}Sr = 2.08%, 0.01 ug/g; ^{137}Ba = 1.84%, 0.01 ug/g; ^{25}Mg = 1.79%, 0.31 ug/g; ^{55}Mn = 2.61%, 0.30 ug/g. LA ICP-MS recoveries were as follows: ^{88}Sr = 97.95%, ^{137}Ba = 99.00%, ^{24}Mg = 95.27%, ^{55}Mn = 102.83%.

Water chemistry samples were analyzed for dissolved trace element concentrations at ALS Environmental Laboratories (Burnaby, BC). Laboratory control samples were analyzed alongside all water samples to assess analytical accuracy, using standardized spikes of target elements. The spike recoveries were ^{43}Ca (98.2%), ^{88}Sr (100%), ^{137}Ba (102%), ^{25}Mg (102%), ^{55}Mn (98.4%).

Statistical Analysis

Outliers, defined as elemental concentrations >2 SD from the mean for each site, were excluded from the dataset, as they likely resulted from issues during the ablation process, such as incomplete ablation of the otolith or premature otolith fragmentation (Loeppky & Davoren, 2018). A total of 1–3 otoliths were removed per site, resulting in 41 otoliths removed from the final dataset of 198 otoliths (5-10 otoliths remaining per site; Table 2.1). All otolith concentrations of all four elements (Sr, Ba, Mg, Mn) were log transformed to improve normality and meet the underlying parametric assumptions.

Statistical analyses were performed in RStudio (RStudio Team, 2021) and JMP Student Edition (version 18.2.0). Multivariate analysis of variance (MANOVA) tests were performed in R using the manova() function to assess whether trace element concentrations (Sr, Ba, Mg, Mn; continuous response variables) varied among bays (categorical fixed factor) and among sites (categorical fixed factor) nested within a bay within each year separately. To calculate the classification success of each capelin larva to their natal bay based on trace element concentrations, linear discriminant function analyses (DFAs) were conducted in JMP for each year (2023, 2024) separately and included all the trace elements (Sr, Ba, Mg, Mn) as response variables and bay as the grouping factor with sites within bays pooled. To assess classification success on a broader spatial scale, as done in other studies (e.g., Stanley et al., 2016), DFAs were repeated for each year (2023, 2024) with NAFO divisions as the grouping factor (2023: 3K, 3L; 2024: 3K, 3L, 3Ps), and bays and sites within bays pooled within each NAFO division (Figures 2.1 and 2.2). Multivariate analyses were followed up by conducting ANOVAs in R using the aov() function to assess which trace elements (Sr, Ba, Mg, Mn) differed among bays and sites nested within bays (categorical fixed factors) for each year separately.

To determine whether otolith trace element concentrations differed among years at each site, separate MANOVAs were performed in R using the manova() function for each site with multi-year sampling, which included Bellevue (2023, 2024), Big Chance Cove (2023, 2024), St Michael’s Capelin Cove (2023, 2024), Middle Cove (2022, 2023, 2024) and Outer Cove (2022, 2023, 2024). For sites with significantly different trace element concentrations across years, ANOVAs were performed in R using the aov() function on each element (Sr, Ba, Mg, Mn) to identify which element differed across years.

Results

MANOVAs revealed that otolith trace element concentrations differed significantly among bays in each year (2023: $F_{12, 174.91} = 8.35$, $p < 0.001$; 2024: $F_{20, 286.18} = 3.83$, $p < 0.001$) and among sites within bays in each year (2023: $F_{24, 231.46} = 3.10$, $p < 0.001$; 2024: $F_{24, 301.23}= 2.25$, $p < 0.001$). These site differences within bays likely resulted in lower overall classification success of larvae to their natal bay within each year (classification success: 55.7% in 2023, 32.7% in 2024; Table 2.2, Figure 2.2). Classification success varied for each bay ranging from 5.9% to 73.3% (Table 2.2), showing that some bays had higher classification success (e.g., Trinity Bay and Witless Bay) than other bays (e.g., Tor Bay; Figure 2.1). When classified to the incorrect bay, larvae often were assigned to bays that were farther apart than close by, such as high misclassification of Tor Bay to Placentia Bay, and Conception Bay to Notre Dame Bay (Figure 2.1).

Univariate analyses revealed that differences in otolith chemistry across bays resulted from significant differences in all otolith trace element concentrations in 2023 and 2024, except for Mn in 2024 (Table 2.3, Figure 2.3). Post hoc results indicated that Trinity Bay and Witless Bay differed significantly in all otolith trace element concentrations except for Sr in 2023 (Figure 3). In 2024, otolith Sr and Mg concentrations differed between Trinity Bay and Placentia Bay (Figure 2.3). Significant differences in each otolith trace element concentration between sites within bays varied across years. In 2023, otolith Sr and Mg concentrations differed significantly between sites within bays, while Ba and Mn did not (Table 2.3). In 2024, otolith Sr and Ba concentrations differed significantly between sites within bays, whereas Mg and Mn did not (Table 2.3).

At the broader spatial scale, DFAs revealed that otolith trace element concentrations did not differ significantly between NAFO divisions (3K, 3L) in 2023 ($F_{4,74} = 0.92$, $p = 0.17$), but did differ among NAFO divisions (3K, 3L, 3Ps) in 2024 ($F_{8, 190} = 0.63$, $p < 0.001$) with a classification success of 65.4% (66.7% for 3K, 64.2% for 3L, 68.8% for 3Ps; Figure 2.2).

Interannual Comparisons

MANOVAs revealed that otolith trace element concentrations differed significantly across years at Bellevue Beach ($F_{4,10} = 15.11$, $p < 0.001$), Big Chance Cove ($F_{4,13} = 9.36$, $p < 0.001$), and Middle Cove ($F_{8, 38} = 4.60$, $p < 0.001$), while concentrations did not differ across years at Outer Cove ($F_{8,44} = 1.30$, $p = 0.268$) and St. Michael's Capelin Cove ($F_{4,12} = 1.74$, $p = 0.206$). ANOVAs performed on datasets from Bellevue, Big Chance Cove, and Middle Cove revealed that otolith Mg concentrations differed significantly at each site across the years, Sr differed significantly across years for Bellevue and Middle Cove, and Ba significantly differed across years at Big Chance Cove (Table 2.4). Post-hoc tests conducted for Middle Cove indicated that otolith Sr concentrations were significantly higher in 2022 than in both 2023 ($p < 0.001$) and 2024 ($p = 0.014$) and Mg was significantly higher in 2023 compared to 2022 ($p = 0.031$).

Discussion

After characterizing the trace element concentrations in the pre-hatch otolith region of capelin larvae throughout NAFO Divisions 3KL and 3Ps, we found partial support for our hypothesis that there are bay-specific otolith chemical signatures. Otolith chemical signatures differed significantly among bays; however, classification success was generally low (2023: 31.3-73.3%; 2024: 5.9-61.1%). Interestingly, classification success did not improve at the broader spatial scale of NAFO divisions, as found in a previous study on Atlantic cod (Stanley et al. 2016). These findings suggest that fine spatial scale otolith chemistry differences between sites within each bay, likely due to site-specific microhabitat environmental conditions, may play a larger role in shaping pre-hatch otolith chemistry than the broader spatial variation in environmental conditions among bays, thereby decreasing accurate assignment of larval capelin to their natal bay or NAFO Division. Further, the inter-annual variation in otolith chemical signatures observed within some sites suggested that bay-specific otolith chemical signatures likely differ across years. Overall, findings suggest that chemical signatures in the otolith pre-hatch region of capelin may be a tool to assign adult capelin to their natal bay, but an annual- and bay-specific (i.e. multiple sites within a bay pooled) otolith reference collection will likely be needed.

Variation in environmental conditions among sites within the same bay may mask broader differences among bays, reducing classification success within bays and, thus, the ability of chemical signatures in the otolith pre-hatch region to reliably distinguish natal bay. Indeed, environmental factors experienced throughout incubation, such as water chemistry, salinity, and temperature (Table 2.5), are known to influence otolith trace element concentrations in fish (Campana, 1999; Elsdon & Gillanders, 2004) and in the pre-hatch otolith region of larval capelin (Davoren et al., 2015; Loeppky & Davoren, 2018). In this study, larval capelin were sampled at intertidal spawning sites, which may have distinct site-specific microclimate conditions that result in distinct otolith chemical signatures among sites in a bay. For instance, variation in salinity among intertidal sites could arise due to varying exposure to freshwater input, such as streams (i.e., Anchor Brook; Table 2.5) and rainfall, which would influence primarily non-essential elements (i.e., Sr and Ba; Elsdon & Gillanders, 2004). Water and air temperatures also are known to vary among intertidal sites (Crook et al., 2017; Penton et al., 2012). As temperature influences somatic growth, otolith trace element concentrations may be affected due to the higher rate of incorporation under warmer conditions of essential elements, such as magnesium and manganese (Sturrock et al., 2014; Woodcock et al., 2012), as well as with non-essential elements, such as strontium and barium (Bath et al., 2000, Chowdhury & Blust, 2001). Temperature and salinity also may interact in complex ways to influence chemical signatures in embryonic capelin otoliths (Loeppky & Davoren, 2018). Indeed, similar studies on coastal juvenile fish have noted lower classification success when examining embryonic otolith chemistry in sample sites with higher microclimate variability due to closer proximity to the coast, compared to sites in deeper, and hence, more stable water temperature and salinity (Fodrie & Levin, 2008; Stanley et al., 2016).

Variation in pre-hatch otolith chemistry at the fine-scale (i.e., site-level) may also partially reflect maternal investment of trace elements into the yolk sac (Loeppky et al., 2018). As the yolk sac is formed while female capelin are maturing offshore, trace elements that reflect site-specific ambient water chemistry during gonadal development may be incorporated into the developing otolith of larvae during incubation (Limburg et al., 2001; Loeppky et al., 2018; Volk et al., 2000; Zimmerman & Reeves, 2002). For lab-reared larval capelin, family-specific otolith chemical signatures, especially barium otolith concentrations, have been identified within the pre-hatch region, likely due to maternal investment (Loeppky et al., 2018). Therefore, if mature female capelin from one offshore region spawn at one intertidal site and those from a different offshore region with different water mass characteristics (e.g., water chemistry, temperature) spawn at another intertidal site within the same bay, this could have caused larvae to have distinct site-specific pre-hatch otolith chemistry and reduced the classification success at the broader spatial scale of the bay.

Our overall lower classification success for individual larval capelin to their natal bay and NAFO division across both studied years (Figure 2.1, Table 2.2) could also be due to lower differences in environmental conditions at these broader spatial scales relative to fine-scale (i.e. site-level) variation (Gillanders, 2002; Rogers et al., 2019; Stanley et al., 2016). Previous work in coastal Newfoundland, however, generally reported the opposite. Indeed, Stanley et al. (2016) and Tripp et al. (2020) found high variation in otolith chemistry at broad scales (bay or coast), which supported high region-specific classification success in both juvenile Atlantic cod and larval capelin, respectively. However, in both studies, site-level variability was minimized; Stanley et al. (2016) pooled juvenile cod caught at different sites within bays and Tripp et al. (2020) sampled capelin larvae at only one site per bay. In the case of Tripp et al. (2020), the high classification success to natal bay may reflect site-level classification rather than bay-level classification. By contrast, our inclusion of multiple sites per bay revealed significant within bay variation in otolith chemistry, suggesting that these site-level differences may mask broader-scale differences in otolith chemistry.

Despite limited classification success, otolith chemical signatures in the pre-hatch region did differ significantly among bays in each year and some bays performed with moderate-high classification success. For example, Trinity Bay and Witless Bay had the highest classification success out of all the studied bays in 2023 (Figure 2.1, Table 2.2), which was also found in another study on Newfoundland larval capelin otolith chemistry (Tripp et al., 2020). The moderate-high classification success within these bays is likely due to Trinity and Witless Bay having distinct environmental conditions (Figure 2.1). Trinity Bay was the only studied bay in 2023 with a sheltered, semi-enclosed embayment (Figure 2.1). This feature may reduce exposure to the Labrador Current, which governs temperature and salinity patterns on the Newfoundland Shelf (Cyr, 2022), and, thus, may result in unique water mass properties (e.g., temperature, salinity) in Trinity Bay relative to other bays in this year. In support, additional semi-enclosed bays, such as Placentia and Conception bays, were sampled during 2024 and resulted in lower classification success of Trinity Bay. All of these bays share similar cliff coastal morphology, characterized by weathering and erosion that transport sediments along the shoreline (Catto, 2012), potentially influencing otolith chemistry (Hegg et al., 2013; Rachel et al., 2008) and, thus, decreasing the uniqueness of Trinity Bay. In contrast to Trinity Bay, the intertidal sites sampled near Witless Bay (Figure 2.1) were on the exposed southeast coast of Newfoundland and, thus, might be more strongly influenced by the Labrador Current. Interestingly, sites sampled within Notre Dame Bay are also on the exposed northeast coast and strongly influenced by the Labrador Current, but larvae from Witless Bay and Notre Dame Bay were rarely misclassified as each other. Overall, these findings suggest that under certain conditions, otolith chemistry can show bay-specific chemical signatures, likely driven by larger spatial variation in environmental factors that can influence otolith elemental incorporation (Bath et al., 2000; Coussau et al., 2023; Elsdon & Gillanders, 2002; Gillanders, 2002; Loeppky & Davoren, 2018).

