DETERMINING THE NATAL ORIGIN OF BEACH VERSUS DEMERSALLY REARED LARVAL CAPELIN, *MALLOTUS VILLOSUS*, OFF THE NORTHEAST NEWFOUNDLAND COAST USING OTOLITH CHEMICAL SIGNATURES

by

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Abstract

Identifying the natal origin of marine fish is important to understand the connectivity and productivity among populations in different habitats. Capelin, *Mallotus villosus*, is a key marine forage fish species that spawns at both beach and deep-water (15-40 m) habitats along the northeast Newfoundland coast. I investigated the ability to identify the natal habitat of larval capelin using otolith trace element concentrations quantified via LA ICP-MS. Otolith signatures of individual larvae reared under identical field and laboratory conditions were highly variable, resulting in an inability to reliably classify individuals into natal habitat or treatments (~50% misclassification). To investigate whether maternal investment was responsible for this variability among individuals, I reared artificially fertilized eggs from nine families (1 male + 1 female) under controlled temperature and salinity treatments. Otolith chemical signatures could reliably classify individuals into families with high success (83.4%), suggesting maternal investment may be confounding our ability to identify natal origin. A spiking experiment, whereby enriched ^{137}Ba was added to the rearing water of eggs, however, revealed that trace elements from the ambient water are being incorporated into developing otoliths. These findings suggest that moderate-high differences in water chemistry and environmental conditions among rearing habitats are required to identify the natal origin of individual capelin larvae using otolith chemistry.

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General Introduction

Connectivity within a species refers to the degree of exchange of individuals, or 'mixing', among geographically separated regions (Cowen et al., 2000). Most marine species experience a dispersive stage during their early life history where they are subjected to a potentially extensive transport via ocean currents (Cowen et al., 2000). Dispersal refers to the movement away from the natal site and/or the movement among regions of suitable environmental conditions (Di Franco et al., 2012), and for many marine invertebrates and fish species is achieved during the larval stage via both active behavior and passive drift throughout the species' geographic range (Stanley et al., 2012). Indeed, dispersal is one of the fundamental life history traits that influences connectivity of spatially structured local populations within a metapopulation (Di Franco et al., 2012). Despite high potential for dispersal, there is growing evidence that populations of marine species are structured as relatively isolated geographic units, likely resulting from patchy distributions of suitable environmental conditions and resources (Roberts and Hawkins, 1999; Kobayashi, 2006). Understanding connectivity and metapopulation structure (e.g., source and sink populations) is essential to estimate the stability and resilience of local populations, as well as the ability of a species to colonize new regions (Thorrold et al., 2001; Gillanders, 2002), which have important conservation implications, including designing marine protected areas and monitoring impacts of climate change (Thorrold et al., 2001).

Identifying the natal origins of marine fish larvae with high dispersal rates to assess connectivity, however, is difficult due to the challenge of conducting mark-recapture studies on large numbers of small pelagic larvae that experience high mortality rates (Thorrold et al., 2001; Davoren and Halden, 2014). Instead of using artificial tags, chemical signatures in the otoliths of teleost fish have proven useful as natural tags to identify natal habitats of marine fish (e.g.,

Thorrold et al., 2001). Otoliths, or 'ear stones', are calcified structures formed within the head of teleost fish and are used for balance and hearing (Campana, 1999). These paired structures are formed by accumulating elemental deposits throughout an individual's lifespan and are composed primarily of aragonite deposited on a proteinaceous matrix (Campana, 1999; Thorrold et al., 2001). Trace elements and stable isotopes are incorporated from the surrounding seawater into the calcium carbonate of otoliths in layers (Elsdon and Gillanders, 2003). Studying otolith chemistry is a useful tool for identifying the natal origin of fish as the abundance and combination of elements form an otolith 'signature' which can be used to reconstruct the environment the fish experienced through various life history stages (Elsdon and Gillanders, 2003). Otoliths are metabolically inert and, therefore, record and preserve the timing of elemental deposition from egg incubation until death (Campana, 1999; Elsdon and Gillanders, 2003). Overall, otolith chemical signatures have provided a powerful tool to identify natal habitats (e.g., Thorrold et al., 2001), as well as to reconstruct movement patterns throughout the lifespan of marine fish species (Campana, 1999; Elsdon and Gillanders, 2003).

The ability to reconstruct environmental histories of fish using otolith microchemistry relies mainly on predictable responses of trace element incorporation rates to environmental variables (Elsdon and Gillanders, 2003). As larvae, juveniles, and adults, inorganic elements from the water enter the blood plasma via the gills (freshwater fish) or intestine (marine fish) and are transferred into the endolymph fluid in the sac around the otolith and crystallized in the calcium carbonate surface of the otolith (Campana, 1999). Fish regulate their solute to water balance and, thus, their calcified structures (e.g., bone, scale, otoliths) tend to reflect ambient water chemistry (e.g., Bath et al., 2000); however, elemental incorporation rates into otoliths can also be influenced by environmental conditions, such as temperature, salinity, pH, and dissolved

oxygen (Campana, 1999; Elsdon and Gillanders, 2003). Changes in temperature influence the balance between the organic matrix and calcium carbonate formation (Campana, 1999). The crystal precipitation process as well as the growth rate of fish otoliths is likely influenced by changes in temperature (Elsdon and Gillanders, 2003) and thus, is reflected in the otolith chemistry of fish (Romanek and Gauldie, 1996). Indeed, otolith strontium (Sr) to calcium (Ca) ratios have been the most promising when inferring temperature gradients in marine fish species. The rate of Sr:Ca incorporation into inorganic aragonite typically varies inversely with temperature in marine species (Townsend et al., 1992; Campana, 1999; DiMaria et al., 2010). As an essential element, Magnesium (Mg) is involved in regulating the activity of enzymes and Ca transport channels (Loewen et al., in press). Therefore, Mg incorporation rates may be directly linked to higher growth rates under warmer conditions and, thus, a need for higher Ca transport to allow for the rapid larval growth. The environmental salinity will also influence the availability of elements to be incorporated into the endolymph and protein matrix surrounding the otolith (Elsdon and Gillanders, 2003) and has been found to be related to elements such as barium (Ba; negative relationship; e.g., Miller, 2011) and Sr (positive relationship; e.g., Tzeng, 1996; Zimmerman and Reeves, 2002). Otolith manganese (Mn) concentrations tend to vary independently of temperature and salinity and, thus, may not be as useful in determining environmental life histories in comparison to other trace elements that have been studied to a higher degree (Elsdon and Gillanders, 2002).

To use otolith chemistry to identify the natal origin of fish, whose larvae disperse away from natal site immediately after hatch, it is important to understand how trace elements are incorporated into the embryonic otolith developing within the egg. This pre-hatch otolith region is often called the ‘primordium’ or the ‘nucleus’ and refers to elemental deposition during egg

incubation. Although little is known about the pathway of elements from the environment into the egg, and then into the embryonic otolith, it is likely a multi-stage process characterized by elemental and ion transport characteristics (Campana, 1999). Teleost fish eggs have complex and highly permeable chorions that swell and become thinner immediately after fertilization (Davenport et al., 1986; Warren-Myers, 2015). Water, inorganic ions, and small molecules are absorbed across the chorion into the egg throughout incubation (Shephard et al., 1989). Due to physiological regulating processes, trace elemental incorporation rates into otoliths vary among species, resulting in species-specific relationships between otolith and ambient trace element concentrations, which can be modified by varying environmental conditions (e.g., temperature, salinity; Elsdon and Gillanders, 2003).

Otolith elemental concentrations in the pre-hatch growth region may not reflect conditions in the natal habitat, but instead can reflect conditions in the mother's environment during the time the yolk sac was formed (e.g., Volk et al., 2000). To illustrate, even when the eggs of some anadromous fish species (e.g., salmonids) are reared under identical conditions, otolith trace element (e.g., Sr, Ba) concentrations in the primordial region of individual offspring may vary widely when mothers experienced either freshwater or saltwater environments while gametes were developing (Limburg et al., 2001; Volk et al. 2000). For instance, Zimmerman and Reeves (2002) also reported that the primordial region of the otoliths of rainbow trout, *Oncorhynchus mykiss*, had elevated Sr concentrations when mothers were in seawater during gonadal development. In addition, certain trace element (e.g., Mn, Ba, Mg) concentrations are much higher in the primordial region of many species (e.g., sprat, *Sprattus sprattus*, Brophy et al., 2004; painted greenling, *Oxylebius pictus*, Ruttenberg et al., 2005 rockfish *Sebastes atrovirens*, Kuroki et al., 2010). In particular, elevated concentrations of Mn occur in the pre-

hatch otolith region of many fish species (Ruttenberg et al., 2005). During embryonic development of the respiratory system in the egg, Mn is actively absorbed for enzyme activation, which may result in a distinct Mn spike seen in the primordial region of larval otoliths (Brophy et al., 2004). Overall, elevated concentrations of these trace elements in the primordium, together with maternal investment, may overwhelm the influence of environmental conditions on otolith chemistry and, thus, hinder our ability to identify natal origin (Ruttenberg et al., 2005). Despite maternal investment as a possible confounding factor, other studies have found a correlation between elevated trace element concentrations in the ambient environment and the primordial region of otoliths of several fish species (e.g., juvenile weakfish, *Cynoscion regalis*, Thorrold et al., 2001). In addition, elemental marking of incubating eggs has been used to successfully distinguish different groups of individuals and is frequently used to assess the proportion of wild versus hatchery-reared stocks to investigate the abundance, stock structure, and contribution to populations (e.g., clown-fish, *Amphiprion melanopus*, serranid, *Centropristis striata*, Thorrold et al., 2006; Atlantic salmon, *Salmo salar*, Warren-Myers, 2015). During elemental marking, fish eggs or larval fish are exposed to water containing enriched concentrations of rare stable isotopes (e.g., ^{86}Sr, ^{137}Ba) at any life-history stage, including egg incubation (Munro et al., 2008; Warren-Myers, 2015). Due to physiological regulating processes, trace elemental incorporation rates into otoliths vary among species (Elsdon and Gillanders, 2003). Overall, understanding whether the primary source of trace elements for the developing larval otolith during egg incubation is from the mother, or the environment, is needed to determine whether identifying the natal origin of a particular species is possible using otolith chemical signatures.

Study species

Capelin, *Mallotus villosus*, is a small, short-lived (3-6 years) schooling fish found in Arctic and sub-Arctic zones in the Atlantic and Pacific Oceans (Carscadden et al., 2013). They are considered a key forage species, as they are an important prey source for many top predators, especially during the summer spawning season off the northeast coast of Newfoundland (Davoren et al., 2006). In this region, capelin typically spawn on sandy beaches in June and July; however, spawning at deep-water ('demersal') habitats was recently discovered (Nakashima and Wheeler, 2002; Davoren et al., 2006). Fertilized capelin eggs adhere to the substrate at spawning sites, which become their rearing habitat. The warm, beach (intertidal) and cooler, deep-water (subtidal, 15-40 m) habitats co-occur in close proximity (<20 km) and have significant environmental differences (Davoren et al., 2015). Beach sites are highly variable in comparison to the demersal sites due to wave action, tidal inundation, solar radiation, salinity, temperature, and oxygen concentration variability (Penton and Davoren, 2013). Demersal sites are 5-10°C cooler (and less saline) and experience less variable temperatures and a consistent replenishment of oxygen from ocean currents (Penton and Davoren, 2013). After hatch, capelin larvae begin to disperse almost immediately from their coastal spawning/rearing sites in late summer to offshore nursery areas near the shelf edge in late fall (Davoren and Halden, 2014). Larvae undergo metamorphosis and remain offshore until reaching sexual maturity at two to three years of age (Davoren and Halden, 2014), after which capelin return to coastal areas to spawn.

Several early life history characteristics differ between offspring reared in the two habitats including egg mortality, developmental rates, hatching success and larval emergence mechanisms (Penton and Davoren, 2008; Penton et al., 2012). Survival and developmental rate of eggs are largely determined by temperature (Frank and Leggett, 1981; Penton et al., 2012),

with faster egg development at higher temperatures at beaches, but also higher mortality rates due to abnormal egg development (Penton et al., 2012). Longer incubation (~1 month) in colder waters at deep-water sites, however, results in an extended exposure to predators and delayed hatching in late summer-early fall, both of which may reduce the probability of larvae reaching a critical size prior to winter and therefore decrease their survivorship (Davoren et al., 2015).

Overall, the relative contribution of offspring from demersal and beach habitats to the spawning population, or 'recruitment', in coastal Newfoundland is unknown. This is important to determine as capelin are predicted to use demersal spawning sites to a higher degree as ocean climate warms (Nakashima and Wheeler, 2002; Penton et al., 2012; Davoren, 2013). To illustrate, tagged male capelin moved between spawning habitats based on temperature during spawning seasons (Davoren, 2013). Most tagged capelin (76%), however, were detected in only one spawning habitat within a given year (Davoren, 2013) and high classification success (71%) of beach and demersal spawners during the larval period, inferred from otolith chemistry (Davoren and Halden, 2014), suggested that fish may be connected to a particular spawning habitat (e.g., natal philopatry). Despite these indications, genetic analyses combined with common garden experiments indicated undifferentiated populations (Penton and Davoren, 2013; Penton et al., 2014). Therefore, Newfoundland capelin are currently treated as one stock (i.e. management unit) even though connectivity between the two spawning habitats is unknown (Davoren, 2013; Penton et al., 2014).

Thesis Objectives

The primary objective of my thesis was to examine whether otolith chemical signatures can be used to determine the natal habitat of larval capelin (i.e. beach vs. demersally reared). To examine this objective, I conducted a series of experiments under varying environmental

conditions including controlled laboratory conditions as well as uncontrolled conditions in the field. First, I examined whether environmental conditions, including temperature and salinity, influenced incorporation rates of trace elements into the embryonic otoliths of lab-reared and field-reared capelin eggs (Chapter 1). Second, I examined the influence of both maternal investment and ambient water chemistry on the otolith chemistry of offspring by conducting two additional experiments (Chapter 2). In this chapter I first lab-reared artificially fertilized offspring of multiple families to hatch under varying temperature and salinity conditions. Second, I lab-reared naturally fertilized eggs in water spiked with a rare barium isotope to investigate whether trace elements from the environment are being incorporated into the developing larval otolith during egg incubation. By examining the influence that both environmental factors (i.e. temperature, salinity, ambient water chemistry) and maternal investment have on otolith chemical signatures in the primordial region of capelin larvae, I provide insight into whether otolith chemistry can be used as a tool to identify the natal habitat of larval capelin.

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Chapter 1. Temperature and salinity influence the otolith chemistry of capelin during lab and field egg incubation experiments

Introduction

Connectivity within a species refers to the degree of exchange of individuals, or 'mixing', among geographically separated regions (Cowen et al., 2000). Most marine species experience a dispersive stage during their early life history where they are subjected to a potentially extensive transport via ocean currents (Cowen et al., 2000). Dispersal refers to the movement away from the natal site and/or the movement among regions of suitable environmental conditions (Di Franco et al., 2012), and for many marine invertebrates and fish species, this is achieved during the larval stage via both active behavior and passive drift throughout the species' geographic range (Stanley et al., 2012). Indeed, dispersal is one of the fundamental life history traits that influences connectivity of spatially structured local populations within a metapopulation (Di Franco et al., 2012). Despite high potential for dispersal, there is growing evidence populations of many marine species are structured as relatively isolated geographic units, likely resulting from patchy distributions of suitable environmental conditions and resources (Roberts and Hawkins, 1999; Kobayashi, 2006). Understanding connectivity and metapopulation structure (e.g., source and sink populations) is essential to estimate the stability and resilience of local populations, as well as the ability of a species to colonize new regions (Thorrold et al., 2001; Gillanders, 2002), which have important conservation implications, for designing marine protected areas and monitoring impacts of climate change (Thorrold et al., 2001).

Identifying the natal origins of marine fish to assess connectivity, however, is difficult due to the challenge of conducting mark-recapture studies on large numbers of small pelagic larvae that experience high mortality rates (Thorrold et al., 2001). Instead of using artificial tags,

chemical signatures in the otoliths of teleost fish have proven useful as 'natural' tags to identify natal habitats of marine fish (e.g., Thorrold et al., 2001). Otoliths, or 'ear stones', are calcified structures formed within the head of fish and are used for balance and hearing (Campana, 1999). These paired structures are formed by accumulating elemental deposits throughout an individual's lifespan and are composed primarily of aragonite deposited on a proteinaceous matrix (Campana, 1999; Thorrold et al., 2001). Trace elements and stable isotopes are incorporated from the surrounding seawater into the calcium carbonate surface of otoliths in layers as the fish grows. Otoliths are metabolically inert and, therefore, record and preserve the timing of elemental deposition from egg incubation until death (Campana, 1999; Elsdon and Gillanders, 2003).

To use otolith chemistry to identify the natal origin of larval fish, it is important to understand how trace elements are incorporated into the embryonic otolith developing within the egg. Although little is known about the pathway of elements from the environment into the egg and then into the embryonic otolith, it is likely a multi-stage process characterized by elemental and transport characteristics (Campana, 1999). Teleost fish eggs have complex, highly permeable, chorions that swell and become thinner immediately after fertilization (Davenport et al., 1986; Fletcher et al., 2015). Water, inorganic ions, and small molecules are absorbed across the chorion into the egg throughout incubation (Shepherd et al., 1989). Due to physiological regulating processes, trace elemental incorporation rates into otoliths vary among species, resulting in species-specific relationships between otolith and ambient trace element concentrations, which can be modified by varying environmental conditions (e.g., temperature, salinity; Elsdon and Gillanders, 2003).

Essential elements such as Calcium (Ca^{2+}) and magnesium (Mg^{2+}) are physiologically regulated and, thus, may not reflect ambient concentrations in the environment (Loewen et al., in press). For example, an influx of intracellular Ca^{2+} into the egg immediately after fertilization stimulates embryonic development (Coward et al., 2002). In contrast, otolith concentrations of nonessential elements, such as strontium (Sr^{2+}) and barium (Ba^{2+}), tend to reflect ambient water chemistry (e.g., Bath et al., 2000) as they are inadvertently transported across biological membranes via calcium channels owing to their chemical similarities (Loewen et al., in press). As ambient concentrations of Sr and Ba vary with salinity, otolith Sr and Ba concentrations often vary predictably with salinity gradients (e.g., Elsdon and Gillanders, 2002; Miller, 2009). Otolith incorporation rates of these nonessential elements can also be influenced by environmental conditions, such as temperature, pH, and dissolved oxygen (Campana, 1999; Ellsdon and Gillanders, 2003). Indeed, Sr:Ca ratios in marine fish otoliths typically vary inversely with temperature (Campana, 1999). Temperature also effects fish growth rates and, thus, otolith growth rates which may influence the rate at which some trace elements (e.g., Sr, Mg) are incorporated into the otolith (Romanek and Gauldie, 1996; Campana, 1999; Elsdon and Gillanders, 2003). Overall, an understanding of the key environmental factors influencing species-specific trace element incorporation rates into otoliths is fundamental to reconstructing the natal origin of larval fish using otolith microchemistry (Elsdon and Gillanders, 2003.

Capelin, *Mallotus villosus*, is a small, short-lived (3-6 years) schooling fish found in Arctic and sub-Arctic zones in the Atlantic and Pacific Oceans (Carscadden et al., 2013). They are considered a key forage species, as they are an important prey source for many top predators, especially during the summer spawning season off the northeast coast of Newfoundland (Davoren et al., 2008). In this region, capelin typically spawn on sandy beaches in June and July;

however, spawning in deep-water ('demersal') habitats was recently discovered in coastal embayments (Nakashima and Wheeler, 2002; Davoren et al., 2006). Fertilized capelin eggs adhere to the substrate at spawning sites, which become their rearing habitat. The warm, beach (intertidal) and cooler, deep-water (15-40 m) habitats co-occur in close proximity (<20 km) and environmental conditions vary greatly between the habitats (Penton et al., 2012; Davoren et al., 2015). The beach habitat is characterized by widely varying solar radiation, salinity, temperature, and oxygen concentrations relative to the demersal habitat due to wave action and tidal inundation (Penton et al., 2012). In contrast, the demersal habitat is 5-10°C cooler (and more saline) with less variable temperature and salinity, along with a consistent replenishment of oxygen from ocean currents (Penton et al., 2012; Davoren et al., 2015). The relative contribution of offspring from demersal and beach habitats to the spawning population, or recruitment, in coastal Newfoundland is unknown. This is important to determine as capelin are predicted to use demersal spawning habitats to a higher degree as ocean climate warms (Nakashima and Wheeler, 2002; Penton et al., 2012, Davoren, 2013). Although some studies have suggested that capelin may be connected to a particular spawning habitat (e.g., natal philopatry; Davoren, 2013; Davoren and Halden, 2014), genetic analyses combined with common garden experiments suggested undifferentiated populations (Penton and Davoren, 2013; Penton et al., 2014). Therefore, Newfoundland capelin are currently treated as one stock (i.e. management unit) even though connectivity between the two spawning habitats is unknown (Davoren, 2013; Penton et al., 2014).

The goal of this study was to investigate whether otolith chemical signatures in recently hatched capelin larvae can be used to successfully identify the natal or rearing habitat by describing how species-specific concentrations of trace element (i.e. Sr, Ba, Mg, Mn) vary when

eggs were reared under varying temperature and salinity conditions. To do this, I first lab-reared capelin eggs under controlled temperature and salinity conditions, similar to those experienced at beach and demersal habitats. I hypothesized that temperature and salinity influence otolith chemical signatures during development of individual larval capelin. I predicted that individual capelin eggs reared at low temperatures and high salinities (i.e. demersal habitat) will have higher otolith Sr concentrations and lower otolith Ba concentrations upon hatch relative to those reared at warmer temperatures and lower salinities (i.e. beach habitat), similar to previous findings for bulk samples of capelin larvae (Davoren et al., 2015) and other cold-adapted marine species (e.g., Townsend et al., 1995; Elsdon and Gillanders, 2002, 2003; DiMaria et al., 2010). In addition, I tested the same hypothesis by field-rearing eggs within each spawning habitat located ~20 km apart. Determining the influence of rearing temperature and salinity conditions on otolith chemistry of individuals during egg incubation will determine whether chemical signatures in an individual's otolith can be used to successfully identify the natal habitat of dispersed capelin larvae in the wild and, thus, determine the relative contribution of each habitat to recruitment as well as the connectivity between spawning habitats.