The interannual variation in otolith chemistry observed at some of the sites with multi-year sampling (Table 2.4) indicates that otolith chemical signatures reflecting the natal site can vary across years. Other capelin studies have also noted interannual variability in pre-hatch otolith chemical signatures of field-reared capelin at a site (Davoren et al., 2015; Tripp et al., 2020). Shifts in water mass structure, air and water temperatures, and/or spawning adult composition across years may alter the chemical signatures at a site (Coussau et al., 2023; Elsdon & Gillanders, 2004; Gillanders, 2002), complicating efforts to accurately assign adults to their natal site. Interannual fluctuations in temperature are especially important to consider, as temperature influences trace element incorporation rates in complex ways within the pre-hatch region of capelin otoliths (Loeppky & Davoren, 2018). Interestingly, the summer of 2024 was much warmer than in 2023, ranking as the fourth warmest summer recorded in Canada (Environment and Climate Change Canada, 2024) with record-breaking sea surface temperatures on the Newfoundland Shelf (Coyne et al., 2025; DFO, 2025). As interannual variation in ocean temperature is likely to increase (Coyne et al., 2025; Soontiens et al., 2025), otolith chemistry will likely not consistently differ among bays across years and, thus, reliably assigning adults to their natal bay using otolith chemistry will likely involve knowledge of pre-hatch otolith chemical signatures corresponding to the adults' natal year.

Conclusion

Our findings indicate that, while our hypothesis was partially supported, the interannual variation and lower classification success at both the bay and regional (i.e. NAFO Division) levels in otolith chemistry in the pre-hatch (embryonic) region of capelin suggest this technique should be applied with caution when attempting to determine natal origins of adult capelin. Indeed, otolith chemistry may need to be used in unison with other methodologies to improve natal origin assignment and to determine relative productivity of bays, such as tracking studies on adults (Bradbury et al., 2008; Catalano et al., 2001; King et al., 2024; Robichaud & Rose, 2001), along with morphometric and genetic techniques (Milton & Chenery, 2001). Otolith chemistry also may be used in conjunction with other otolith traits, such as with back-calculated growth rates (Rogers et al., 2019) or morphology (Couillard et al., 2022) to improve natal origin assignments. Regardless of the partial support of our hypothesis, this study offers valuable insight into the challenges and limitations of using otolith chemistry in pre-hatch otolith region to determine the natal origin of fish. Indeed, by showing distinct site-specific otolith chemical signatures that may mask broad-scale differences among bays that decrease the ability to classify larvae at broader spatial scales, we identify a key constraint of using otolith chemistry to determine natal origin in marine fish. Therefore, when site-specific otolith chemical signatures of natal sites are unknown, we emphasize the need for careful interpretation of chemical signatures in otolith regions that represent early life stages when identifying natal origin.

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Tables

Table 2.1. Total number of larval capelin otoliths analyzed at each site across bays and collection years. Sites are grouped per bay. Counts reflect the number of otoliths ablated after outliers were removed. In total 198 otoliths were analyzed.

Region	Site	Year	Otolith Count
Conception Bay	Bryants Cove	2024	7
	Fresh Water	2024	9
Notre Dame Bay	Anchor Brook	2024	8
	Capelin Cove	2023	9
	Deadmans Bay	2023	9
	Mussel Shells	2023	8
	Shalloway	2024	10
Placentia Bay	Fox Cove	2024	8
	Tides Cove	2024	8
Trinity Bay	Bellevue	2023	7
	Bellevue	2024	8
	Big Chance Cove	2023	8
	Big Chance Cove	2024	10
Tor Bay	Middle Cove	2022	8
	Middle Cove	2023	7
	Middle Cove	2024	9
	Outer Cove	2022	10
	Outer Cove	2023	9
	Outer Cove	2024	8
Witless Bay	Bauline East	2023	8
	Lower Pond	2024	8
	Tors Cove	2023	5
	St.Micheal's Capelin Cove	2023	9
	St.Micheal's Capelin Cove	2024	8

Table 2.2. Classification success of larval capelin to their natal bay based on otolith trace element concentrations (Sr, Ba, Mg, Mn) in the pre-hatch region, based on linear discriminant function analyses (DFA) conducted separately for 2023 and 2024.

Year	Bay	Classification Success	No. of Otoliths	Overall Classification Success
2023	Notre Dame Bay	46.2%	26	
	Trinity Bay	73.3%	15	
	Witless Bay	72.7%	22	
	Tor Bay	31.3%	23	55.7%
2024	Notre Dame Bay	61.1%	18	
	Trinity Bay	59.1%	22	
	Placentia Bay	52.9%	17	
	Conception Bay	43.8%	16	
	Tor Bay	5.9%	17	
	Witless Bay	31.3%	16	32.7%

Table 2.3. Summary of ANOVA results assessing differences in each otolith trace element concentration among bays and sites nested within bays (site/bay) for larval capelin collected at intertidal sites in 2023 and 2024. Significant p-values ($p < 0.05$) are bolded.

Year	Element	Effect	df	df_{error}	F ratio	p-value
2023	Sr	Bay	3	69	3.72	**0.015**
		Site/Bay	6	69	4.50	**<0.001**
	Ba	Bay	3	69	13.88	**<0.001**
		Site/Bay	6	69	0.62	0.715
	Mg	Bay	3	69	29.27	**<0.001**
		Site/Bay	6	69	7.35	**<0.001**
	Mn	Bay	3	69	4.92	**0.004**
		Site/Bay	6	69	1.86	0.101
2024	Sr	Bay	5	89	2.91	**0.018**
		Site/Bay	6	89	2.53	**0.026**
	Ba	Bay	5	89	10.73	**<0.001**
		Site/Bay	6	89	4.61	**<0.001**
	Mg	Bay	5	89	2.68	**0.026**
		Site/Bay	6	89	1.08	0.378
	Mn	Bay	5	89	0.65	0.663
		Site/Bay	6	89	1.43	0.214

Table 2.4. Summary of ANOVA results assessing interannual differences in otolith trace element concentrations (Sr, Ba, Mg, Mn) in larval capelin collected at Bellevue (2023, 2024), Big Chance Cove (2023, 2024), and Middle Cove (2022, 2023, 2024) beaches. Significant p-values ($p < 0.05$) are bolded.

Site	Element	df	df_{error}	F ratio	p-value
Bellevue	Sr	1	13	59.53	**< 0.001**
	Ba	1	13	1.05	0.324
	Mg	1	13	12.23	**0.004**
	Mn	1	13	1.80	0.203
Big Chance Cove	Sr	1	16	0.96	0.342
	Ba	1	16	29.4	**< 0.001**
	Mg	1	16	14.19	**0.002**
	Mn	1	16	1.62	0.221
Middle Cove	Sr	2	21	9.70	**0.001**
	Ba	2	21	2.78	0.085
	Mg	2	21	3.77	**0.040**
	Mn	2	21	1.78	0.193

Table 2.5. Summary of water chemistry, water temperature, salinity (psu) collected at all sampling sites across bays and years. Values represent a single point measurement at each site. Concentrations of dissolved elements (in mg/L) are indicated.

Year	Region	Site	Date	Water Temperature (°C)	Salinity	Strontium (mg/L)	Barium (mg/L)	Magnesium (mg/L)	Manganese (mg/L)
2023	**Trinity Bay**	Bellevue	July 21	4.1	28.3	6.88	0.0059	1140	<LOD
		Big Chance Cove	July 22	5.5	27.8	7.21	0.0076	1170	0.0040
	Notre Dame Bay	Capelin Cove	July 29	19.0	26.5	6.90	0.0077	1080	0.0053
		Mussel Shells	July 18	16.9	26.7	6.46	0.0061	1060	<LOD
		Deadmans Bay	August 16			1.67	0.0026	262	0.0098
	Tor Bay	Outer Cove	July 22	16.3	27.7	6.85	0.0056	1100	0.0060
		Middle Cove	July 22	16.0	9.2	6.95	0.0061	1100	0.0122
	Witless Bay	Tors Cove	July 22	12.5	24.3	6.03	0.0053	967	0.0048
		Bauline East	July 22	8.5	26.6	6.32	0.0054	1020	0.0145
		St. Michael's Capelin Cove	July 22	9.5	27.7	6.95	0.0060	1140	0.0087
2024	**Trinity Bay**	Bellevue	July 18th	7.7	27.5	6.79	0.0077	1170	<LOD
		Big Chance Cove	July 18	4.6	25.3	6.81	0.0097	1140	0.0050
	Notre Dame Bay	Shalloway	July 24	21.2	26.3	6.38	0.0066	1060	<LOD
		Anchor Brook	July 24	22.0	12.2	3.40	0.0040	559	0.0027
	Tor Bay	Outer Cove	July 15	14.2	26.1	6.66	0.0061	1110	0.0046
		Middle Cove	July 15	13.6	26.4	6.01	0.0047	984	<LOD
	Witless Bay	Lower Pond	July 15	16.3	17.6	3.45	0.0043	581	0.0101
		St. Michael's Capelin Cove	July 15	15.5	25.1	6.50	0.0057	1100	<LOD
	Conception Bay	Freshwater Cove	August 3	16.6	21.3	6.62	0.0066	1130	0.0028
		Bryant's Cove	July 18	9.2	25.9	5.72	0.0049	993	0.0086
	Placentia Bay	Tide's Cove	July 25	11.7	27.7	6.71	0.0066	1150	0.0134
		Fox Cove	July 25	13.7	27.9	6.48	0.0058	1060	0.0705

Figures

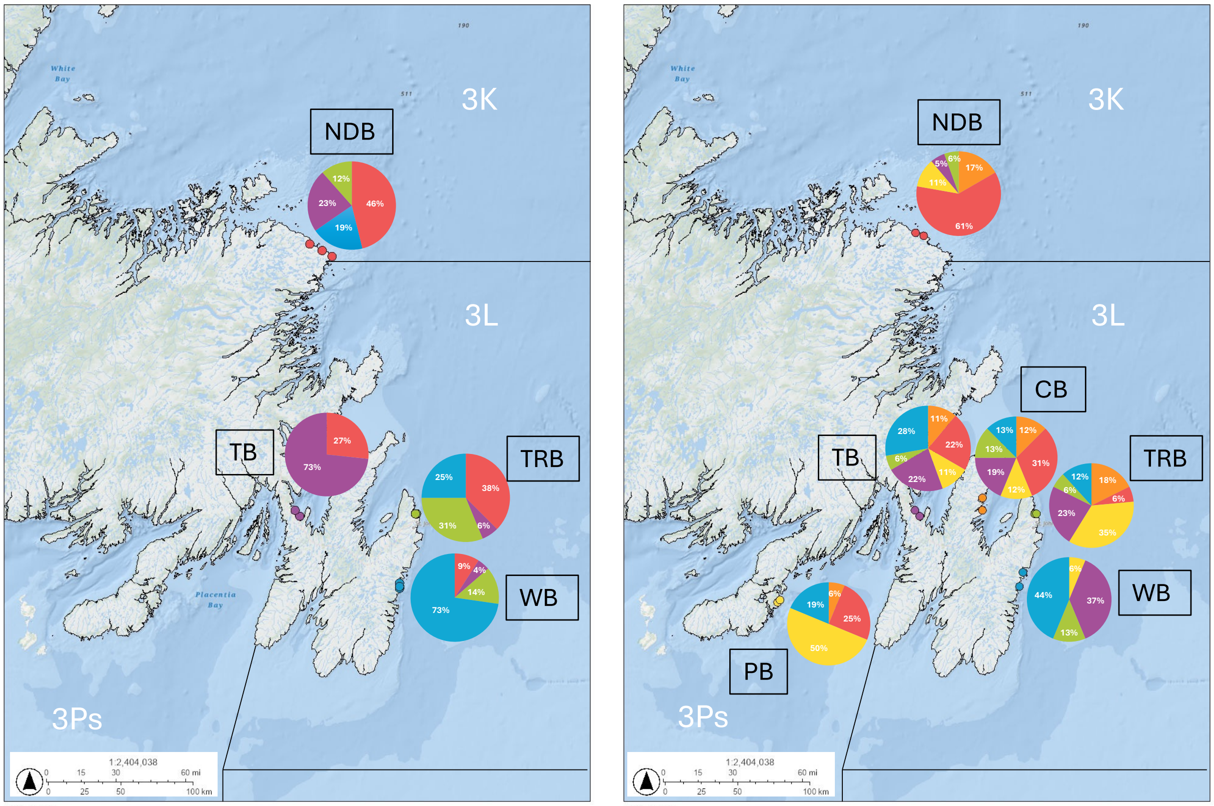

Figure 2.1. Map illustrating larval capelin sampling sites within bays (NDB: Notre Dame Bay; TB: Trinity Bay; TRB: Tor Bay; WB: Witless Bay; CB: Conception Bay; PB: Placentia Bay) within Northwest Atlantic Fisheries Organization (NAFO) divisions (3K, 3L, 3Ps) during each year (2023 on left, 2024 on right). Pie charts indicate the percentage of larvae sampled within each bay that were assigned to each sampled bay within a year based on discriminant function incorporating otolith chemistry. Each bay is represented by a different color.

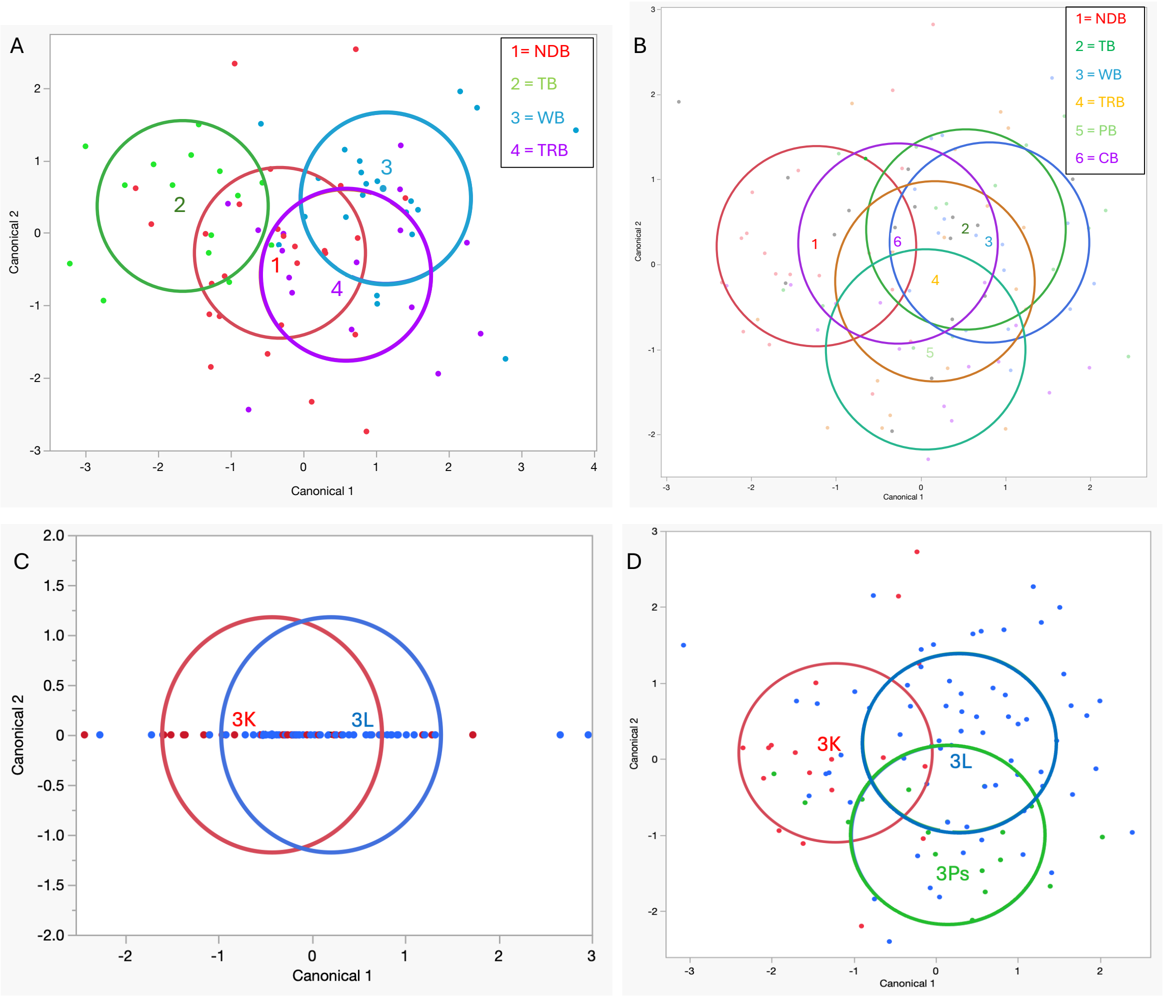

Figure 2.2. Linear discriminant function analysis (DFA) of larval capelin otolith trace element concentrations (Sr, Ba, Mg, Mn) collected during July–August of 2023 (A, C) and 2024 (B, D). Each point represents an individual larva, color-coded by collection region (A, B: Notre Dame Bay (NDB), Trinity Bay (TB), Witless Bay (WB), Tor Bay (Tor Bay), Placentia Bay (PB), and Conception Bay (CB)) or Northwest Atlantic Fisheries Organization (NAFO) Divisions (C, D: 3L, 3K, 3Ps). Ellipses represent 95% confidence contours around group centroids, indicating the multivariate spread of individuals within each bay.

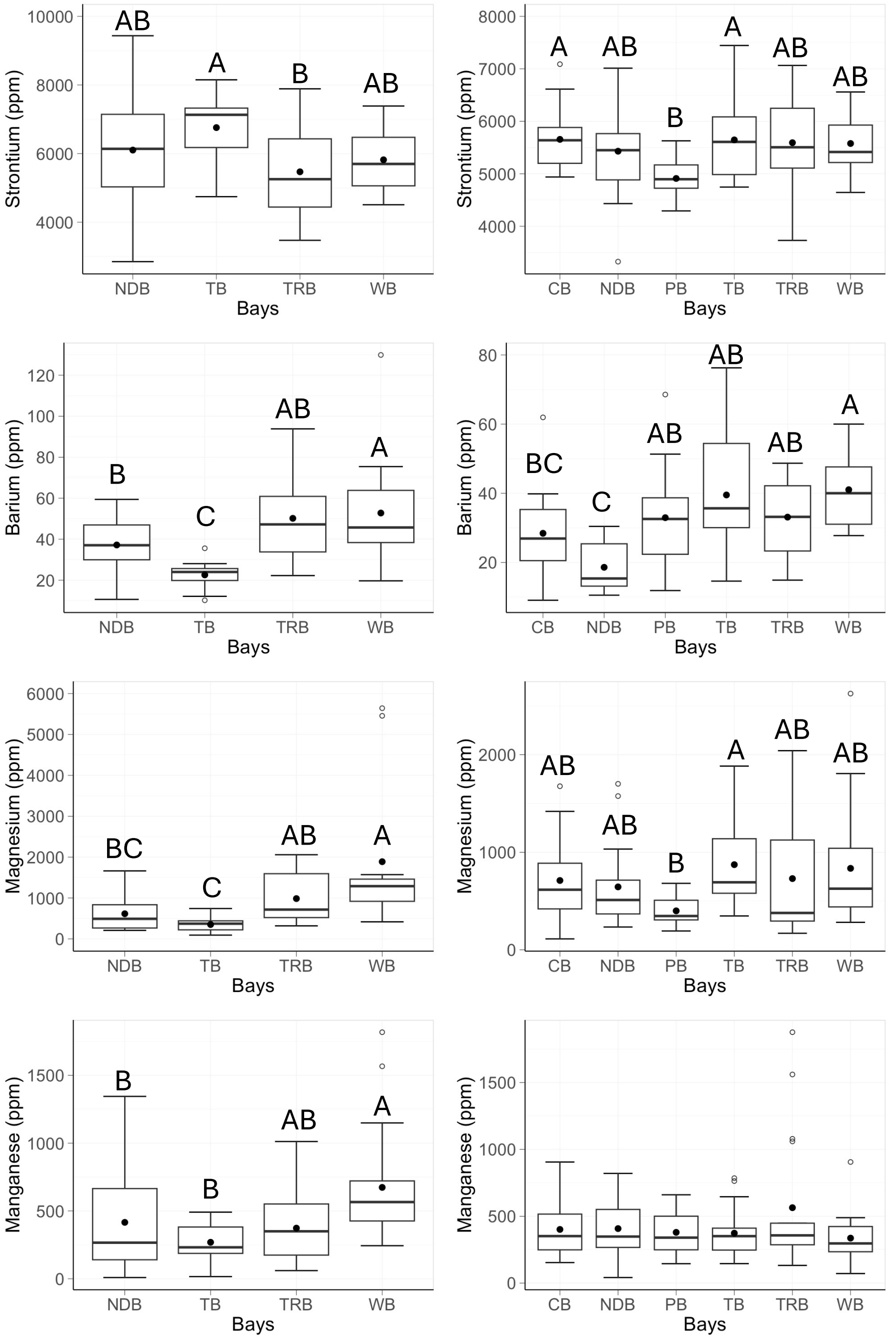

Figure 2.3. Otolith trace element concentrations in parts per million (ppm) for Strontium, Barium, Magnesium, and Manganese in larval capelin collected across bays in 2023 (left column) and 2024 (right column). Bays included are Notre Dame (NDB), Trinity (TB), Tor Bay (TRB), Witless (WB), Conception Bay (CB), and Placentia Bay (PB). Each row represents a different element (top to bottom: Sr, Ba, Mg, Mn). Letters above each bay indicate significant differences based on Tukey HSD post-hoc tests ($p < 0.05$). Boxes represent the interquartile range (IQR), horizontal lines within the boxes indicate medians, whiskers are 1.5 x IQR, and black points are the means. Outliers are shown as open circles.

Chapter Three: Connectivity of sand lance (*Ammodytes dubius*) on the Newfoundland Shelf

Abstract

Connectivity involves the exchange and movement of individuals among regions within a species' geographic range. Forage fish species play a vital role in pelagic marine food webs, serving as prey for numerous commercially and ecologically important species. Despite their ecological significance, dispersal and connectivity of forage fish remain largely unknown in the Northwest Atlantic (NWA), especially for species that are not commercially exploited, such as sand lance species (*Ammodytes dubius*). *Ammodytes* species in other regions (Pacific, Northeast Atlantic) are known to disperse as larvae and settle as juveniles at sites with sandy sediment, where they remain throughout their life. Our research aimed to investigate connectivity of *A. dubius* on the Newfoundland Shelf, Canada in the NWA using otolith chemistry of age 1 adults collected from inshore and offshore regions during 2019 and 2020. We quantified age-specific otolith concentrations of a variety of trace elements (strontium, barium, manganese, magnesium) using Laser Ablation Inductively Coupled Plasma-Mass Spectrometry. Chemical signatures were less distinct between inshore and offshore regions in the otolith region representing the larval period (i.e., otolith region near-core) than the otolith regions representing the juvenile, post-settlement phases, suggesting that there is lower connectivity post-settlement, as observed for other *Ammodytes* species. This research not only sheds light on the connectivity patterns of sand lance species in the NWA but suggests that otolith chemistry may be an important tool to develop effective conservation strategies (e.g., protection of productive areas) to ensure the long-term persistence of these vital forage fish species.

Introduction

Demographic connectivity (hereafter referred to as ‘connectivity’) involves the exchange and movement of individuals among regions (Cowen et al., 2006; Taylor et al., 1993; Turgeon et al., 2010) and the degree of connectivity is determined by whether individuals can successfully disperse to other regions (Cowen et al., 2006; Cowen & Sponaugle, 2009; Palumbi, 2003). Understanding connectivity of marine fish is critical for defining management units, or stocks, which is crucial for designing effective management regulations as well as establishing effective Marine Protected Areas (MPAs) (Sinclair & Smith, 2002; Stephenson, 2002).

For marine fish, the larval phase is the dominant life phase during which dispersal, or movement away from natal origins, occurs (Cowen et al., 2006; Cowen & Sponaugle, 2009). Determining the degree of dispersal across regions (‘connectivity’), poses challenges for marine fish because larvae are often too small for external tagging. Consequently, alternative tools are required to examine connectivity within these species (Cowen & Sponaugle, 2009). Otolith microchemistry is one tool that can be used to discriminate between different contingents of a population that may have minimal mixing, thereby providing key information to define stocks for fisheries management (Edmonds et al., 1991). Otoliths are inner ear stones that allow fish to hear, orientate, and balance (Campana & Neilson, 1985). Otoliths grow continuously throughout the fish's life and are metabolically inert, meaning all deposited trace elements become permanent within the otoliths. This chronological chemical record provides valuable insights into the environmental conditions the fish experienced throughout its lifespan, thereby providing information regarding an individual’s natal origins as well as dispersal and movement patterns (Campana & Neilson, 1985; Fowler et al., 1995). When otolith chemical signatures are compared across capture sites, differences or similarities can shed light on the degree of mixing among local population at different ages/stages of life. For example, natal origins and connectivity patterns have been uncovered using otolith chemistry in a number of forage fish species, including sandeels (*Ammodytes marinus*; Gibb et al., 2017), Atlantic silverside (*Menidia menidia*; Clarke et al., 2009), and capelin (*Mallotus villosus*; Tripp et al., 2020).

Environmental factors, including salinity, temperature, and ambient water chemistry, influence otolith trace element concentrations (Campana, 1999; Elsdon & Gillanders, 2002). As these factors can vary across regions within the same year, they influence the rate at which certain elements are incorporated onto the otolith, resulting in distinct region-specific otolith chemical signatures (Campana, 1999; Rogers et al., 2019). Otolith trace elements widely studied include strontium, barium, manganese, and magnesium, as they occur at detectable concentrations and reliably track environmental variation (Campana, 1999, 2005). In particular, strontium and barium are non-essential elements for fish and, as such, are not under physiological control during incorporation into the otolith (Campana, 1999). Therefore, their concentrations primarily vary with ambient water chemistry, influenced by factors such as bedrock and sediment composition, salinity, temperature, pH, and dissolved oxygen levels (Bath et al., 2000; Campana, 1999; Chowdhury & Blust, 2001; Hegg et al., 2013). By contrast, essential elements (e.g., magnesium, manganese) are under physiological control (Loewen et al., 2016) and typically vary less with ambient water chemistry. However, because temperature influences growth, otolith magnesium and manganese concentrations may increase under warmer conditions due to higher rates of incorporation into the body and, thus, the otoliths (Sturrock et al., 2014; Woodcock et al., 2012).

In the Northwest Atlantic (NWA), sand lance (*Ammodytes dubius* and *A. americanus*) are important forage fish species, providing prey for many top predators (Staudinger et al. 2020). *A. americanus* occurs near beaches, while *A. dubius* typically occurs away from beaches and farther offshore (Jones et al., 2023; Nizinkski et al., 1990; Scott, 1968). Although *Ammodytes* species are commercially fished in the Northeast Atlantic (Engelhard et al., 2014), they are not on the Newfoundland and Labrador Shelf. As such, connectivity along with the general ecology of sand lance species in this region remain poorly understood (Staudinger et al., 2020). NWA *Ammodytes* appear to spawn between late fall and winter, producing demersal, adhesive eggs that attach to sandy substrate and develop over approximately 1-3 months, depending on temperature (2-10℃) (Smigielski et al., 1984; Winters, 1983). After hatching, the larvae are planktonic and remain in the water column for several months (Norcross et al., 1961; Scott, 1968). Based on studies of *Ammodytes* species in other regions, including A. *marinus* and A. *tobianus* in the Northeast Atlantic and A. *hexapterus* in the Pacific, sand lance then settle into demersal habitats as juveniles at sites with suitable habitat characteristics, where they remain resident for their lifespan, rarely venturing more than 15 km (Gauld, 1990; Kunzlik et al., 1986; Robinson et al., 2013). These restricted movement and dispersal patterns from settlement sites are likely driven by the limited availability of suitable patches of coarse sand habitats with minimal silt (<10%), a habitat requirement likely resulting in the necessity to bury when they are not feeding to minimize energy expenditure and avoid predation due to their lack of a swim bladder (Engelhard et al., 2008; Robinson et al., 2013; van Der Kooij et al., 2008; Wright et al., 2000). Such suitable sediment patches are often limited and patchily distributed across broader regions, promoting strong site fidelity and constraining movement among patches (Robinson et al., 2013; Wright et al., 2000). Consequently, this reduced dispersal appears to result in minimal mixing and connectivity among regions at this life stage, as illustrated by distinct otolith trace element concentrations among sampling areas in the northeast Atlantic (Gibb et al., 2017; Laugier et al., 2015; Wright et al., 2018). As otolith chemistry studies on sand lance (*Ammodytes dubius*) on the Newfoundland Shelf have not been conducted (Staudinger et al., 2020), it is unclear whether the same patterns occur in this region.