Methods

Lab-rearing experiment

Naturally fertilized capelin eggs adhered to substrate were collected by hand from a beach spawning habitat (Site C, Fig. 1.1B) July 8th, 2014. Eggs (~300) were placed in 20 mL plastic canisters perforated with holes and covered with 0.250 mm Nitex mesh sleeves ('incubation canisters'). Incubation canisters (n=90) were shipped overnight in a cooler from Gander, Newfoundland to Winnipeg, Manitoba. Upon arrival, eggs were removed from canisters and placed in individual glass jars (120 mL) filled with seawater. Rearing jars and associated

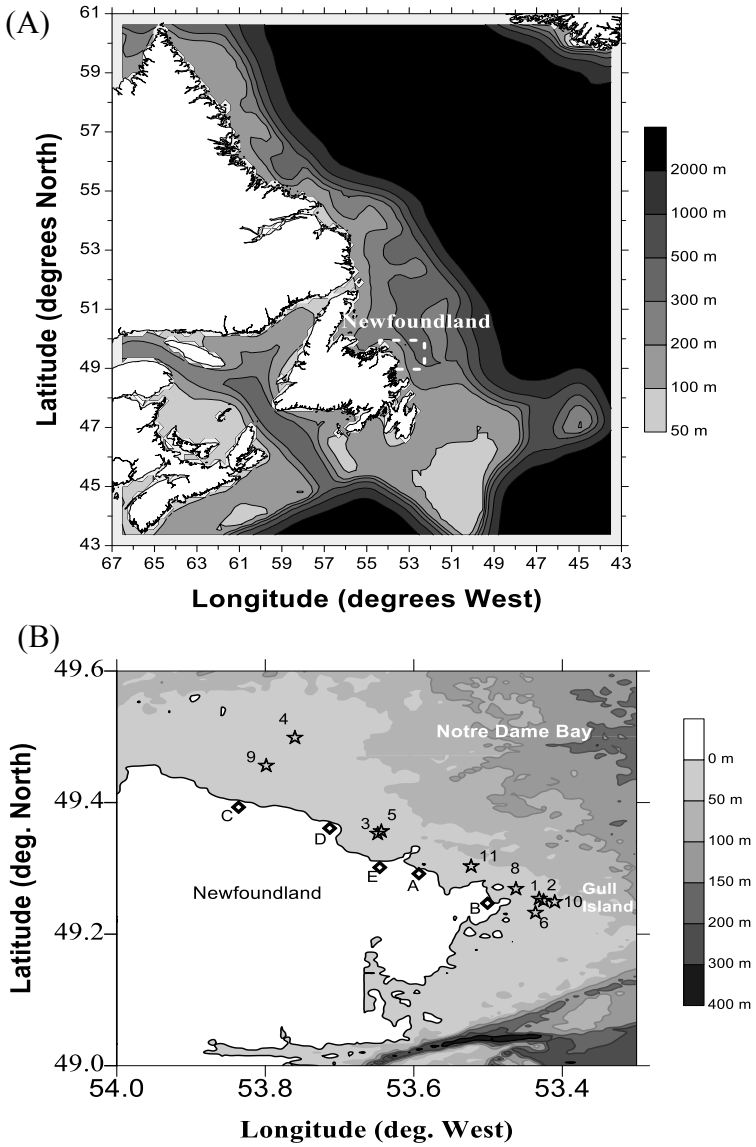

Figure 1. 1 Map of the northeast coast of Newfoundland (A) with study area indicated by the white dashed box. Study area (B) where stars indicate the location of demersal (deep-water) spawning sites of capelin and diamonds indicating beach-spawning sites. Four annually persistent demersal habitats (1, 2, 6, and 10) are located near Gull Island. Sites used in the field-rearing experiment are indicated at site B (Lumsden Beach, beach habitat), and site 6 (Turr Rock, demersal habitat).

equipment were cleaned by soaking in ~10% NaClO prior to the experiment and seawater was prepared by filtering purified distilled water (19L) using a *Lifeguard Aqua Step* UV Filter. *Seachem Marine Salt*^{TM} was added until the desired salinity was met by measuring conductivity using a multi-parameter YSI Pro30 probe. Eggs were then incubated and reared to hatch under three temperature (4, 8, and 12 °C) and three salinity (10, 20, and 30 psu) treatments, with five replicate jars per treatment (Fig. 1.2), within controlled environmental chambers at the University of Manitoba. Conditions were chosen to mimic natural rearing conditions for capelin spawned at both beach and demersal habitats on the northeast Newfoundland coast (Penton et al., 2012, Davoren et al., 2015).

Within each environmental chamber, jars with eggs were retained in a plastic tray half-filled with water to aid in maintaining a constant temperature. Each environmental chamber was set to the desired temperature, and light settings were programmed to provide a 14-hour light to 9-hour dark cycle as would be experienced during natural incubation in Newfoundland. Water changes were conducted every 2-3 days by gently discarding ~90% of the water from each jar and replacing with new water of the desired treatment. When larvae were present, water from each jar was gently poured over a 0.232 mm sieve to collect larvae. Larvae were then transferred into a pre-labeled 20 mL glass vial filled with 95% ethanol. Larvae were collected and counted once every second day until all eggs hatched (Table 1.1).

Throughout the experiment, incubation jars were monitored daily for temperature, salinity, pH, and dissolved oxygen using a multi-parameter YSI Pro30 (Table 1.2A). In addition, environmental chambers were each equipped with *Hobo Water Temp Pro V2* loggers set to record temperature hourly. To monitor ambient trace element concentrations, water samples

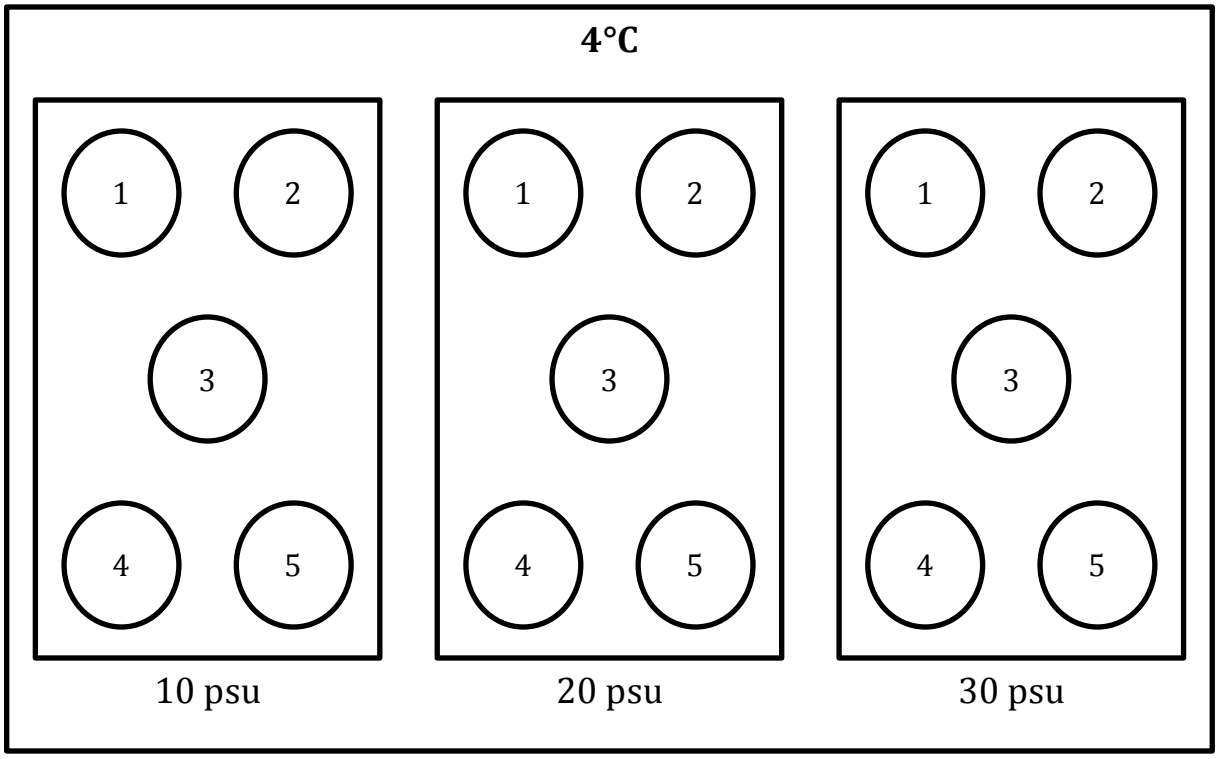

Figure 1. 2 Diagram of lab-rearing experiment indicating 5 replicates within each temperature and salinity treatment. Approximately 300 eggs were placed in each replicate jar. Similar set up was repeated for the 8 and 12 °C temperature chambers.

Table 1. 1 Number of larvae (i.e. 'count) that hatched and was collected from each temperature and salinity treatment from the lab-rearing experiment.

Temp.	Salinity	Jar	Count	Temp.	Salinity	Jar	Count	Temp.	Salinity	Jar	Count
4°C	10 psu	1	10	8°C	10 psu	1	854	12°C	10 psu	1	227
		2	16			2	479			2	48
		3	20			3	694			3	32
		4	43			4	806			4	108
		5	102			5	929			5	769
	20 psu	1	111		20 psu	1	337		20 psu	1	972
		2	18			2	684			2	337
		3	139			3	525			3	615
		4	148			4	773			4	652
		5	102			5	139			5	1090
	30 psu	1	20		30 psu	1	664		30 psu	1	589
		2	42			2	13			2	833
		3	228			3	952			3	450
		4	64			4	1038			4	233
		5	50			5	740			5	212

Table 1. 2 Average experimental conditions (±SE) for the (A) lab- and (B) field-rearing experiments, including temperature (°C), salinity (psu), pH, depth (m), along with average water elemental concentrations (±SE ppm) with the Me/Ca ratios in brackets. Trace elements below the limit of detection (LOD) are indicated.

(A) Lab-Reared

Treatment		Conditions			Water Chemistry				
Temp.	Sal.	Temp.	Sal.	pH	Ca	Sr	Ba	Mg	Mn
4°C	10 psu	4.2±0.4	9.8±0.4	8.4±0.2	-	-	-	-	-
	20 psu	4.1±0.4	19.7±0.8	8.4±0.1	-	-	-	-	-
	30 psu	4.2±0.4	28.9±1.8	8.3±0.1	-	-	-	-	-
8°C	10 psu	8.4±0.5	10.6±0.9	8.3±0.3	-	-	-	-	-
	20 psu	8.2±0.5	19.9±0.7	8.3±0.2	-	-	-	-	-
	30 psu	8.3±0.4	29.6±0.3	8.3±0.1	-	-	-	-	-
12°C	10 psu	12.1±0.4	10.5±0.3	8.2±0.2	134.3 ±11.9	2.79 ±0.2 (0.02)	0.007 ±0.0007 ($5.2\ e^{-5}$)	335.0 ±43.1 (2.3)	0.01 ±0.0007 ($7.4e^{-5}$)
	20 psu	12.0±0.5	19.8±0.4	8.3±0.2	255.3 ±13.1	5.2 ±0.15 (0.02)	0.013 ±0.0015 ($5.1\ e^{-5}$)	644.0 ±81.5 (2.5)	0.02 ±0.0012 ($7.8\ e^{-5}$)
	30 psu	12.2±0.5	29.0±0.9	8.4±0.1	406.0 ±54.0	8.1 ±0.81 (0.02)	0.019 ±0.0015 ($4.6\ e^{-5}$)	982.3 ±114.1 (2.4)	0.03 ±0.0028 ($7.4\ e^{-5}$)

(B) Field-Reared

Habitat		Conditions			Water Chemistry				
Year	Site	Temp.	Sal.	Depth	Ca	Sr	Ba	Mg	Mn
2014	Beach	6.4±0.1	30.1±0.04	7.5 ±0.02	352.5 ±6.5	6.28 ±0.14 (0.02)	0.0006 ±0.0001 ($1.8\ e^{-5}$)	1090 ±20.0 (3.09)	0.0001 ±0.0002 ($3.5e^{-6}$)
	Demersal	4.9±0.4	31.4±0.08	23.4±0.03	386.5 ±0.4	7.02 ±0.37 (0.02)	0.0071 ±0.0004 ($1.8\ e^{-5}$)	1200 ±40.0 (3.10)	0.0015 ±0.0005 ($3.8e^{-6}$)
2015	Beach	9.1±0.2	28.9±0.03	12.6±0.02	338.0 ±3.0	6.17 ±0.08 (0.02)	0.0065 ±0.0004 ($1.9\ e^{-5}$)	1040 ±14.1 (3.07)	< LOD
	Demersal	5.7±0.7	31.1±0.17	18.3±0.04	339.5 ±4.5	6.10 ±0.20 (0.02)	0.0006 ±0.0005 ($1.8\ e^{-5}$)	1045 ±3.08 (3.08)	< LOD

were collected from each salinity treatment every time new saltwater was made. Water samples were collected using 60 mL acid-washed polypropylene syringes and transferred through 0.45 μm filters into 50 mL acid cleaned polyethylene bottles, preserved with 3 mL of 33% HNO_3, and stored until analysis.