Objectives

The objective of this chapter was to examine the connectivity among sand lance (*A. dubius*) in different regions across northeastern (inshore) and southeastern (offshore) regions of the Newfoundland Shelf using otolith chemistry. I hypothesized that larvae mix over a large geographic region but remain where they settle as juveniles throughout their lifespan, similar to *Ammodytes* species in other regions (Gibb et al., 2017). As such, I predicted that adult sand lance collected from inshore and offshore regions will show less distinct otolith chemistry signatures in the otolith region representing larval dispersal but otolith chemistry will be more distinct post-settlement, demonstrating limited connectivity as adults.

Methods

Sand lance were sampled from two broad regions on the Newfoundland Shelf, an inshore and offshore region within NAFO Division 3L during 2019 and 2020 (Figure 3.1). The inshore samples were collected at one site during July-August using a 23 cm^2 Ponar Bottom grab system (Morrison & Davoren, 2024), whereas the samples from the offshore region were collected at seven sites (2019: 4 sites; 2020: 3 sites) using a Campelen 1800 shrimp trawl (mesh size (knot centre): 80 mm (wings), 40-60 mm (bellies), codend lined with 12.5 mm knotless nylon mesh) by Fisheries and Oceans Canada during their annual spring (April – June) and autumn (September – December) multi-species bottom-trawl surveys. Standardized fishing sets were distributed in proportion to the stratum area (Rideout & Ings, 2018). Sites were approximately 15 to 220 km apart and sand lance used in this study were primarily caught in the autumn multi-species survey (Table 3.1). In the spring, up to 75 sand lance were randomly selected from trawl catches, and in the autumn, up to 25 sand lance were randomly selected from trawl catches. All fish were frozen within 4 hours of sampling. In the lab, fish were thawed and total length (mm), sex, and maturity index (0 = unknown, 1 = immature, 2= maturing, 3 = ripe, 4 = partially spent, 5 = spent, 6 = recovering) were recorded for up to 25 sand lance from each bottom grab/ autumn trawl and up to 50 sand lance from the spring survey before the otoliths were removed, cleaned, and placed in dry coin envelopes.

Otoliths were imaged and aged. First, the right sagittal otolith of each sand lance was immersed in water and photographed (4 to 5.6x) under reflected light with a dissecting microscope (Olympus SZX7). From the photographs, two readers independently aged all the otoliths. A pair of opaque (summer) and translucent (winter) bands, were considered to represent one year of growth, as described in the literature (Matta & Baker, 2020; Wright et al., 2018). These age readings were based on reference sand lance otoliths from published material (ICES, 1995; Laugier et al., 2015; Wright et al., 2018). Each reader assigned an ageing confidence score (i.e., good, fair, poor) to each otolith. Age estimates were compared between readers and if the ages differed, and a consensus could not be made based on the reference material, the otolith was not used further in the analysis. Additionally, total lengths of all confidently aged individuals were plotted by estimated age to summarize length-at-age (Figure S3.1). As the modal age was 1 across all regions and years, we focused otolith chemistry analysis on age 1 fish to ensure they represented the same cohort in each year.

Otoliths were cleaned in a 95% ethanol bath and then placed in epoxy (Epoxicure, Buehler). Once cured, a transverse cut was made on the rostrum side of the otoliths with an IsoMet 1000 Precision saw (with 15LC Wafering Blade, Buehler). The otoliths were polished with a series of polishing grits (16, 23, 36, and 53 µm) to reach the otolith core (i.e. pre-hatch region). Once the core was reached, a 0.1 µm polishing paper and aluminum polishing solution were used to gently remove any remaining impurities on the sample. A final transverse cut was made on the unpolished side of the otolith with a Precision saw to remove excess epoxy. The otoliths were then be mounted on a glass microscope slide, affixed with double sided tape (Scotch^{TM}), in the dimensions necessary to fit into the Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS) cell, with the polished core pointing upwards for ablation (a method modified from Fairclough et al., 2011). Prior to chemical analyses, photographs were taken of each sectioned otolith.

The otoliths were ablated with a line technique and a 30 µm laser diameter, repetition rate of 5 Hz, 40% output, and the beam traveled at 2 µm s^{-1} from the dorsal to ventral side of the otolith, passing through the core, when visible (Figure 3.2). Although a line spanning the entire cross-section of the otolith was ablated, we focused on the half of the transect from the core to the marginal edge on the longer side. We quantified the following elements with LA ICP-MS: Lithium (Li), Boron (B), Aluminum (Al), Potassium (K), Sodium (Na), Copper (Cu), Rubidum

(Rb), Molydenum (Mo), Lead (Pb), Uranium (U), Strontium (Sr), Barium (Ba), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Calcium (Ca).

Seasonal increment widths were then measured for each otolith with Image-J (version 1.54i 03) from the sectioned otolith images, which corresponded to different sections of the laser ablation transect extending from the core to the edge. These seasonal increments were identified optically, with opaque zones corresponding to periods of higher growth, presumably the summer, and translucent zones corresponding to periods of lower growth, presumably the winter (ICES, 1995). Four otolith growth increments were identified: near-core (larval dispersal), summer age 0 (S0; recently settled), winter age 0 (W0; settled), and summer age 1 (S1; settled) (Figure 3.2). The near-core region encompassed both the pre-hatch and subsequent larval winter growth and the S0 region represents recent settlement (Wright et al., 2018). In cases where the pre-hatch region was not clearly visible in the imaged otoliths (<15%), I examined elemental concentrations from the full line transect to identify the center of the mirror-imaged elemental profiles (a feature of full transects). The near-core region was defined around this central mirrored zone, where elemental peaks (e.g., manganese) were typically observed (Ben-Tzvi et al., 2007). As sand lance on the Newfoundland Shelf spawn between fall and winter (Richards, 1982; Staudinger et al., 2020, Winters 1983), the midpoint of the first winter increment (W0) would include an individual's first birthday (arbitrarily set to January 1) and, thus, technically encompasses year 0 and year 1 growth. As sand lance caught offshore were primarily sampled in September-November, whereas inshore sand lance were sampled in July-August (Table 3.1), offshore otoliths contained an additional 1-3 months of late-season growth (less than 230 um) beyond the S1 boundary (Figure 3.2). To ensure comparability, this excess otolith material was excluded from analysis. Therefore, S1 was the final increment examined in all individuals.

Data Analysis

All analyses were conducted in RStudio (R Core Team, 2025). Elemental concentration of each 30 µm ablation pit along each transect were assigned to a specific seasonal increment (near-core, S0, W0, S1) based on the increment width measurements from the sectioned otolith images. All 30 µm otolith trace element concentration measurements along the transect were examined for outliers (i.e. exceeded the 99.9th percentile from each trace element) caused by instrument error or surface contamination. Outliers were subsequently removed to reduce their influence on the average otolith trace element concentrations for each seasonal increment. Elements with concentrations frequently below or near the limit of detection were excluded from this study (i.e., Zn, Li, B, Al, Cu, Rb, Mo, Pb, U), along with elements (i.e., Na, K) that contributed little information to the otolith chemical patterns of interest. Therefore, the final analyses focused on otolith concentrations of Sr, Ba, Mg, and Mn, which were consistently above detection limits, as found in other studies on forage fish otolith chemistry papers on the Newfoundland Shelf (Davoren et al., 2015; Tripp et al., 2020). Limits of detections (LODs) were defined as three times the standard deviation of the blank signal, which were averaged across all blank analyses for Sr, Ba, Mg, and Mn. The LODs were as follows for the studied elements: ^{88}Sr = 0.01 ug/g; ^{137}Ba = 0.01 ug/g; ^{25}Mg = 0.06 ug/g; ^{55}Mn= 0.15 ug/g. The mean concentration of each trace element (Sr, Ba, Mn, Mg) was determined for each seasonal increment (near core, S0, W0, S1), similar to other studies (e.g., Davoren & Halden, 2014; Fowler et al., 2005; Steer et al., 2009).

To test my hypothesis, I assessed whether sand lance collected in different regions showed distinct season-specific (near core, S0, W0, S1) otolith trace element concentrations. First, I tested whether each response variable (i.e., otolith element concentrations and seasonal increment widths, IW) were normally distributed with homogenous variances using Shapiro-Wilks and Levene's tests, respectively. All response variables were log transformed to improve normality and meet the underlying parametric assumptions. I used repeated-measures LMMs (lmer function, lme4 package; Bates et al., 2015) to test whether IW and mean trace element (Sr, Ba, Mg, Mn) concentrations (response variables) were influenced by both predictor variables: region (inshore vs offshore) and seasonal increment (near core, S0, W1, S1), as well as their interaction (region x seasonal increment). Individual ID was a random effect to account for repeated measures on each otolith and separate analyses were run for each year. Prior to LMMs, we examined whether offshore sites could be pooled in our comparison of inshore versus offshore regions. Using only data from the offshore sites, LMMs were run using the same model structure described above to assess if offshore sites differed in increment widths (IW) and otolith trace element concentrations (response variables), with separate tests for 2019 and 2020. We pooled offshore sites in subsequent analyses as there were minimal differences in IWs and otolith trace element concentrations among offshore sites (Figure S3.2; Table S3.1 and S3.2), along with relatively low classification success across all and within each seasonal increment (Table S3.2). Unequal sample sizes between regions were accommodated by fitting linear mixed models that are robust to unbalanced designs. The influence of each predictor variable was assessed using F-tests (anova function, lmerTest package; Kuznetsova et al., 2017). Post hoc pairwise comparisons were conducted using EMMs (emmeans function; emmeans package) to compare regional differences (inshore vs pooled offshore sites) across each seasonal increment and differences across seasonal increments within each region.

We also used a multivariate approach, where principal components analyses (PCA; PCA function, FactoMineR package; Lê et al., 2008) were performed separately for 2019 and 2020 during data exploration to examine if the response variables (elemental concentrations across seasonal increments) explained the majority of the variation in each year's dataset. Further, discriminant function analyses (DFAs) were performed separately for each otolith seasonal increment and year to test for regional differences (inshore vs pooled offshore sites) in multi-element signatures (Sr, Ba, Mg, Mn; response variables). DFA models were validated using jackknifed (leave-one-out) cross-validation (lda function, MASS package; Ripley & Venables, 2009). This jackknifing procedure was used to reduce bias during classification, ensuring that each fish was classified using a model built without its own data, hence, the "leave-one-out" method (Quinn & Keough, 2002). This approach was particularly important given the relatively small and uneven sample sizes across the regions, which reduced overfitting and provided a more realistic measure of how well the otolith elemental signatures classify among regions. As groups with larger sample sizes tend to perform better (i.e. larger group is classified disproportionately higher) because their means and variances are estimated more precisely, we addressed this imbalance by equally weighting the groups *a priori* (argument: prior = equal within lda function; MASS package; Ripley & Venables, 2009) to ensure that classification of each region (inshore and pooled offshore sites) reflected the otolith element concentration differences rather than the sample size imbalance.

Results

For the inshore region, 8 to 9 otoliths of age 1 fish were available in each year (Table 3.1). For the offshore region, 6 to 10 otoliths of age 1 fish were available in each year from each site (Table 3.1). Overall, 77 otoliths from aged 1 fish were used in this study and all fish were immature (maturity index '1') or maturing (index '2'; Table 3.1).

In 2019 and 2020, IW differed significantly between inshore and offshore regions (except in 2020), among seasonal increments, and the interaction was also significant (Table 3.2). Post hoc comparisons revealed IW differed between regions across all seasonal increments in 2019, and between S0 and S1 for 2020 (Figure 3.3). The S0 increment had a significantly larger IW compared to the other seasonal increments within inshore and offshore sand lance, but other increments differed slightly in their width within regions and years (Figure 3.3).

In 2019, region (inshore vs. pooled offshore sites) significantly influenced all otolith trace element concentrations (Sr, Ba, Mg, Mn), but only Mg and Mn in 2020. Seasonal increments (near core, S0, W1, S1) significantly affected all otolith trace element concentrations but Ba in 2019, and all but Sr in 2020 (Table 3.2). Significant interactions occurred for all otolith trace element concentrations except Mg in 2019 and Ba and Mn in 2020 (Table 3.2). For both years, post hoc tests revealed no significant regional differences for otolith Sr concentrations at the near core or S0 increments; however, otolith Sr concentrations were significantly higher in fish caught offshore than inshore for W0 and S1 in 2019, and S1 in 2020 (Figure 3.3). Otolith Sr concentrations did not significantly differ among the seasonal increments within inshore sand lance in either year; however, Sr differed across seasonal increments for offshore sand lance in both years, with concentrations increasing with age (Figure 3.3). For otolith Ba concentrations, sand lance caught offshore in 2019 had significantly higher otolith concentrations than inshore fish at the near core, S0, and W0 increments, whereas no regional differences were found for S1, and concentrations did not differ among the seasonal increments within inshore or offshore sand lance (Figure 3.3). In 2020, otolith Ba concentrations did not differ between regions, but were significantly higher in the near-core region relative to W0 and S1 within the offshore fish (Figure 3.3). For otolith Mg concentrations, inshore fish in 2019 had significantly higher concentrations than offshore fish at S0 and S1, which was similar to higher concentrations found in inshore fish at W0 and S1 in 2020 (Figure 3.3). Otolith Mg concentrations differed significantly among most seasonal increments within inshore and offshore sand lance in both years, showing a general decrease in concentrations from near-core to S1 (Figure 3.3). For otolith Mn concentrations, concentrations were significantly higher inshore than offshore in all seasonal increments for fish caught in 2019 and W0 and S1 in 2020 (Figure 3.3). Otolith Mn concentrations also differed significantly among all seasonal increments within inshore and offshore sand lance in 2019 and most increments in 2020, again showing a general decrease in concentrations from near-core to S1 (Figure 3.3). Otolith Mn concentrations were also significantly higher inshore relative to offshore at all seasonal increments in 2019 and in W0 and S1 in 2020 (Figure 3.3).

In 2019, the PCA revealed five components with eigenvalues greater than 1, explaining 80.0% of the total variance in the otolith trace element concentrations (PC1 = 36.8%; PC2 = 17.3%; PC3 = 9.6%; PC4 = 9.2%; PC5 = 7.0%; Table 3.3, Figure 3.4). The response variables with the highest correlations (i.e., loadings) with PC1 were the S1 Mn concentration (r = 0.87) and W0 Mg and Mn (r =0.86), PC2 was near-core Ba concentrations (r = 0.71), PC3 was near-core Mn concentrations (r = 0.65), PC4 was near-core Sr concentrations (r = 0.71), and PC5 was near-core Mg concentrations (r = 0.60; Table 3.3, Figure 3.4). In 2020, the PCA revealed four components with eigenvalues over 1, explaining 74.0% of the total variation (PC1 = 27.4%, PC2 = 21.1%; PC3 = 14.7%; PC4 = 10.8%; Table 3.3, Figure 3.4). The response variables with the highest correlation with PC1 was W0 Mn concentrations (r = 0.94) and Mg S1 concentrations (r = 0.90), PC2 was S0 Sr (r = 0.78) and W0 concentrations (r = 0.76), PC3 was near-core Mn concentrations (r = 0.78), PC4 was near-core Ba concentrations (r = 0.68) (Table 3.3, Figure 3.4).

In 2019, DFAs including otolith concentrations of trace elements in all seasonal increments that compared fish caught inshore and pooled offshore sites revealed an overall significant difference, with a jackknifed-validated classification success of 78.6% when all seasonal otolith increments were pooled (Table 3.4, Figure 3.5). DFAs examining otolith concentrations of trace elements within each seasonal increment showed a significant difference in combined otolith trace element concentrations between the regions at all seasonal increments, with classification success ranging from 66.7-88.1 % (Table 3.4, Figure 3.5). In 2020, DFAs including otolith concentrations of trace elements in all seasonal increments that compared fish caught inshore and pooled offshore sites revealed an overall significant difference, with a jackknifed-validated classification success of 62.1% when all seasonal otolith increments were pooled (Table 3.4, Figure 3.5). DFAs examining otolith concentrations of trace elements within each seasonal increment revealed a lack of significant regional differences at the near-core and S0, but significant regional differences for W0 and S1, with classification success varying between 51.7- 72.4% (Table 3.4, Figure 3.5).

Discussion

After comparing the mean otolith trace element concentrations across season-specific increments and between age 1 sand lance caught inshore and offshore during two years, where inshore and offshore individuals within each year belonged to the same cohort, we found support for our hypothesis that sand lance larvae may mix during larval dispersal, but do not appear to mix once they settle as juveniles. Indeed, otolith chemistry was more distinct between sand lance caught inshore and offshore during post-settlement (i.e., otolith regions S0, W0, S1) relative to during larval dispersal (i.e. near-core otolith region), as evidenced by more significant differences in most otolith trace element concentrations and higher classification success of chemical signatures (especially in 2020) in otolith increments after the near-core (i.e. after larval dispersal). The lack of mixing post-settlement between inshore and offshore regions is further supported by significant differences in growth (i.e., seasonal increment width, IW) across all life-stages for sand lance sampled in 2019, and in the summers of fish sampled in 2020, which suggested regional differences in environmental conditions (e.g., temperature) and development during these periods of growth (Hüssy et al., 2003). These trends are similar to previous studies on sand lance species in other regions, where near-core otolith chemistry was less distinct than in later stages (*A. tobianus,* Laugier et al., 2015; *A. marinus,* Wright et al., 2018). Overall, these findings suggest that sand lance on the Newfoundland Shelf exhibit similar biology and behaviour, including higher connectivity as larvae and high site-fidelity post-settlement as juveniles and adults, to the better-studied *Ammodytes* species across the northeast Atlantic (Gibb et al., 2017; Laugier et al., 2015; Wright et al., 2018).

Most evidence suggests that sand lance caught inshore and offshore were likely mixing during larval dispersal or occupying regions with similar environmental conditions prior to settlement (Hüssy et al., 2003). Across both years, near-core otolith Sr and Mg concentrations did not differ, but otolith Ba and Mn concentrations and increment width (proxy of growth) differed between inshore and offshore fish in the near-core otolith region for fish caught in 2019. Differences in otolith Ba and Mn concentrations in the near-core region, however, may not always reflect environmental conditions during larval dispersal. Indeed, studies have shown these elements tend to be the most consistently enriched in the larval core, possibly due to differing maternal contributions, rapid early growth, and differences in crystalline structure within the newly formed otolith (Brophy et al., 2004; Loeppky et al., 2018; Ruttenberg et al., 2005). For instance, elevated Mn concentrations in the otolith core of lab-reared and wild-caught Atlantic herring (*Clupea harengus*) and wild-caught sprat (*Sprattus* sprattus) were attributed to embryological development (i.e., maternal investments, early growth patterns, higher calcite composition within the core), rather than environmental variables (i.e., water chemistry, spawning substrate), as elevated otolith Mn concentrations were consistent in the core regardless of egg incubation substrate (e.g., gravel or glass; Brophy et al., 2004). Additionally, Ba concentrations within the otolith pre-hatch region of lab-reared larval capelin showed family-specific concentrations, suggesting differing maternal investment during gonadal development (Loeppky et al., 2018). Therefore, regional differences in Ba and Mn concentrations in the near-core otolith region observed in this study might not indicate that larvae do not mix during dispersal or originated from different sources (i.e. locations). This interpretation is consistent with the generally low classification success of fish from offshore and inshore regions based on otolith chemical signatures during larval dispersal (Table 3.4, Figure 3.3).

Although otolith chemistry differences were minimal in the near-core region, it remains unclear whether all larvae originated from the same source or from multiple sources with similar water mass features (e.g., temperature, salinity, water chemistry). By contrast, studies on *Ammodytes* species in other regions reported variable elemental concentrations in the near-core region, which allowed classification of larvae into multiple unidentified sources using unsupervised cluster analyses (Gibb et al., 2017; Laugier et al., 2015; Wright et al., 2018). These studies, however, also noted overlap in near-core chemical signatures among natal sources, suggesting larval mixing, or similar site-specific environment conditions (Gibb et al., 2017; Wright et al., 2018). Overall, more research will be needed to resolve whether inshore and offshore sand lance on the Newfoundland shelf originate from the same or multiple natal sources.

Inshore and offshore sand lance otolith chemistry and growth (increment width) differed more post-settlement (i.e. after the near-core otolith region) than during larval dispersal (i.e. near-core region), suggesting that sand lance caught inshore and offshore likely occupied different sites with distinct water mass features (i.e. did not mix) post-settlement. Indeed, otolith chemical signatures distinguished inshore and offshore fish with high classification success (Table 3.4), and more otolith trace elements differed significantly in concentration between regions as age increased for fish caught in both years. Interestingly, otolith Sr concentrations were significantly higher in offshore compared to inshore fish during post-settlement, especially in S1, and Sr increased with age in the offshore but not the inshore fish. As Sr is a non-essential element, varying otolith concentrations have been commonly used to indicate occupancy in different waterbodies in other studies, with higher concentrations often associated with higher salinity (Bath et al., 2000; Campana, 1999; Elsdon & Gillanders, 2004; Hegg et al., 2013). Therefore, one interpretation of these patterns is that offshore fish settled in areas of higher salinity after mixing within inshore fish as larvae, whereas inshore fish remained in areas similar to those experienced as larvae. The lower salinity of the inshore fish throughout their seasonal increments and offshore fish in the near-core region suggests a possible nearshore natal source, given the higher potential for lower salinity in coastal areas from freshwater input. This aligns with the literature describing the salinity gradient along the Newfoundland Shelf, where inshore regions typically have lower salinity than offshore regions (Cyr et al., 2022).

Interestingly, otolith Mn and Mg concentrations were higher for inshore relative to offshore fish during post-settlement in both years. As both Mn and Mg are essential elements (i.e., physiologically regulated), these findings suggest persistent physiological differences between regions (Sturrock et al., 2014; Woodcock et al., 2012). For instance, as concentrations of both elements tend to increase with temperature and, thus, growth rate, these elevated concentrations suggest that inshore sand lance experienced warmer, faster-growth conditions post-settlement (Sturrock et al., 2014; Woodcock et al., 2012). Our increment widths support this interpretation, being greater inshore than offshore post-settlement in S0 for fish caught in

2019 and S1 for those caught in 2020, reflecting spatially distinct growth patterns particularly in the summers. Additionally, studies have found that otolith Mn concentrations are typically higher at coastal sites (*A. tobianus;*Laugier et al., 2015; *Solea solea;* Tanner et al., 2011), as the bottom waters within inshore regions may be more enriched in Mn due to terrigenous inputs (i.e., coastal erosion, sediment and nutrient transport; Laës et al., 2007; Shiller, 1997). Higher dissolved Mn concentrations also are often associated with hypoxic conditions in bottom waters and sediments, where Mn released from anoxic sediments can become bioavailable for uptake into the otoliths of benthic fish (Limburg et al., 2011, 2015; Macdonald & Gobeil, 2012). As anoxic conditions may be higher in coastal regions, where nutrient concentrations may be higher (i.e., from land runoff), leading to a higher probability of algae blooms and oxygen depletion near the seafloor relative to offshore regions (Limburg et al., 2011; Middelburg & Levin, 2009), these conditions may explain higher otolith Mn concentrations in sand lance caught inshore relative to offshore in our study. Overall, our findings are similar to previous studies on sand lance species in other regions indicating that otolith Mn concentration is an important element driving the classification of fish from different sites (*A. tobianus*, Laugier et al., 2015; *A. marinus*, Wright et al., 2018).

Fish from both regions showed similar general trends in otolith chemistry across seasonal increments. Indeed, otolith concentrations of both essential elements (Mg, Mn) consistently declined from near-core to S1 (Figure 3.3), and concentrations of these elements explained most of the variation in the dataset (Table 3.3, Figure 3.4). These trends were similarly found in *A. tobianus* (Laugier et al., 2015) and *A. marinus* (Wright et al., 2018) with Mg otolith concentrations being the main element differing among age and seasonal otolith increments. Consistent declines in otolith Mg and Mn concentrations with age may reflect a corresponding decrease in somatic growth throughout the lifespan (Hüssy et al., 2021).

Conclusion

Our findings indicate support for the hypothesis that sand lance (*A. dubius*) larvae likely mix during the pelagic dispersal phase but show strong regional fidelity after settlement as juveniles. This research provides the first otolith chemistry-based assessment of connectivity of NWA sand lance and offers new insight into the spatial structure of this poorly studied forage fish on the Newfoundland Shelf. Otolith chemistry proved to be an effective tool for detecting broad-scale patterns, where the near-core elemental signatures showed minimal regional differences, consistent with larval mixing or shared water mass characteristics, whereas post-settlement otolith chemistry and increment widths indicated region-specific differences. Knowledge gaps, however, persist concerning whether all larvae originated from the same source or from multiple sources with similar water mass features (e.g., temperature, salinity, water chemistry). As previous studies on other *Ammodytes* species identified multiple natal sources using unsupervised classification techniques (Gibb et al., 2017; Wright et al., 2018), applying these techniques and targeted larval sand lance surveys would help clarify the location of the natal sources in the NWA. Additionally, expanding the spatial coverage of adult sampling sites used in this study, increasing sample sizes, and incorporating otolith back-calculations of growth, could help better understand NWA sand lance connectivity. Given the critical role of forage fish in marine food webs, continued research on sand lance populations and connectivity is essential to inform the conservation and ecosystem-based fisheries management (Cury et al., 2000; Pikitch et al., 2012; Staudinger et al., 2020).

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Tables

Table 3.1. The age 1 sand lance examined in this study (would have turned two years old in January), including the year and month fish were collected, the region (inshore or offshore), the site name, how many age 1 fish were available, and their total length range (mm). The percentage of females (i.e. sex ratio) is also provided. All fish were either immature or maturing.