Field-rearing experiment

Naturally fertilized capelin eggs from beach and demersal spawning sites were used to determine baseline otolith trace element concentrations during the summers of 2014 and 2015. Known beach spawning sites within the study area (Fig. 1.1B) were monitored daily for the presence of capelin eggs. Eggs were collected by hand from Shalloway beach (Site C, Fig. 1.1B) July 8, 2014 and using a 0.3-m^2 *Ponar Benthic Grab* system from Turr Rock (Site 6, Fig. 1.1B) July 20, 2015. Approximately 300 eggs adhered to sediment were placed in 20 mL plastic canisters as described above. Twenty canisters were placed in a mesh bag and moored at Lumsden Beach (Site B, Fig. 1.1B) by securing the bag to an anchor line. A 0.3-m^2 *Ponar Benthic Grab* system was used to collect eggs from Turr Rock July 19th, 2014 and July 20th, 2015 and placed in incubation canisters. Canisters were moored at Turr Rock (Site 6, Fig. 1.1B) as described above. An oceanographic device (*Star oddi* DST CTD, *Hobo Water Temp Pro V2* logger) set to record temperature and salinity every hour was attached to the anchor line near the bag of canisters. The timing of hatch was estimated for each habitat using equations describing time to hatch of capelin eggs and average incubation temperature (e.g., Frank and Leggett, 1994; Penton et al., 2012). Upon estimated hatch, canisters were removed and the larvae from each canister were collected and preserved in 95% ethanol. Water samples were collected twice at each site during incubation approximately 1 m from the ocean bottom to monitor ambient chemistry using a *Van Dourne* water sampler.

Otolith Chemistry Analyses

Trace elemental concentrations in otoliths of recently hatched larvae were analyzed via laser ablation inductively couple plasma mass spectrometry (LA ICP-MS, *Perkin-Elmar DRCII*), similar to Lazartigues et al. (2014). For the lab-rearing experiment, 10 larvae per replicate were randomly chosen from three of the five replicate jars and ablated (n=30 per treatment). Twenty-five larvae from each site per year (n=50 per site) were randomly chosen for analysis. Prior to otolith removal, equipment and microscope slides were acid washed with 2% nitric acid. Larvae were placed on a microscope slide and rinsed in 2-3 drops of milliQ water. Each larva was dissected under an *Olympus SZX7* dissecting microscope mounted with *Olympus SZX-PO* polarizers and the left sagittal otolith was removed (Fig. 1.3A). The otolith was rinsed in a drop of milliQ water and allowed to dry for 30 seconds. Each microscope slide had a one centimeter square grid divided into 25 equal squares and covered with a piece of double-sided (*Scotch*^{TM}) tape (Fig. 1.3B). Each otolith was fixed within one of the squares and then photographed to increase location efficiency prior to laser ablation.

Slides with 25 otoliths each were placed separately in the ablation cell and the otoliths were located by referencing previously taken photographs. A 60 μm transect was drawn across the center of the planar view of each otolith for the lab-reared experiment (Fig. 1.3C). The following laser parameters were used during ablation for lab-reared larvae: 80% energy, 5 Hz repetition rate, 2 μm/s speed, and 8-μm beam size. Ablation first was performed on NIST SRM 610 reference glass material to calibrate the LA ICP-MS and to provide an external standard. A 30 s gas blank was run prior to ablating each otolith to ensure the previous sample was cleared and to determine background trace element concentrations for estimating elemental detection

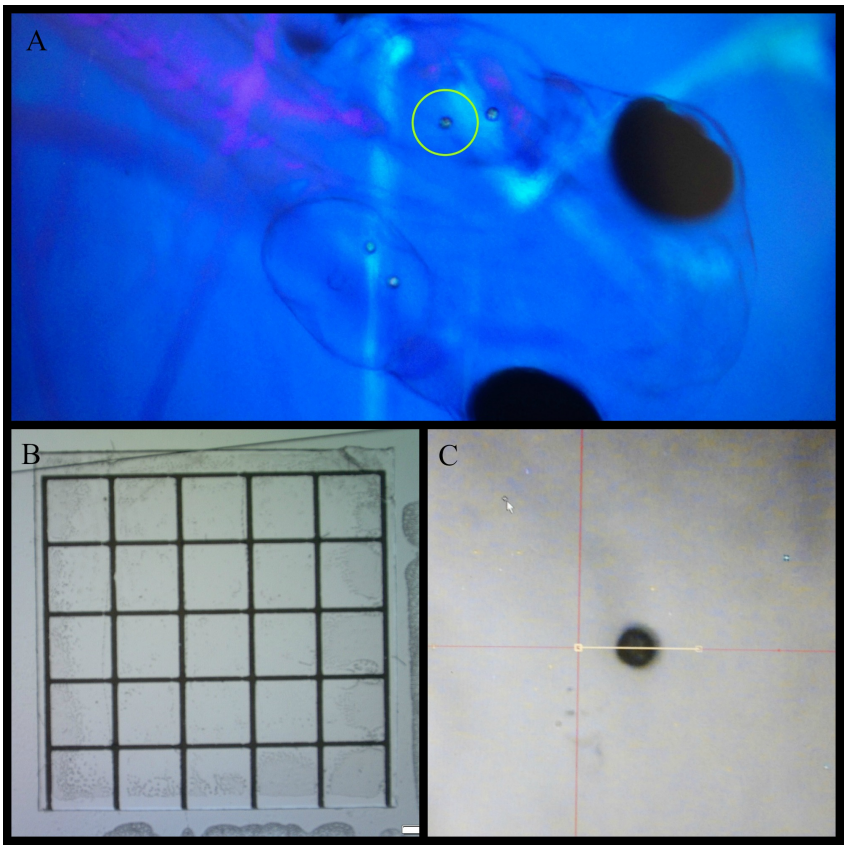

Figure 1. 3 Otoliths (A) in a larval capelin with the left sagittal otolith indicated (yellow circle). The 1cm by 1cm grid (B) placed on a microscope slide and covered with double-sided tape where individual otoliths were mounted. Otolith in ablation cell (C) with 60 μm transect drawn through the center of the planar view.

limits. Once all otoliths on a slide were ablated, the reference glass material was ablated again to correct for any changes during analysis.

Field-reared larvae were ablated using a spot analysis technique rather than a line transect, as otoliths appeared to be blasting apart partway through the ablation transect, resulting in increased variation in elemental concentrations. By ablating down through the otolith (rather than across the surface), we were able to achieve more consistent trace element concentrations while continuing to distinguish the nucleus region. The line transect and spot analysis techniques yielded similar trace elemental concentrations, revealing that methods were comparable. The following parameters were used for the spot analyses: 65% energy, 1 Hz repetition rate, and 30-μm beam size.

Data reduction (i.e. mapping, calculations of concentrations and limits of detection (LOD)) were performed using *Igor Pro* graphing software with an *Iolite* version 2.21 package for LA ICP-MS. Ca counts per second (CPS) were used to determine an internal standard to correct for the change in ablation volume. The mean (± SE) concentration of each trace element was then calculated by averaging across the entire transect for each otolith. Water samples were analyzed at ALS Environmental Laboratories (Burnaby, BC) following EPA Methods 6010B. Major elements (e.g., Ca) were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Thermo iCap 6500) after a 10x dilution. Trace elements (e.g., Ca, Sr, Ba, Mg, Mn) were analyzed by ICP-MS (Table 1.2).

Data analysis

Mean otolith trace elemental concentrations that were greater than two standard deviations from the mean were identified as outliers and removed from the dataset (lab-reared: n=57, field-reared: n=14). Concentrations of each trace element (response variables, μg/g) were

examined separately for normality using the Shapiro-Wilk's W test, and for homogeneity of variance using the Levene's test. All response variables were log transformed to meet the underlying assumptions of parametric statistics. To test whether otolith trace element concentrations differed significantly among treatments in the lab-rearing experiment, multifactor ANOVAs were conducted with temperature (°C) and salinity (psu) as fixed independent factors. Jar (replicates) was used as a nested random factor to control for variation among replicates, while testing the main factors of interest (i.e. temperature, salinity). To test whether otolith trace element concentrations differed between beach and demersal spawning sites in the field-rearing experiment, multifactor ANOVAs were performed with rearing habitat and year as fixed independent factors. Post-hoc Tukey HSD tests were performed to identify which factor levels were different. In addition, a linear discriminant function analysis (DFA) was performed, where all response variables were combined to determine whether treatment-specific (lab-reared) or habitat-specific (field-reared) otolith chemical 'signatures' could be defined.

Results

Throughout the lab-rearing experiment, temperature and salinity were held constant within each treatment (Table 1.2A). Water chemistry (i.e. metal/calcium, 'Me/Ca' ratios) was similar among all treatments and, thus, otolith trace element concentrations (μg/g) were presented rather than Me/Ca ratios. Mean otolith trace element concentrations of individuals were highly variable across all temperature and salinity treatments (Fig. 1.4). Otolith trace element concentrations differed significantly among temperature treatments, with the exception of Ba (Table 1.3A). Post-hoc Tukey HSD tests revealed that otolith Sr concentrations differed significantly among all temperature treatments, being highest at higher temperatures (Fig. 1.4A). Similarly, mean otolith Mg concentrations were significantly higher in the highest temperature

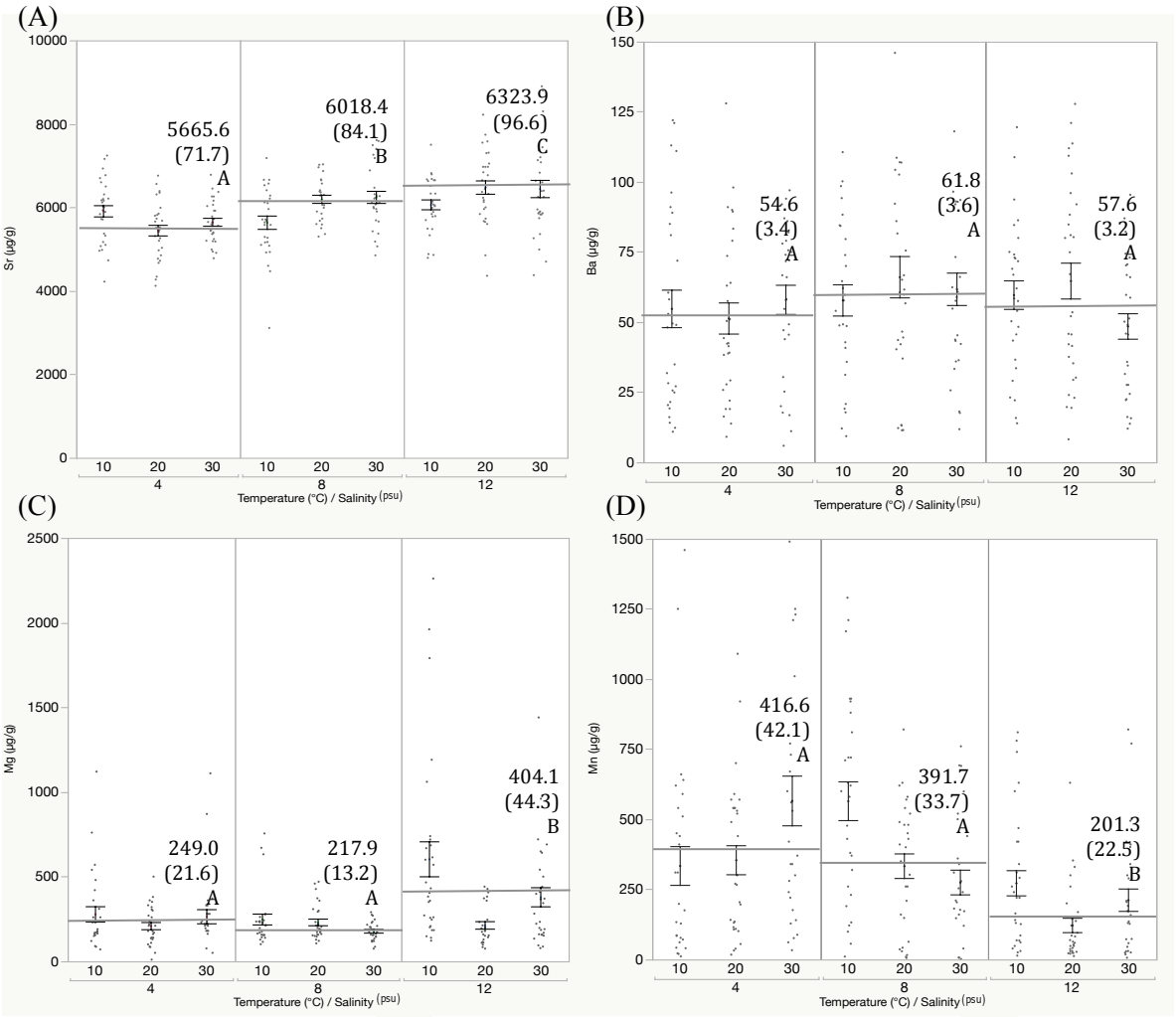

Figure 1. 4 Mean concentration (±SE) of otolith strontium (A), barium (B), magnesium (C), and manganese (D) within combined temperature and salinity treatments of the lab-reared experiment. Points represent average elemental concentrations of individual otoliths. The horizontal grey lines indicate the grand mean for each temperature treatment with the mean value above (± SE). Temperature treatments not sharing the same letter are significantly different.