Year	Month	Region	Site	Latitude	Longitude	# of Otoliths	Length Range (mm)	% of Females
2019	July-August	Inshore	Gull 3	49.249	-53.410	9	119-180	44.4%
2020	July-August	Inshore	Gull 3	49.249	-53.410	8	120-195	62.5%
2019	November	Offshore	Set 33 – (S29)	46.963	-50.217	8	138-161	75.0%
2019	October	Offshore	Set 12- (S29)	46.603	-50.145	10	121-196	60.0%
2019	October	Offshore	Set 60 – (U30)	46.603	-48.212	6	138-159	66.7%
2019	June	Offshore	Set 88 – (U29)	46.845	-48.883	9	136-194	44.4%
2020	September	Offshore	Set 10- (V29)	46.946	-47.948	6	140-197	66.7%
2020	October	Offshore	Set 47- (V29)	46.721	-50.800	8	160- 191	37.5%
2020	October	Offshore	Set 55- (U29)	46.822	-48.698	7	143-180	57.1%

Table 3.2. Results of repeated-measures linear mixed-effects models (LMMs) testing the effects of region (inshore vs. pooled offshore sites), seasonal increment (season: near core, S0, W1, S1), and their interaction on increment widths (IW) and on otolith chemical concentration (Sr: strontium; Ba: barium; Mg: Magnesium; Mn: manganese) for 2019 and 2020. Significant effects ($p < 0.05$) are bolded

Year	Response	Effect	df	df_{error}	F ratio	p-value
2019	Increment Width	Region	1	40	11.21	**p = 0.002**
		Season	3	120	138.52	**p < 0.001**
		Region x Season	3	120	6.69	**p < 0.001**
	Sr	Region	1	40	6.13	**p = 0.018**
		Season	3	120	3.04	**p = 0.032**
		Region x Season	3	120	5.48	**p = 0.001**
	Ba	Region	1	40	18.04	**p < 0.001**
		Season	3	120	0.99	p = 0.397
		Region x Season	3	120	3.73	**p = 0.013**
	Mg	Region	1	40	5.09	**p = 0.030**
		Season	3	120	320.99	**p < 0.001**
		Region x Season	3	120	0.25	p = 0.861
	Mn	Region	1	40	26.25	**p < 0.001**
		Season	3	120	88.85	**p < 0.001**
		Region x Season	3	120	2.88	**p = 0.039**
2020	Increment Width	Region	1	27	0.02	p = 0.89
		Season	3	81	39.70	**p < 0.001**
		Region x Season	3	81	3.53	**p = 0.018**
	Sr	Region	1	27	2.83	p = 0.104
		Season	3	81	2.65	p = 0.054
		Region x Season	3	81	4.02	**p = 0.010**
	Ba	Region	1	27	3.27	p = 0.082
		Season	3	81	5.66	**p = 0.001**
		Region x Season	3	81	0.55	p = 0.648
	Mg	Region	1	27	6.61	**p = 0.019**
		Season	3	81	145.37	**p < 0.001**
		Region x Season	3	81	4.72	**p = 0.004**
	Mn	Region	1	27	4.92	**p = 0.035**
		Season	3	81	31.31	**p < 0.001**
		Region x Season	3	81	1.86	p = 0.143

Table 3.3. Principial component loadings for 2019 and 2020 based on otolith trace element concentrations (Sr, Ba, Mg, Mn) across seasonal increments (near core, summer 0, winter 0, summer 1). Bolded values indicate the variable with the strongest correlation per principal component (PC). Variance explained by each principal component is shown in parentheses.

Variable	2019					2020			
	PC1 (36.8%)	**PC2** (17.3%)	**PC3** (9.6%)	**PC4** (9.2%)	**PC5** (7.0%)	**PC1** (27.1%)	**PC2** (21.1%)	**PC3** (14.7%)	**PC4** (10.8%)
Sr: Near Core	0.02	0.54	-0.19	**0.71**	-0.15	0.07	0.66	0.00	0.10
Ba: Near Core	-0.24	**0.71**	-0.26	-0.39	-0.05	-0.33	0.40	-0.03	**0.68**
Mg: Near Core	0.67	0.11	0.21	0.08	**0.60**	0.46	0.07	0.67	0.29
Mn: Near Core	0.59	-0.35	**0.65**	-0.12	-0.20	-0.02	-0.48	**0.78**	-0.11
Sr: Summer 0	0.27	0.42	0.15	0.57	-0.36	0.48	**0.78**	0.06	-0.08
Ba: Summer 0	-0.11	0.65	0.10	-0.48	-0.18	-0.19	0.44	0.35	0.61
Mg: Summer 0	0.72	0.05	-0.10	0.05	0.50	0.72	-0.01	0.37	0.40
Mn: Summer 0	0.66	-0.31	0.56	0.06	-0.20	0.17	-0.55	0.75	-0.19
Sr: Winter 0	-0.71	0.29	0.38	0.29	0.15	-0.08	0.76	0.34	-0.21
Ba: Winter 0	0.37	0.66	0.25	-0.19	-0.17	0.26	0.49	0.30	-0.32
Mg: Winter 0	0.86	0.22	-0.18	-0.05	0.07	**0.94**	0.03	-0.13	0.01
Mn: Winter 0	0.86	0.03	-0.16	-0.04	-0.10	0.76	-0.29	-0.08	-0.14
Sr: Summer 1	-0.72	0.23	0.23	0.22	0.37	-0.54	0.45	0.22	-0.24
Ba: Summer 1	0.09	0.68	0.47	-0.12	0.18	-0.03	0.56	0.22	-0.58
Mg: Summer 1	**0.87**	0.23	-0.17	-0.02	0.05	0.90	0.10	-0.14	0.12
Mn: Summer 1	0.75	0.09	-0.22	0.21	-0.06	0.78	0.19	-0.37	-0.15

Table 3.4. Multivariate analysis of variance (i.e. DFA) results testing for regional differences (inshore vs pooled offshore sites) in otolith trace element concentrations (Sr, Ba, Mg, Mn) across all increments and within each seasonal increment (i.e., near-core, summer 0, winter 0, and summer 1) for 2019 and 2020. Discriminant function analysis results showing total, inshore and offshore classification success. Significant p-values are bolded.

Year	**Grouping Factor**	**Wilks λ**	**Approx. F**	**num Df**	**den Df**	**p-value**	**Total Classification**	**Inshore Classification**	**Offshore Classification**
2019	All Increments	0.68	19.38	4	163	**$p < 0.001$**	78.6%	77.8%	78.8%
	Near-Core	0.50	9.18	4	37	**$p < 0.001$**	83.3%	77.8%	84.8%
	Summer 0	0.40	13.70	4	37	**$p < 0.001$**	88.1%	88.9%	87.9%
	Winter 0	0.63	5.51	4	37	**$p = 0.001$**	73.8%	77.8%	72.7%
	Summer 1	0.67	4.50	4	37	**$p = 0.005$**	66.7%	55.6%	69.7%
2020	All Increments	0.87	3.99	4	111	**$p = 0.005$**	62.1%	65.6%	60.7%
	Near-Core	0.86	0.98	4	24	$p = 0.436$	62.1%	62.5%	61.9%
	Summer 0	0.90	0.65	4	24	$p = 0.631$	51.7%	37.5%	57.1%
	Winter 0	0.67	2.94	4	24	**$p = 0.041$**	69.0%	75.0%	66.6%
	Summer 1	0.54	5.03	4	24	**$p = 0.004$**	72.4%	62.5%	76.2%

Figures

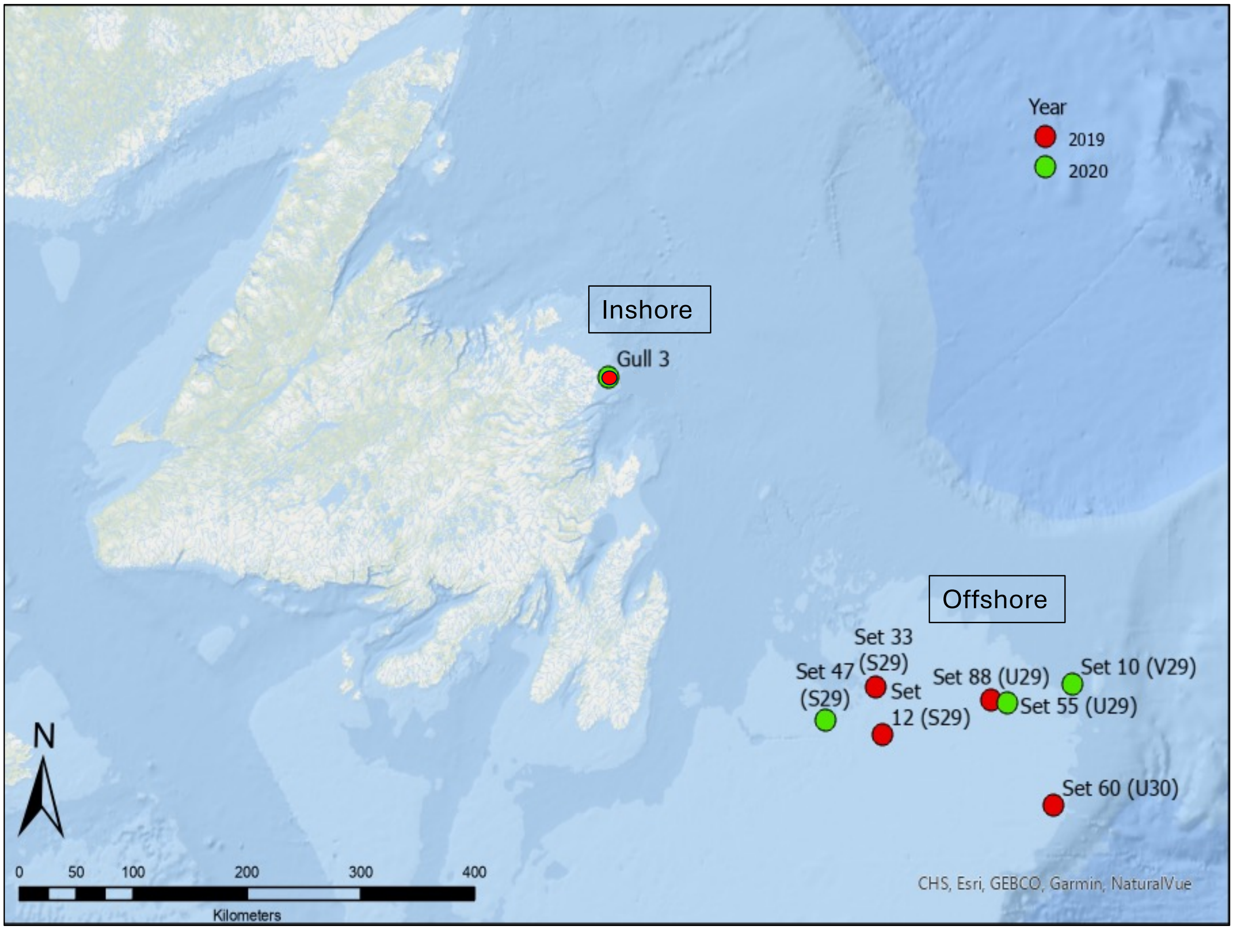

Figure 3.1. Sand lance sampling sites during 2019 and 2020 on the Newfoundland Shelf, Canada, with the study regions labeled (inshore and offshore). Red circles represent the sample year 2019 and green circles represent 2020. Created in ArcGIS. See Table 3.1 for sampling details.

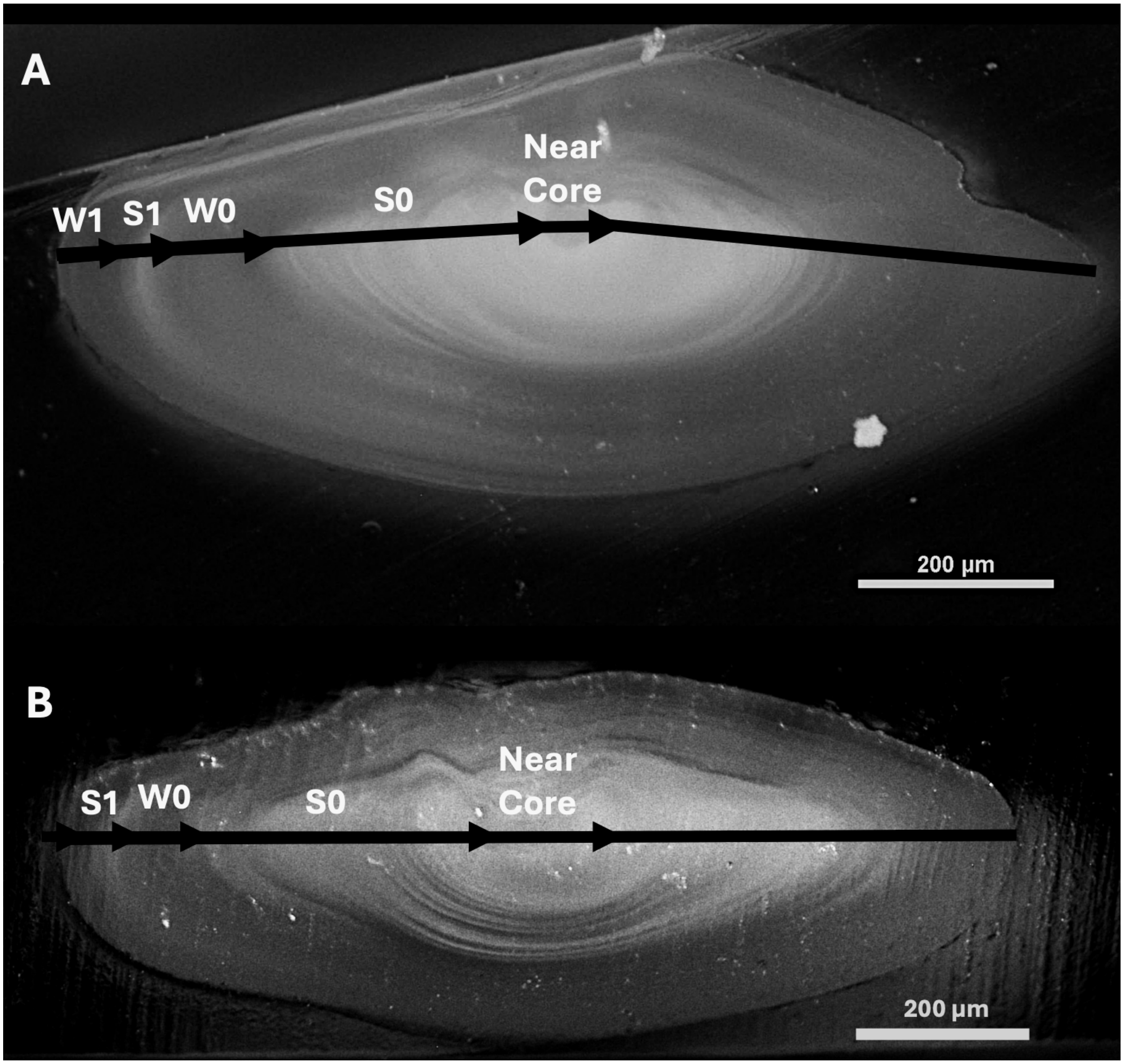

Figure 3.2. Images of two sand lance otoliths, one collected offshore (A) and one collected inshore (B) during 2019. Labeled are the measured seasonal increment regions (from arrow tip-to-tip): near core, summer age 0 (S0), winter age 0 (W0), and winter age 1 (W1). Note: the offshore sand lance (A) was collected in October and, thus, had W1 extra growth compared to the inshore sand lance (B).

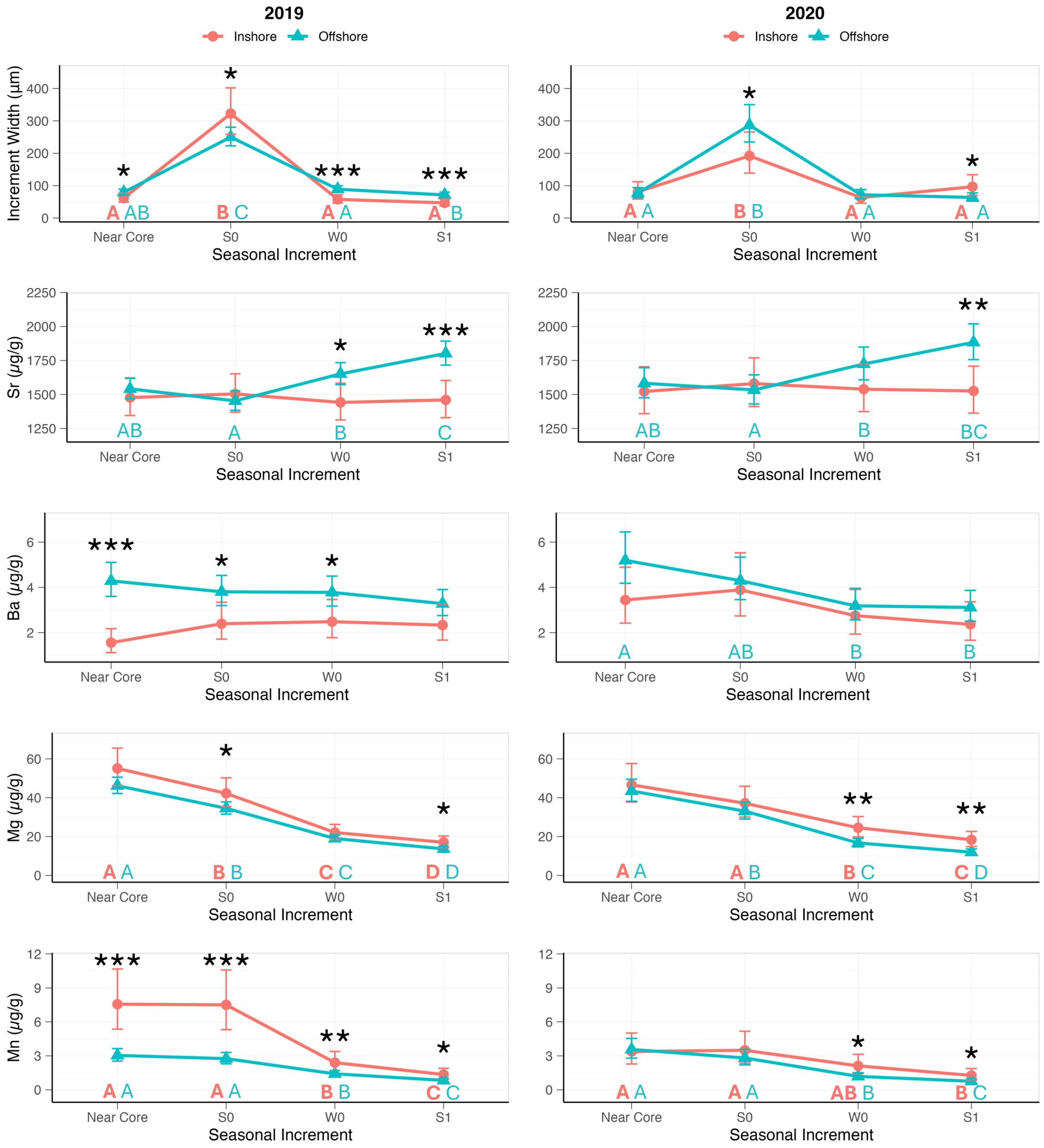

Figure 3.3. Estimated marginal means (EMMs; back transformed from log scale) of increment width (µm) and otolith trace element (Sr: strontium; Ba: barium; Mg: Magnesium; Mn: manganese) concentrations (µg/g) of sand lance collected from inshore (red circle) versus pooled offshore sites (blue triangle) for each seasonal otolith region (near core; S0 = summer age 0; W0 = winter age 0; S1 = summer age 1) during 2019 (left) and 2020 (right). Letters represent significant differences within a seasonal increment from within an individual color-coded region. Whiskers represent 95% confidence intervals. Asterisks indicate a significant difference in otolith trace element concentrations during a specific seasonal increment between regions based on Tukey-adjusted post hoc tests (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

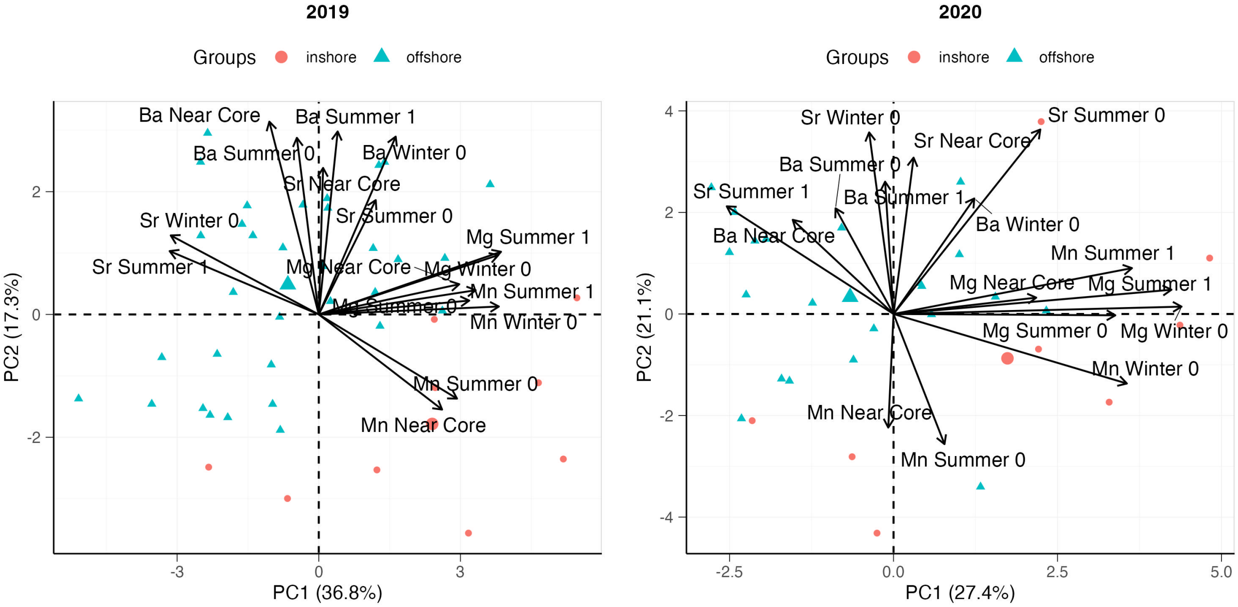

Figure 3.4. Principal component analysis biplots of otolith trace element concentrations (log-transformed: Sr, Ba, Mg, Mn) within each seasonal increment (near core, summer 0, winter 0, summer 1) for sand lance collected in 2019 (left) and 2020 (right). Arrows represent variable vectors, indicating direction and magnitude of each otolith trace element concentration across seasonal increment's contribution to the principal components.

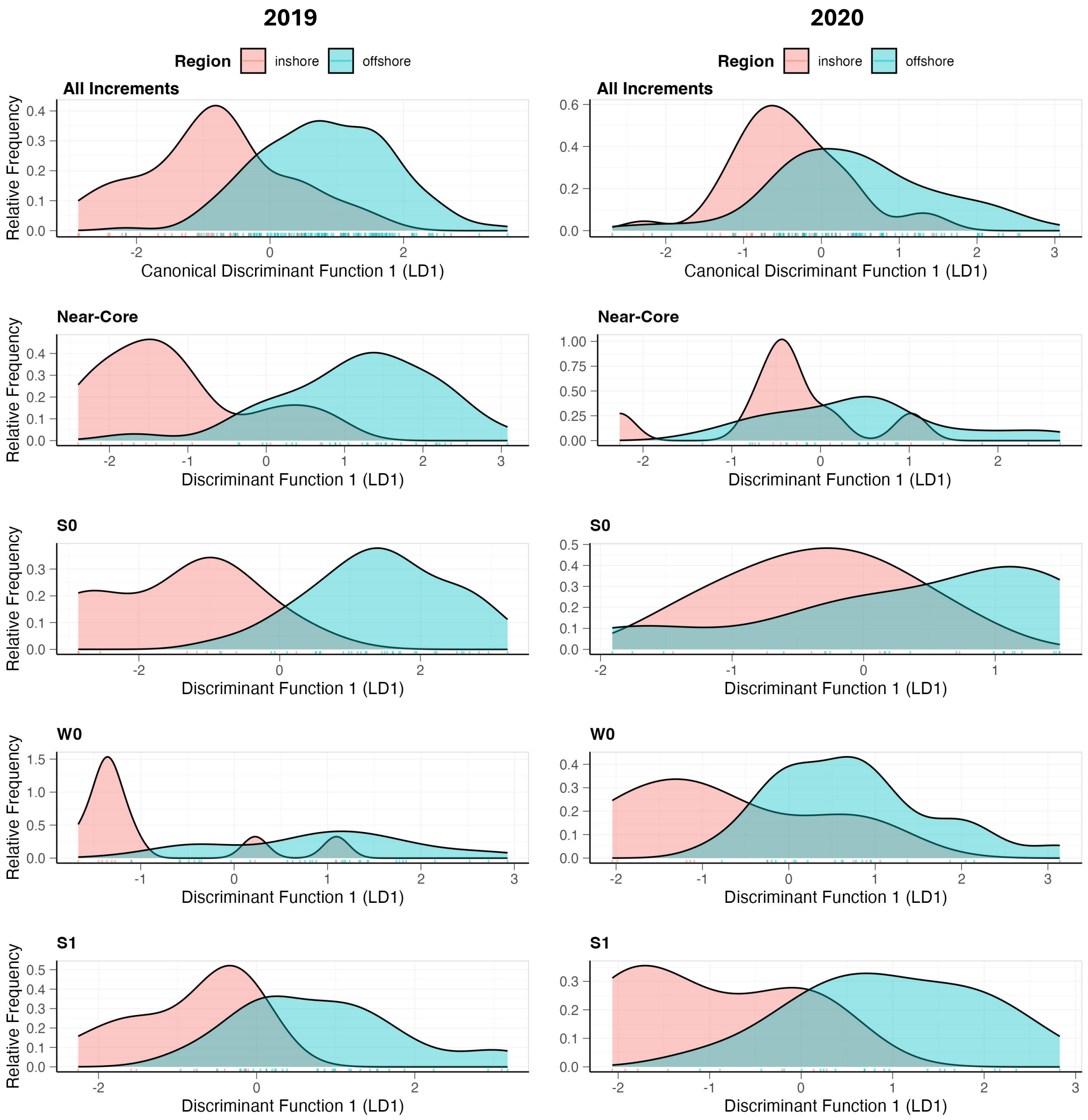

Figure 3.5. Discriminant function analysis of otolith trace element concentrations (strontium, barium, magnesium, manganese) across otolith seasonal increments (near-core, S0= summer age 0, W0 = winter age 0, S1 = summer age 1) for sand lance collected from inshore (red) and pooled offshore (blue) regions during 2019 (left) and 2020 (right). Density plots show distributions of LD1 scores representing regional separation and overlap based on multielement otolith signatures for otolith increment. The y-axis represents the relative frequency (i.e., probability density), scaled so that total area under each curve equals one. Note that peaks exceeding 1.0 on the y-axis represent tightly cluster discriminant score, not that the values exceed 100% frequency.

Supplementary Material

Table S3.1. Results of repeated-measures linear mixed-effects models (LMMs) testing the effects of offshore sites, seasonal increment (season: near core, S0, W1, S1), and their interaction on increment widths (IW) and on otolith chemical concentration (Sr: strontium; Ba: barium; Mg: Magnesium; Mn: manganese) for 2019 and 2020. Significant effects ($p < 0.05$) are bolded.