Table 1. 3 Statistical results of two-factor ANOVAs from lab- (A) and field-rearing (B) experiments. P-values less than 0.05 (bolded) indicate significant differences.

(A) Lab-Reared					
Element	Treatment	df	df_{error}	F ratio	p-value
Sr	Temperature	2	202	6.91	**<0.0001**
	Salinity	2	202	6.46	0.1302
	Temp. x Salinity	4	202	6.50	**0.0027**
Ba	Temperature	2	247	1.00	0.3882
	Salinity	2	247	1.07	0.5534
	Temp. x Salinity	4	247	0.83	0.2985
Mg	Temperature	2	239	9.97	**<0.0001**
	Salinity	2	239	8.72	**0.0004**
	Temp. x Salinity	4	239	4.01	**0.0020**
Mn	Temperature	2	243	10.61	**<0.0001**
	Salinity	2	243	2.78	**0.0293**
	Temp. x Salinity	4	243	3.59	**0.0008**
(B) Field-Reared					
Element	Treatment	df	df_{error}	F ratio	p-value
Sr	Habitat	1	97	0.04	0.8496
	Year	1	97	0.14	0.7081
	Site x Year	1	97	0.06	0.8123
Ba	Habitat	1	95	0.04	0.8393
	Year	1	95	0.40	0.5282
	Site x Year	1	95	0.26	0.6147
Mg	Habitat	1	92	42.94	**<0.0001**
	Year	1	92	20.99	**<0.0001**
	Site x Year	1	92	28.70	**<0.0001**
Mn	Habitat	1	94	0.15	0.6992
	Year	1	94	8.49	**0.0045**
	Site x Year	1	94	2.42	0.1231

treatment relative to the other treatments, but the opposite was found for otolith Mn concentrations (Fig. 1.4C,D). Although mean otolith Sr and Ba concentrations did not differ significantly among salinity treatments (Table 1.3), Mg concentrations were significantly higher at lower salinity relative to the two higher salinity treatments. Similarly, otolith Mn concentrations were significantly higher at lower salinity relative to the mid-salinity treatment (Fig. 1.4). When all four otolith trace element concentrations were considered together, larvae could be successfully classified into temperature treatments (linear DFA, $F_{8,354}$=5.89 p<0.0001) and salinity treatments ($F_{8,354}$=4.75 p<.0001); however, classification success was low, with only 55.1% and 42.3% of larvae being correctly classified into each temperature and salinity treatment, respectively.

Throughout the field-rearing experiment, mean daily temperature was significantly higher at the beach relative to the demersal rearing habitat in 2015 ($F_{1,47}$=35.41 p<0.0001) but not in 2014 ($F_{1,50}$=2.49 p=0.12). Mean daily salinity differed significantly between rearing habitats in both years (2014: $F_{1,50}$=51.68 p<0.0001; 2015: $F_{1,47}$=476.0 p<0.001). Temperature and salinity also differed between years within the beach habitat (temperature: $F_{1,47}$=17.43 p<0.0001; salinity: $F_{1,47}$=416.72 p<0.0001) but not at the demersal habitat (temperature: $F_{1,51}$=0.59 p=0.45; salinity: $F_{1,51}$=3.83 p=0.6; Table 1.2B). Water chemistry Me/Ca ratios were similar between habitats in each year (Table 1.2B) and, thus, otolith trace element concentrations (μg/g) were presented rather than Me/Ca ratios. Mean otolith Mg concentrations differed significantly between rearing habitats and between years, while Sr and Ba did not, and otolith Mn concentrations only differed significantly between years (Table 1.3B; Fig. 1.5). When all four otolith trace elements were considered together, larvae could not be successfully classified into

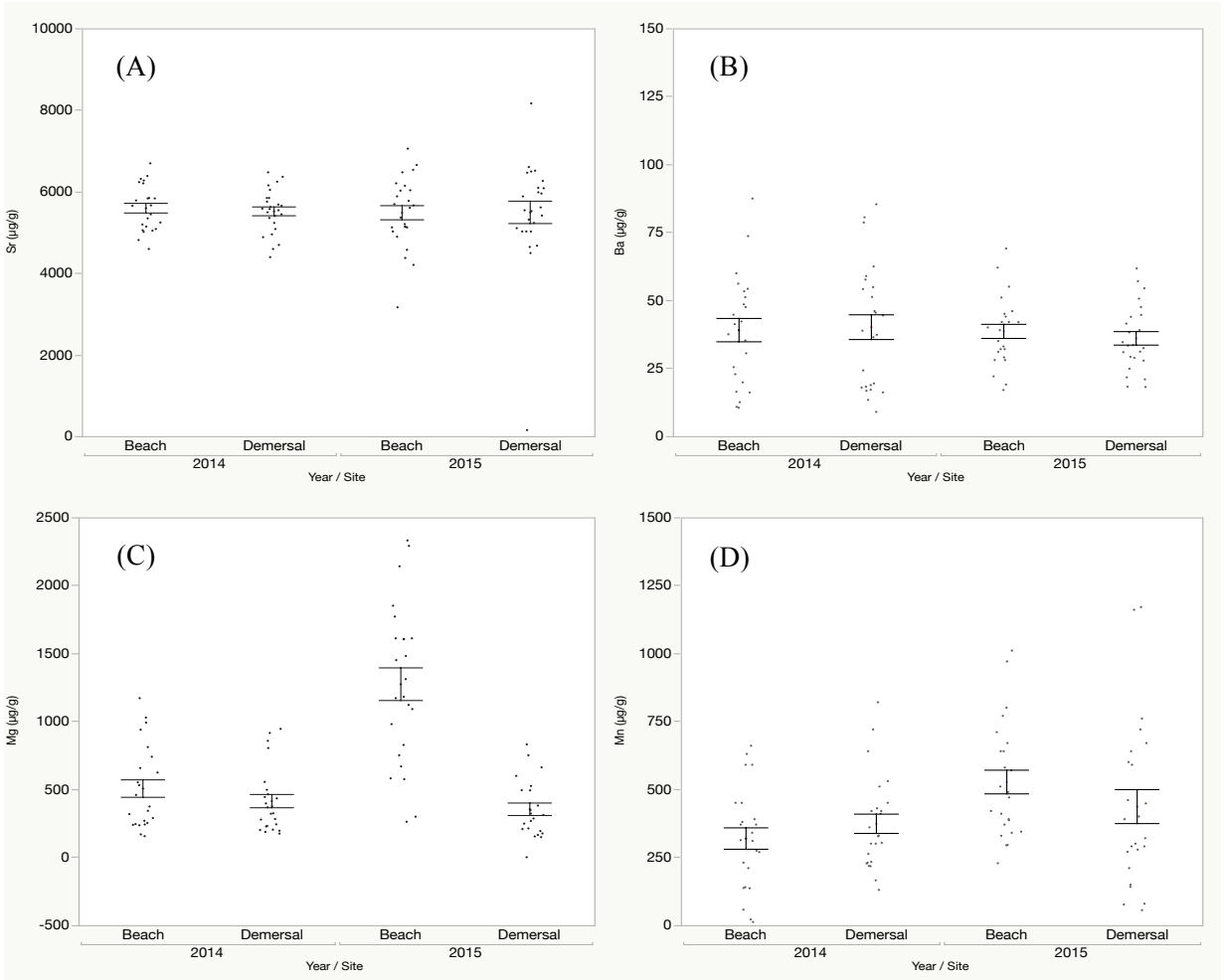

Figure 1. 5 Mean concentration (±SE) of otolith strontium (A), barium (B), magnesium (C), and manganese (D) within combined year and site treatments of the field-rearing experiment. Points represent average elemental concentrations of individual otoliths.

rearing habitat in 2014 (linear DFA, $F_{4,38}$=0.84 p=0.51), but could in 2015 ($F_{4,38}$=10.17 p<0.0001) with a classification success of 86.1%. Individual larvae could be successfully classified into year at the beach habitat ($F_{4,37}$=10.47 p<.0001) with a classification success of 83.33%, but could not at the demersal site ($F_{4,39}$=0.48 p=0.75).

Discussion

Otolith trace element concentrations were highly variable among capelin larvae reared under the exact same controlled conditions in the lab and natural conditions in the field. Despite this high variability, otolith concentrations of some trace elements (primarily Sr, Mg, and Mn) of lab-reared larvae differed among some temperature and salinity treatments. These results are similar to lab-rearing studies on other marine species, where otolith trace elemental concentrations differed among individuals raised under different temperature, salinity and/or ambient trace elements (e.g., larval spot, *Leiostomus xanrhurus*, Bath et al. 2000; juvenile black bream, *Acanthopagrus butcheri*, Elsdon and Gillanders 2002; larval Pacific cod, *Gadus macrocephalus*, DiMaria et al., 2010). Classification success of otolith chemical signatures into experimental treatments, however, was generally low (42-55%), suggesting a low predictive capacity. Similarly, larvae reared within both spawning habitats in the field in close proximity (<20 km) could not be reliably classified (53% success), based on habitat-specific otolith chemical signatures, in one year when only salinity differed between habitats, but could be reliably classified (86% success) in another year when both temperature and salinity differed. Overall, these findings suggest that when capelin eggs are reared under homogeneous ambient water chemistry (both experiments), moderate-high differences in temperature (i.e. 4-8 °C) and salinity (i.e. 10-20 psu) are needed to successfully distinguish eggs raised in each rearing habitat using otolith chemical signatures.

The high variability of otolith trace element concentrations among individual larvae reared under identical conditions in both the lab and field suggests that there are confounding factors influencing the incorporation of trace elements into the otoliths of larval capelin during egg incubation. One confounding factor may be maternal investment. Even when the eggs of some anadromous fish species (e.g., salmonids) are reared under identical conditions, otolith trace element (e.g., Sr, Ba) concentrations of individual offspring may vary widely, due to mothers experiencing either freshwater or saltwater environments while gametes are developing (Limburg et al., 2001; Volk et al. 2000). The relative influence of environmental conditions experienced by the mother and egg on larval otolith chemistry during capelin egg incubation, however, is unknown. Although capelin eggs have a complex, two-layered chorion, the chorion becomes thinner immediately after fertilization (Davenport et al., 1986). Both layers are perforated with radially arranged pores and canals, which allow for the transport of essential elements (e.g., Ca, Mg) into the egg to fuel the rapidly growing larva (Davenport et al., 1985; Shephard et al., 1989). Therefore, trace elements may enter capelin eggs, but the degree to which this occurs needs further study. Overall, it is possible that our ability to classify the natal origin of larval capelin using otolith chemical signatures may have been confounded by both maternal investment of trace elements during gonadal development as well as the physiological transport characteristics and processes that regulate the timing and rate of trace elements entering the egg and, thus, become incorporated into the developing larval otolith within the egg during incubation.

Despite the high variability among individuals, differences in otolith trace element concentrations were observed among temperature treatments. Indeed, otolith Sr concentrations were higher at higher temperatures. Interestingly, the positive relationship between temperature

and otolith Sr concentrations was opposite of my prediction, as an inverse relationship has been found for other marine, cold-adapted species (e.g., juvenile Atlantic herring, *Clupea harengus*, Townsend et al., 1995; juvenile black bream, *Acanthopagrus butcheri*, Elsdon and Gillanders, 2002; larval Pacific cod, *Gadus macrocephalus,* DiMaria et al., 2010). Positive relationships also have been reported for other marine species (e.g., adult black bream; Elsdon and Gillanders, 2003; spot, Bath et al., 2000), suggesting that the influence of temperature on otolith trace element incorporation rates is species-specific (Campana, 1999; Elsdon et al. 2003). A negative relationship between otolith Sr concentrations and temperature was recently suggested for capelin larvae reared in the field (Davoren et al., 2015). Differences between capelin studies may be explained by the different methods used in each study, including rearing under varying, instead of controlled, environmental conditions (this study) as well as quantifying trace element concentrations from all otoliths of many larvae (~500 individuals) instead of only the left sagittal otolith from each individual (this study). Contrary again to other studies, otolith Sr and Ba concentrations did not vary over salinity treatments. Indeed, otolith Sr and Ba concentrations have previously indicated shifts between marine and freshwater environments in some anadromous fish species (e.g., steelhead trout, *Oncorhynchus mykiss*, Zimmerman and Reeves, 2002) and between regions of lower and higher salinity in some marine species (e.g., spot, Elsdon and Gillanders, 2002; Atlantic cod, *Gadus morhua*, D'Avignon and Rose, 2013). Significant interactions between temperature and salinity, however, suggest that these factors combined to influence capelin otolith trace element incorporation rates as found for other species (e.g., Elsdon and Gillanders 2002, 2004), possibly confounding the influence of each factor separately.

Otolith Mg concentrations were higher at higher temperatures. As an essential element, Mg can influence the activity of enzymes and Ca transport channels (Loewen et al., in press). Embryonic capelin develop and grow more rapidly at higher temperatures (Frank and Leggett, 1981; Penton et al., 2012). Therefore, Mg incorporation rates may be directly linked to higher growth rates under warmer conditions and, thus, a need for higher Ca transport to allow the rapid larval growth. Although otolith concentrations of Mn were lower at higher temperatures, elevated concentrations of Mn in the primordial (i.e. pre-hatch) region of capelin otoliths relative to the outer (i.e. post hatch) region was observed, similar to another study on capelin (Lazartigues et al., 2014) as well as several marine species (e.g., Atlantic hering, *Clupea harengus*; spot, *Sprattus sprattus*, Brophy et al., 2004; painted greenling, *Oxylebius pictus*, Ruttenburg et al., 2005). Elevated concentrations of Mn in the egg during embryonic development of the respiratory system, when Mn is actively absorbed for enzyme activation, likely influences the distinct Mn spike seen in the primordial region of larval otoliths (Brophy et al., 2004). Otolith Mg and Mn concentrations decreased with increasing salinity, similar to other species that have illustrated the influence of salinity on otolith Mg and Mn concentrations (e.g., black cream, *Acanthopagrus butcheri*, Elsdon and Gillanders, 2002; European plaice, *Pleuronectes platessa*, Sturrock et al., 2014). The underlying physiological mechanisms, however, are not well understood (Campana, 1999; Elsdon and Gillanders, 2002).

In conclusion, the high variability in otolith elemental concentrations among individual capelin larvae suggests that some confounding factor is influencing our ability to successfully classify larvae reared under different environmental conditions but similar ambient water chemistry. Studies investigating the influence of maternal investment on the chemical signature of larval otoliths alongside quantifying trace elemental incorporation rates into developing

capelin otoliths during egg incubation would provide further insight into the relative influence of these factors on otolith chemical signatures and, thus, whether otolith chemistry can be used to identify the natal habitat of capelin. Our inability to reliably classify individuals into experimental treatments and rearing habitats with homogeneous ambient chemistry indicates that environmental conditions (e.g., temperature, salinity) need to vary widely to influence otolith chemistry of capelin larvae when reared in close proximity. In support, recently hatched larval capelin could be reliably classified into their natal origin on a broader scale (100-200 km; Lazartigues et al., 2016), with presumably varying ambient water chemistry. Therefore, distinguishing larvae reared in different habitats in close proximity (i.e. similar ambient water chemistry) may only be possible in some years when moderate-high differences in temperature (i.e. 4-8 °C) and salinity (10-20 psu) occur between rearing habitats. Further investigation of differences in otolith chemical signatures on a larger spatial scale, therefore, may also aid in a better understanding of population structure, connectivity and the contribution to recruitment from different spawning regions.

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Chapter 2. Influence of maternal investment and ambient water chemistry on otolith chemical signatures during egg incubation.

Introduction

Determining the natal origin of marine fish is important to elucidate characteristics of population structure, including connectivity. Determining natal origin, however, is difficult due to the challenge of conducting mark recapture studies on large numbers of small pelagic larvae. Chemical signatures in the otoliths of teleost fish have proven useful as natural tags and have been used to successfully identify the natal habitat (e.g., Thorrold et al., 2001; Elsdon and Gillanders 2003) of larval and juvenile marine fish species (e.g., Lazartigues et al., 2016). Otoliths, or 'ear stones', are calcified structures formed within the head of fish by accumulating trace elements on a proteinaceous matrix as the fish grows (Campana, 1999). As otoliths are metabolically inert, the trace elements and stable isotopes incorporated into otoliths from the surrounding water record and preserve the timing of elemental deposition from egg incubation until death (Campana, 1999; Elsdon and Gillanders, 2003).

Otolith elemental ratios in the pre-hatch growth region, often called the 'primordium' or the 'nucleus', may not reflect environmental conditions in the natal habitat, but instead may reflect conditions in the mother's environment during the time the yolk sac was formed (e.g., Volk et al., 2000). To illustrate, even when the eggs of some anadromous fish species (e.g., salmonids) are reared under identical conditions, otolith trace element (e.g., Sr, Ba) concentrations in the primordial region of offspring may vary widely when mothers experienced either freshwater or saltwater environments while gametes were developing and the embryonic otolith was forming (Limburg et al., 2001; Volk et al. 2000). Zimmerman and Reeves (2002) also reported that the otolith primordial region of rainbow trout, *Oncorhynchus mykiss*, had

elevated Sr concentrations when mothers lived in seawater during gonadal development. In addition, certain trace element (e.g., Mn, Ba, Mg) concentrations are much higher in the primordial region of many species (e.g., sprat, *Sprattus sprattus*, Brophy et al., 2004; painted greenling, *Oxylebius pictus*, Ruttenburg et al., 2005 rockfish *Sebastes atrovirens*, Kuroki et al., 2010). These elevated concentrations in the primordium may result from higher incorporation rates of these elements during embryonic otolith development, which together with maternal investment, may overwhelm the influence of environmental conditions in the rearing habitat on otolith chemistry and, thus, the ability to identify natal origin (Ruttenberg et al., 2005).

Despite the possible influence of maternal investment on otolith signatures, other studies have found a correlation between trace element concentrations in the natal or rearing habitat and the otolith primordial region of several fish species (e.g., weakfish, *Cynoscion regalis*, Thorrold et al., 2001; black bream, *Acanthopagrus butcheri*, Elsdon and Gillanders, 2004). In addition, elemental marking of incubating eggs has been used to successfully distinguish different groups of individuals (e.g., clown-fish, *Amphiprion melanopus*, serranid, *Centropristis striata*, Thorrold et al., 2006; Atlantic salmon, *Salmo salar*, Warren-Myers, 2015) and, thus, is frequently used as a marking method to assess the proportion of wild versus hatchery-reared stocks to investigate the abundance, stock structure, and contribution to populations (Warren-Myers, 2015). During elemental marking, fish are exposed to water containing enriched concentrations of rare stable isotopes (e.g., ^{86}Sr, ^{138}Ba) at any life-history stage, including egg incubation (Munro et al., 2008; Warren-Myers, 2015). Due to physiological regulating processes, trace elemental incorporation rates into otoliths vary among species, resulting in species-specific relationships between otolith and ambient trace elemental concentrations and environmental conditions (e.g., temperature, salinity; Elsdon and Gillanders, 2003). Understanding whether the primary source of trace

elements for the developing larval otolith during egg incubation is from the mother or the environment would provide insight into whether identifying the natal origin of a species is possible using otolith chemical signatures.

Capelin, *Mallotus villosus*, is a small, short-lived (3-6 years) schooling fish found in Arctic and sub-Arctic regions of Atlantic and Pacific Oceans (Carscadden et al., 2013). They are considered a focal forage species, as they are an important prey source for many top predators, including during the summer spawning season on the east coast of Newfoundland (Carscadden and Vilhjalmsson, 2002). In the Newfoundland region, capelin typically spawn on sandy beaches during June and July; however, spawning in deep-water ('demersal', 15-40 m) habitats was recently discovered in coastal embayments (Nakashima and Wheeler, 2002; Davoren et al., 2006). Fertilized capelin eggs adhere to the substrate at spawning sites, which become their rearing habitat. The relative contribution of offspring from demersal and beach habitats to the spawning population, or recruitment, in coastal Newfoundland is unknown. Previous studies suggested that otolith chemical signatures may identify the natal, or rearing, habitat of capelin (Davoren et al., 2015, Chapter 1). High variability in otolith chemical signatures among individuals reared under identical conditions in both the lab and field (Chapter 1), however, suggest that some confounding factor may be hindering our ability to successfully classify capelin into rearing habitats.

The goal of this study was to investigate the influence of both maternal investment and ambient water chemistry on otolith chemical signatures of recently hatched capelin larvae. First, I tested the hypothesis that maternal investment influences otolith chemistry of developing larvae during egg incubation. I predicted that otolith signatures of offspring would reflect maternally invested trace element concentrations. To test this, I reared artificially fertilized eggs from

known parents (one male, one female) under controlled temperature and salinity conditions. If otolith chemical signatures of recently hatched larvae differed more among families than within families, then maternal investment may confound our ability to identify rearing habitat using chemical signatures in the otolith primordium. Second, I tested the hypothesis that ambient water chemistry influences otolith chemistry of developing larval otoliths during egg incubation. I predicted that otolith chemistry of larvae would reflect ambient water chemistry of rearing environment. To test this, I reared naturally fertilized eggs in water spiked with a rare stable isotope of Barium (^{138}Ba) for different durations during egg incubation. If otolith chemistry of larvae exposed to water spiked with ^{138}Ba have a lower ratio of ^{138}Ba/^{137}Ba than those from the control, then trace elements are incorporated into the embryonic otolith during egg incubation. Overall, this study will elucidate the influence of ambient water chemistry and maternal investment in otolith chemical signatures in the primordial region of capelin, thereby providing insight into whether otolith chemistry can be used as a tool to identify the natal habitat of capelin.

Methods

Experimental set up

Prior to fish collection and experimental set up, rearing jars and associated equipment were cleaned by soaking in ~10% NaClO. For the maternal investment experiment, treatment water was prepared by adding *Seachem Marine Salt*^{TM} to dechlorinated city water until the desired salinity (~10, 20, 30 psu) was met by measuring conductivity using a multiparamater YSI. For the water spiking experiment, rearing water was collected from the ocean in 19 L polyethylene holding containers and purified using an *Aquastep* UV filter. Enriched treatment

water was created by adding 52.3 µg of 90.75 mg/mL concentrated stable isotope ^{137}Ba to 19 L of natural seawater (diluted concentration = 250 µg/mL).