Year	Response	Effect	df	df_{error}	F ratio	p-value
2019	Increment Width	Site	3	116	2.76	**p = 0.045**
		Season	3	116	135.88	**p < 0.001**
		Site x Season	9	116	3.50	**p < 0.001**
	Sr	Site	3	29	1.77	p = 0.174
		Season	3	87	20.69	**p < 0.001**
		Site x Season	9	87	3.87	**p < 0.001**
	Ba	Site	3	29	1.86	p = 0.158
		Season	3	87	2.78	**p = 0.046**
		Site x Season	9	87	1.96	p = 0.053
	Mg	Site	3	29	4.83	**p = 0.008**
		Season	3	87	421.90	**p < 0.001**
		Site x Season	9	87	2.03	**p = 0.045**
	Mn	Site	3	29	0.88	p = 0.461
		Season	3	87	74.63	**p < 0.001**
		Site x Season	9	87	1.31	p = 0.242
2020	Increment Width	Site	2	72	9.73	**p < 0.001**
		Season	3	72	69.30	**p < 0.001**
		Site x Season	6	72	3.14	**p = 0.008**
	Sr	Site	2	18	0.45	p = 0.646
		Season	3	54	12.69	**p < 0.001**
		Site x Season	6	54	0.86	p = 0.525
	Ba	Site	2	18	1.68	p = 0.213
		Season	3	54	7.41	**p < 0.001**
		Site x Season	6	54	3.31	**p = 0.007**
	Mg	Site	2	18	0.29	p = 0.750
		Season	3	54	171.69	**p < 0.001**
		Site x Season	6	54	0.88	p = 0.512
	Mn	Site	2	18	1.47	p = 0.256
		Season	3	54	48.63	**p < 0.001**
		Site x Season	6	54	0.65	p = 0.687

Table S3.2. Multivariate analysis of variance (DFA) results testing for offshore site differences in otolith trace element concentrations (Sr, Ba, Mg, Mn) across all increments grouped, near-core, summer 0, winter 0, and summer 1 otolith increments for 2019 and 2020. Discriminant function analysis results showing total percent classification success. Significant p-values are bolded.

Year	Grouping Factor	Wilks λ	Approx. F	num Df	den Df	p-value	Total Classification
2019	All Increments	0.76	3.02	12	331	**$p < 0.001$**	39.4%
	Near-Core	0.28	3.58	12	69	**$p < 0.001$**	63.6%
	Summer 0	0.53	1.58	12	69	$p = 0.119$	33.3%
	Winter 0	0.41	2.31	12	69	**$p = 0.015$**	45.5%
	Summer 1	0.34	2.87	12	69	**$p = 0.003$**	42.4%
2020	All Increments	0.81	2.15	8	156	**$p = 0.034$**	40.5%
	Near-Core	0.53	1.39	8	30	$p = 0.241$	23.8%
	Summer 0	0.46	1.77	8	30	$p = 0.121$	47.6%
	Winter 0	0.80	0.44	8	30	$p = 0.884$	19.0%
	Summer 1	0.84	0.34	8	30	$p = 0.945$	23.8%

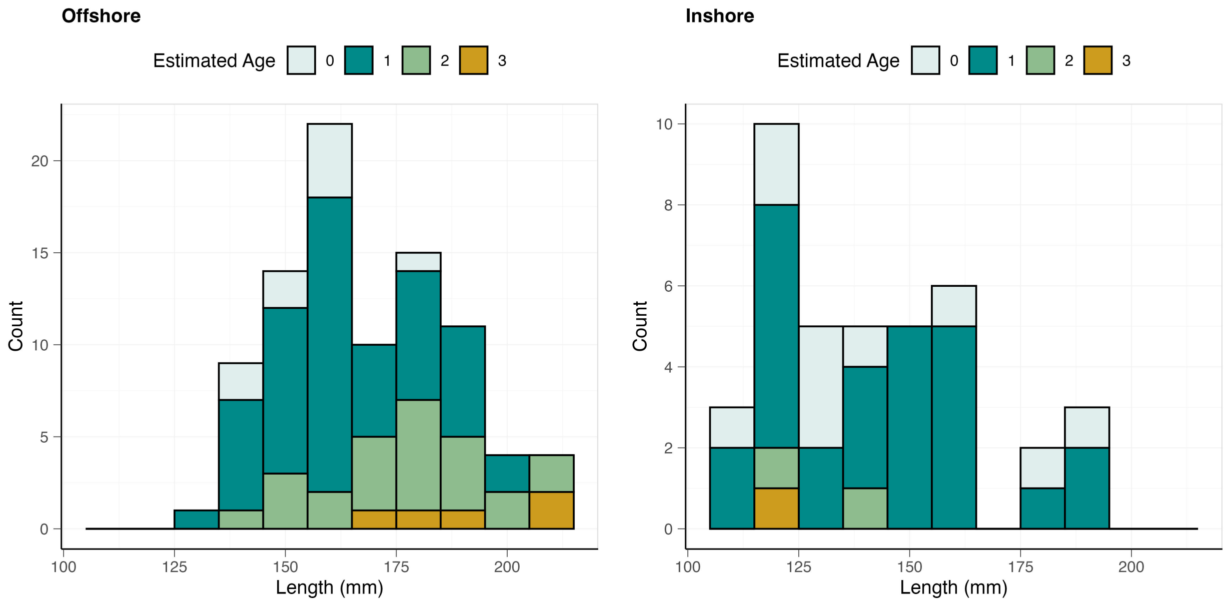

Figure S3.1. Figures showing estimated ages and their lengths (mm) of sand lance from the offshore (left) or inshore (right) region from all of the confidently aged fish.

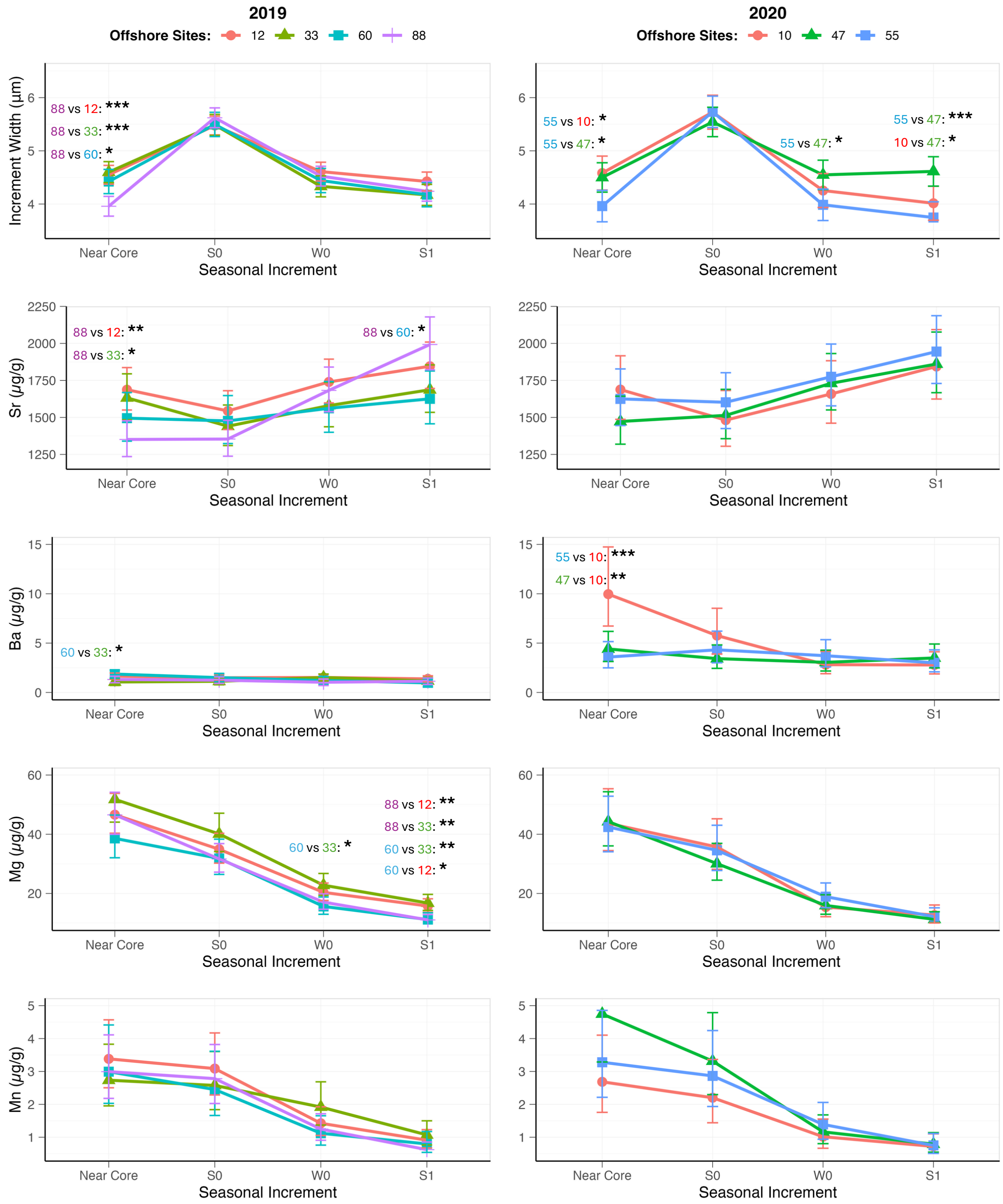

Figure S3.2. Estimated marginal means (EMMs; back transformed from log scale) of increment width (µm) and otolith trace element (Sr: strontium; Ba: barium; Mg: Magnesium; Mn: manganese) concentrations (µg/g) in offshore sites for each seasonal otolith region (near core; S0 = summer age 0; W0 = winter age 0; S1 = summer age 1) during 2019 (left) and 2020 (right). Whiskers represent 95% confidence intervals. Asterisks indicate a significant difference in otolith trace element concentrations during a specific seasonal increment based on Tukey-adjusted post hoc tests (*p < 0.05; **p < 0.01; ***p < 0.001). Overall, IW differed significantly among sites (Table S3.1). Post hoc tests showed that IW in the near core region only differed at one site compared to the other sites in 2019, while in 2020, some sites differed significantly in IW compared to the other sites at the near core, W0, and S1 otolith regions. Additionally, some otolith trace element concentrations differed significantly among offshore sites in both years (Table S3.1). Specifically, otolith Sr concentrations differed among sites in the near-core and S1 in 2019, Ba differed among sites in the near-core in both years and Mg differed among sites in W0 and S1 in 2019.

Chapter Four: Conclusions

The overarching aim of this thesis was to evaluate the effectiveness and limitations of otolith chemistry as a tool for investigating connectivity and/or identifying natal origins in two ecologically important forage fish species on the Newfoundland and Labrador Shelf: capelin (*Mallotus villosus*) and sand lance (*Ammodytes dubius*). Specifically, this work sought to determine the spatial scales at which otolith elemental signatures reliably differentiate natal origins (capelin, Chapter 2), and whether otolith elemental signatures can be used to infer mixing patterns of dispersing larvae and post-settlement stages, along with potential natal origins/sources across the Newfoundland Shelf (sand lance, Chapter 3).

For both forage fish species, our findings revealed that otolith chemistry at early developmental stages (e.g., pre-hatch or near-core otolith regions) showed limited ability to distinguish individuals at broader spatial scales, such as among bays or regions. In chapter 2, variation in otolith chemistry of capelin larvae from closely spaced sites within bays and among years at a site may limit the ability to assign adults to their natal bay. In support, low classification success of otolith chemical signatures was found at both the bay- and NAFO division-level within the pre-hatch (embryonic) region, demonstrating that chemical signatures of the otolith pre-hatch region may be insufficient to reliably identify natal origins of adult capelin alone, as distinct site-specific chemical signatures may overwhelm broader bay-level variation. Therefore, otolith chemistry may need to be combined with complementary approaches such as morphometrics (Couillard et al., 2022), genetics (Milton & Chenery, 2001), growth back-calculations (Rogers et al., 2019) or adult tracking (Bradbury et al., 2008; Catalano et al., 2001; King et al., 2024; Robichaud & Rose, 2001) to improve assignment success and help determine relative productivity among differing natal bays.

In chapter 3, near-core otolith elemental signatures in sand lance showed minimal regional differentiation, consistent with larval mixing during the dispersive pelagic phase or shared water mass characteristics across early-life stages. However, post-settlement otolith chemistry and increment widths revealed more distinct regional differences, supporting the hypothesis that sand lance exhibit strong site fidelity following settlement. This study represents the first otolith chemistry–based assessment of Northwest Atlantic sand lance and contributes new insight into the spatial and connectivity structure of this understudied forage fish. Similar to our capelin findings, however, uncertainty remains about whether natal sources can be determined at the shelf-scale investigated. This uncertainty could be addressed in future research through additional sampling, unsupervised classification techniques, and targeted larval surveys to better characterize natal sources/origins of sand lance.

Management Implications and Future Research

These chapters combined demonstrate the merits and challenges of using otolith chemistry to help determine the connectivity patterns of forage fish while considering the spatial scale, ontogenetic stage, and environmental context of the species. While this study showed that otolith chemistry can effectively detect broad-scale patterns, its utility for resolving natal origins/sources at finer spatial scales is limited on the Newfoundland Shelf when otolith chemical signatures of early life stages may be dominated by site-specific microhabitat conditions. By evaluating the utility of using otolith chemistry of these two species with differing ecology, this thesis highlights both the potential and the constraints of otolith chemistry for connectivity research in marine fish.

Given the essential role of forage fish in marine food webs, improving our understanding of their population structure, early-life history, and connectivity is essential for fisheries management (Cury et al., 2000; Pikitch et al., 2012; Staudinger et al., 2020). The insights gained through this thesis provide valuable direction for future studies and reinforce the need to combine otolith-based studies in a multi-method approach when attempting to identify natal origins or assess connectivity in species with complex early life histories. Continued research integrating otolith chemistry with genetics, morphology, back-calculations of growth, larval sampling surveys, and adult tracking data will be crucial to better understand the spatial and connectivity dynamics of forage fish populations to support informed conservation and management decisions on the Newfoundland and Labrador Shelf.

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