For the maternal investment experiment, mature male and female capelin were collected from Middle Cove Beach, Newfoundland during July 2014. Live fish were transported to the lab at Memorial University of Newfoundland where gametes from nine male-female pairs were artificially fertilized to produce offspring from nine families. For each family, one mature male and one mature female capelin were dried by gently patting with paper towel. Eggs (~1.5 mL) were stripped from the female into a plastic dish and two drops of milt from males was added directly onto the eggs. The eggs and milt were mixed together and left on ice for 30 s. A mixture of 600 mg/L tannic acid (10°C, 30 psu) was then added to the eggs to reduce egg adhesiveness. Eggs were then rinsed three times (30 psu) and ~50 eggs were transferred to 50 mL glass beakers and filled with water of the desired salinity treatment. This process was repeated for a total of nine families. Artificially fertilized eggs of known families were then incubated under two temperature (10 and 15°C) and three salinity (10, 20, and 30 psu) treatments, for a total of six treatments, with two replicate beakers per family per treatment (Fig. 2.1) within controlled environmental chambers at Memorial University of Newfoundland. Water changes were conducted every second day by gently discarding ~50% of the water from each jar and replacing with new water. When larvae were present, water from each jar was gently poured over a 0.232 mm sieve to collect larvae. Larvae were then transferred into a 20 mL glass vial filled with 95% ethanol. Larvae were collected once each day until all eggs hatched (Table 2.1). Ambient trace element concentrations were monitored throughout the experiment by collecting water samples from each salinity treatment at the beginning, midpoint, and end of the study (Table 2.2). Samples were collected using 60 mL acid-washed polypropylene syringes and transferred

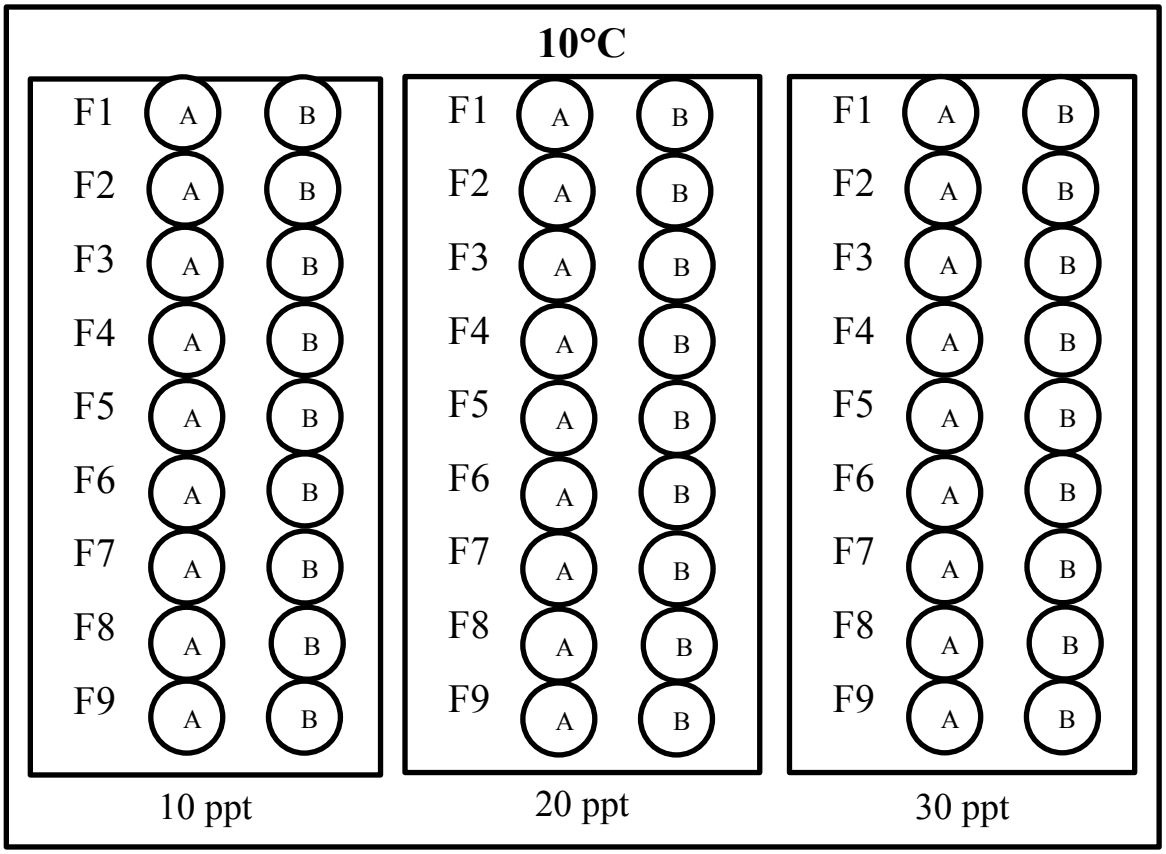

Figure 2. 1 Diagram of the maternal investment experiment indicating two replicates (A-B) for each of the nine families (F1-9) within each temperature and salinity treatment. Similar setup was repeated within the 15 °C temperature chamber.

Table 2. 1 Approximate number of larvae (i.e. count) hatched and collected from each artificially fertilized capelin family from within each replicate of the temperature and salinity treatments. Families with highest hatching success from all treatments were used for analyses (2, 4, 7, and 9).

Family	Temp.	Salinity	Replicate	Count	Family	Temp.	Salinity	Replicate	Count
1	10	10	A	30	**2**	10	10	A	75
		10	B	40			10	B	60
	10	20	A	50		10	20	A	-
		20	B	20			20	B	50
	10	30	A	20		10	30	A	20
		30	B	50			30	B	40
	15	10	A	4		15	10	A	6
		10	B	10			10	B	7
	15	20	A	4		15	20	A	15
		20	B	2			20	B	10
	15	30	A	-		15	30	A	2
		30	B	-			30	B	2
3	10	10	A	5	**4**	10	10	A	20
		10	B	4			10	B	50
	10	20	A	-		10	20	A	20
		20	B	-			20	B	10
	10	30	A	1		10	30	A	-
		30	B	-			30	B	5
	15	10	A	-		15	10	A	-
		10	B	-			10	B	-
	15	20	A	-		15	20	A	-
		20	B	-			20	B	10
	15	30	A	-		15	30	A	-
		30	B	-			30	B	-
5	10	10	A	60	**6**	10	10	A	20
		10	B	20			10	B	10
	10	20	A	75		10	20	A	20
		20	B	30			20	B	30
	10	30	A	50		10	30	A	75
		30	B	50			30	B	50
	15	10	A	30		15	10	A	1
		10	B	30			10	B	-
	15	20	A	10		15	20	A	-
		20	B	10			20	B	1
	15	30	A	1		15	30	A	-
		30	B	5			30	B	-

Table 2.1 continued

Family	Temp.	Salinity	Replicate	Count	Family	Temp.	Salinity	Replicate	Count
7	10	10	A	20	**8**	10	10	A	-
		10	B	30			10	B	-
	10	20	A	50		10	20	A	8
		20	B	20			20	B	-
	10	30	A	40		10	30	A	-
		30	B	40			30	B	-
	15	10	A	6		15	10	A	-
		10	B	10			10	B	-
	15	20	A	15		15	20	A	-
		20	B	-			20	B	-
	15	30	A	1		15	30	A	-
		30	B	1			30	B	-
9	10	10	A	40					
		10	B	40					
	10	20	A	20					
		20	B	20					
	10	30	A	10					
		30	B	40					
	15	10	A	8					
		10	B	5					
	15	20	A	7					
		20	B	10					
	15	30	A	-					
		30	B	-					

Table 2. 2 Mean rearing water elemental concentrations (±SE ppm) with the Me/Ca ratios in brackets for the artificial fertilization experiment.

Artificial Fertilization Experiment						
Treatment		Water Chemistry				
Temperature	Salinity	Ca	Sr	Ba	Mg	Mn
10°C	10 psu	-	-	-	-	-
	20 psu	-	-	-	-	-
	30 psu	-	-	-	-	-
15°C	10 psu	100.9 ±2.10	1.72 ±0.06 ($1.7\ e^{-2}$)	0.004 ±0.0007 ($1.9\ e^{-5}$)	333.0 ±4.00 (3.3)	0.002 ±0.0005 ($1.9\ e^{-5}$)
	20 psu	214.0 ±16.0	3.58 ±0.17 ($1.7\ e^{-2}$)	0.005 ($1.5\ e^{-5}$)	791.0 ±76.0 (3.6)	0.003 ±0.0010 ($1.4\ e^{-5}$)
	30 psu	326.0	5.16 ($1.6\ e^{-5}$)	-	1100.0 (3.4)	-

through 0.45 μm filters into 50 mL acid cleaned polyethylene bottles, preserved with 3 mL of 33% HNO_3, and stored until analysis.

For the water spiking experiment, naturally fertilized eggs were collected on the first day of spawning (July 2015) from a deep-water spawning site (site 5; see Fig. 1.1) using a 0.3-m^2 Ponar Benthic Grab system. Approximately 300 eggs were transferred to glass jars (120 mL) and assigned to a treatment. Treatments were based on the timing of exposure to enriched ^{138}Ba water and included exposure immediately after fertilization (T1), three days after (T2), six days after (T3) and no exposure (T4, control; Table 2.3). Three replicate jars per treatment were placed in a controlled environmental chamber where temperature was held at ~6.5°C (Fig. 2.2). Water changes were performed every second day by gently discarding ~90% of rearing water and replacing with new water while environmental conditions (i.e. temperature, salinity, pH) were monitored daily using a multi-parameter YSI Pro30 probe. Larval collection procedures were the same as the previous experiment. As experimental water used throughout the entire experiment was the same and known concentrations of ^{138}Ba were added to create treatments, water chemistry was not sampled or monitored for changes throughout this experiment.

Otolith Chemistry Analyses

Trace elemental concentrations in otoliths of recently hatched larvae were analyzed via laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS, *Perkin-Elmar DRC II*), similar to Lazartigues et al. (2014). For the maternal investment experiment, families with sufficient hatching success (i.e. n=5 larvae/replicate/treatment) were selected for analysis and five larvae per replicate per treatment were randomly chosen (n=50 larvae/family). For the spiking experiment, 10 larvae per replicate per treatment (n=30 larvae/treatment) were randomly chosen for analysis. Prior to otolith removal, equipment and microscope slides were acid washed

Table 2. 3 as well as average experimental conditions for the spiking experiment (B) including treatment, based on the timing of exposure to enriched ^{138}Ba, as well as water and average (±SE) temperature (°C), salinity (psu), and pH conditions.

Spiking Experiment				
Treatment	Description	Temperature	Salinity	pH
T1	Day 0 - end	6.44 (±0.31)	29.11 (±0.22)	7.33 (±0.09)
T2	Day 3 - end	6.39 (±0.35)	28.62 (±0.44)	7.22 (±0.21)
T4	Day 6 - end	6.59 (±0.47)	29.05 (±0.27)	7.46 (±0.08)
T4	Cont. (no spike)	6.73 (±0.52)	28.82 (±0.33)	7.40 (±0.08)

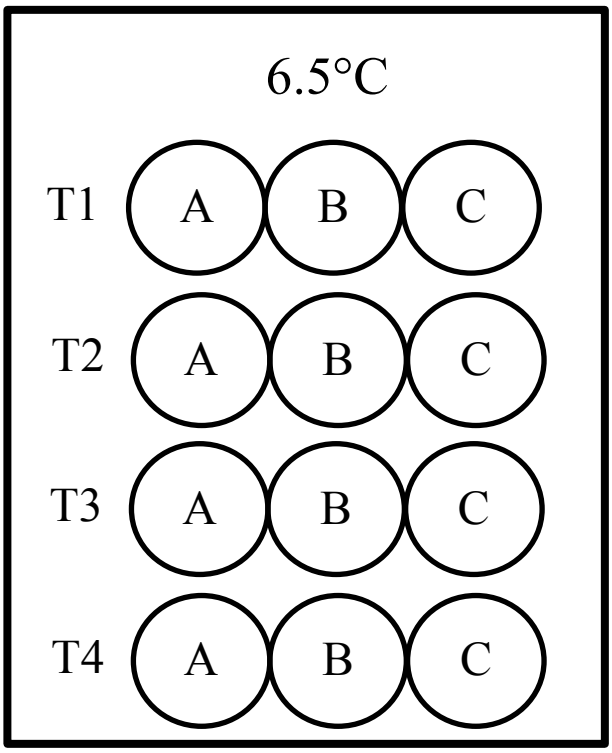

Figure 2. 2 Diagram of the spiking experiment indicating three replicates (A-C) for each treatment. Treatments were based on the timing of exposure to enriched ^{138}Ba water and included exposure immediately after fertilization (T1), three days after (T2), six days after (T3) and no exposure (T4, control).

with 2% nitric acid. Larvae were placed on a microscope slide and rinsed in 2-3 drops of milliQ water. Each larva was dissected under an *Olympus SZX7* dissecting microscope mounted with *Olympus SZX-PO* polarizers and the left sagittal otolith was removed (Fig. 1.3A). The otolith was rinsed in a drop of milliQ water and allowed to dry for 30 seconds. Each microscope slide had a one centimeter square grid divided into 25 equal squares covered with a piece of double-sided (*Scotch*^{TM}) tape. Each otolith was fixed within one of the squares (Fig. 1.3B) and then photographed to increase location efficiency prior to laser ablation.

Slides with 25 otoliths each were placed separately in the ablation cell and the otoliths were located by referencing previously taken photographs. A spot analysis technique was used to ablate down through the otolith (rather than across the surface, Chapter 1) to achieve a consistent signal and quantify trace element concentrations while distinguishing the core region (Fig. 1.3C). The following parameters were used for the spot analysis: 65% energy, 1 Hz repetition rate, and 30 μm beam size. A 30 s gas blank was run prior to ablating each otolith to ensure the previous sample was cleared and to determine background trace element concentrations for estimating elemental detection limits. Once all the otoliths on a slide were ablated, the reference glass material was ablated again to correct for any changes during analysis.

Data reduction (i.e. mapping, calculations of concentrations and limits of detection (LOD)) was performed using *Igor Pro* graphing software with an *Iolite* version 2.21 package for LA ICP-MS. Calcium (Ca) counts per second (CPS) were used to determine an internal standard to correct for changes in ablation volume. The mean (± SE) concentration of each trace element was then calculated by averaging ablation samples for each otolith. Water samples were analyzed at ALS Environmental Laboratories (Burnaby, BC) following EPA Methods 6010B (see Davoren et al., 2015). Major elements (e.g., Ca) were analyzed by inductively coupled plasma

optical emission spectroscopy (ICP-OES, Thermo iCap 6500) after a 10x dilution. Trace elements (e.g., Ca, Sr, Ba, Mg, Mn) were analyzed via ICP-MS (Table 2.2A).

Data Analysis

Mean otolith trace elemental concentrations that were greater than two standard deviations from the mean were identified as outliers and removed from the dataset (maternal investment: n=19; spiking experiment: n=8). Mean concentrations of each trace element (continuous response variables, μg/g) were examined separately for normality using the Shapiro-Wilk's W test, and for homogeneity of variance using the Levene's test. Response variables from the maternal investment experiment were non-normal and, therefore, were log transformed to meet the underlying assumptions of parametric statistics.

In the maternal investment experiment, four families (i.e. 2, 4, 7, 9) had sufficient hatching success and, thus, larvae from these families were used to test whether the mean concentration of each trace element (i.e. Sr, Ba, Mg, Mn) in larval otoliths differed significantly among treatments using mixed multifactor ANOVAs. Fixed factors were temperature (°C), salinity (psu) and family, while replicate jar was used as a nested random factor to control for variation among replicates. In addition to these univariate tests, a linear discriminant function analysis (DFA) was performed where all response variables (i.e. log mean Mg, Mn, Sr, and Ba concentrations) were combined to determine whether treatment-specific otolith chemical 'signatures' could be defined. For the spiking experiment, I tested whether the ratio of $^{138}Ba/^{137}Ba$ in larval otoliths (response variable) differed significantly among treatments using a mixed single factor ANOVA. The natural ratio of $^{138}Ba/^{137}Ba$ in seawater is 6.3 and, thus, any deviation from this natural ratio indicated that the naturally rare isotope (i.e. ^{138}Ba) had been incorporated into the developing embryonic otolith during incubation. Again, rearing treatment

was the fixed factor and jar was a nested random factor to control for variation among replicates. Post-hoc Tukey HSD tests were performed to identify which factor levels were significantly different.

Results

Throughout the maternal investment experiment, temperature and salinity were held constant and water chemistry (i.e. metal/calcium, 'Me/Ca' ratios) was similar among all treatments (Table 2.2A) and, thus, otolith trace element concentrations (μg/g) were presented rather than Me/Ca ratios. Mean otolith concentrations of Ba, Mg, and Mn differed significantly between temperature treatments, while Sr did not (Table 2.4). Mean otolith Ba and Mg concentrations were consistently higher in the 10°C relative to the 15°C treatment. Linear DFA analyses revealed that larvae could be successfully classified into temperature treatments ($F_{12, 469}$=62.76 p≤0.0001), with an 83.2% classification success. Otolith trace element concentrations of Sr, Ba, and Mg differed significantly among salinity treatments while Mn did not (Table 2.4). Post-hoc Tukey HSD tests indicated that mean otolith Sr and Mg concentrations were significantly higher in the 10 psu treatment relative to the 20 and 30 psu treatments (Fig. 2.3). Significant differences in mean otolith Ba concentrations occurred among all salinity treatments and decreased with increasing salinity. Linear DFA analyses revealed that larvae could be successfully classified into salinity treatments ($F_{8,356}$=3.58 p=0.0005) but classification success was low (46.7%).

Significant differences in all otolith trace element concentrations were observed among families (Table 2.4). Indeed, mean otolith trace element concentrations differed significantly

Table 2. 4 Statistical results of three-factor ANOVAs from the maternal investment experiment. P-values less than 0.05 (bolded) indicate significant differences.

Element	Treatment	df	df_{error}	F ratio	p-value
Sr	Family	3	198	32.73	**<0.0001**
	Temperature	1	198	3.62	0.0584
	Salinity	2	198	7.73	**0.0006**
	Temp x Sal	2	198	1.44	0.2403
Ba	Family	3	195	240.14	**<0.0001**
	Temperature	1	195	57.21	**<0.0001**
	Salinity	2	195	4.20	**0.0164**
	Temp x Sal	2	195	9.67	**<0.0001**
Mg	Family	3	187	7.42	**0.0001**
	Temperature	1	187	70.32	**<0.0001**
	Salinity	2	187	12.41	**<0.0001**
	Temp x Sal	2	187	15.41	**<0.0001**
Mn	Family	3	197	29.15	**<0.0001**
	Temperature	1	197	4.91	**0.0278**
	Salinity	2	197	1.73	0.1793
	Temp x Sal	2	197	2.00	0.0137

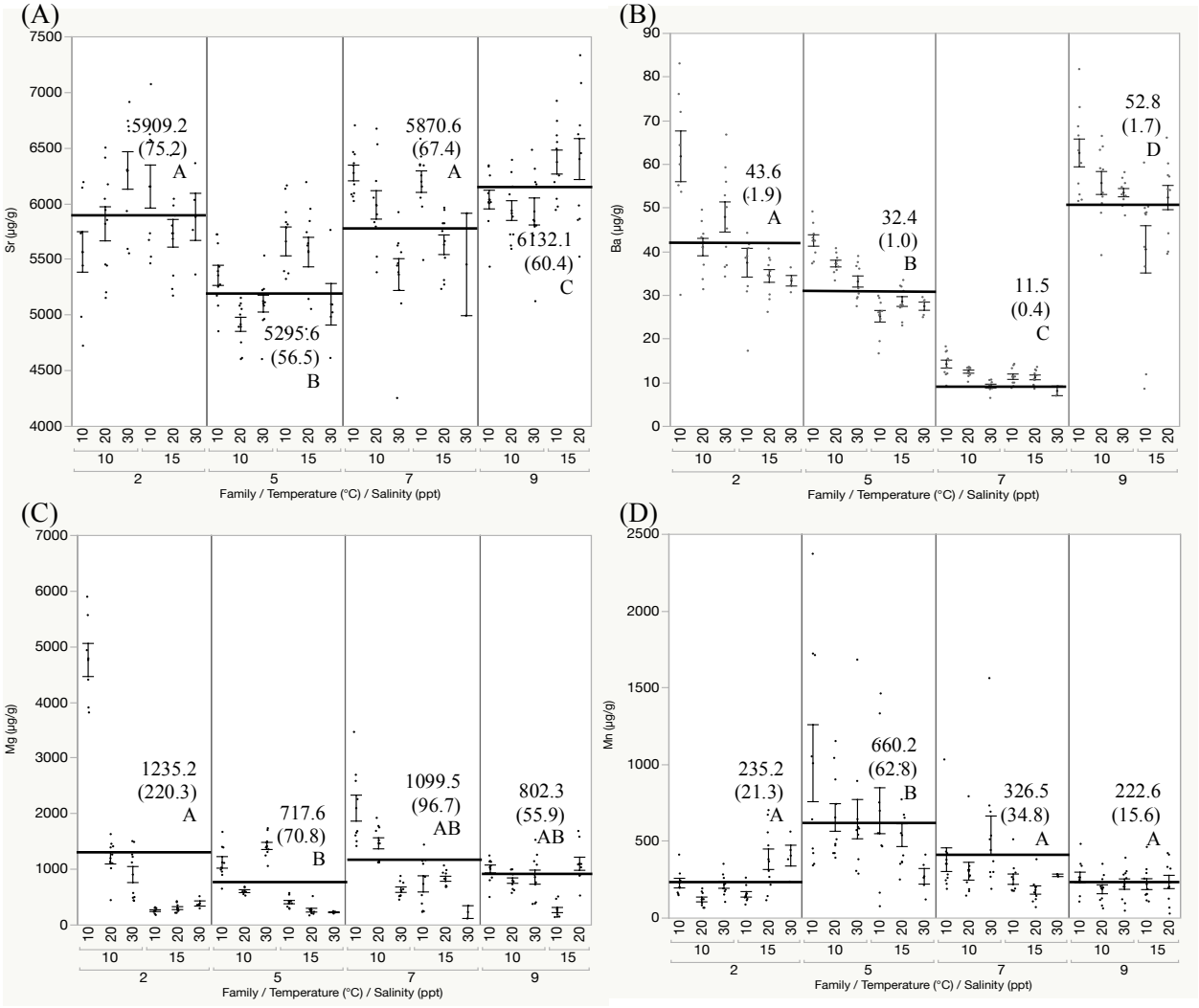

Figure 2. 3 Mean concentration (±SE) of otolith strontium (A), barium (B), magnesium (C), and manganese (D) within combined temperature, salinity, and family treatments of the maternal investment experiment. Points represent average elemental concentrations of individual otoliths. The grand mean (±SE) for each family is indicated by the horizontal grey line with the mean value indicated above. Families not sharing the same letter are significantly different.

among all four families for Ba and among three families for Sr, while only one family differed significantly in mean otolith Mg and Mn concentrations (Fig. 2.3). Linear DFA analyses revealed that larvae could be successfully classified into families ($F_{12,469}$=62.76 $p \leq 0.0001$), with 83.7% of larvae being correctly classified into each family. When otolith Ba concentrations were removed from the DFA analysis, families could be classified ($F_{9,189}$=455.3 $p \leq 0.0001$) but success decreased (51.8%). In contrast, separate removal of otolith Sr, Mg, and Mn concentrations from the DFA analysis did not influence classification success to a great degree (77.6-81.8%), suggesting that otolith Ba concentrations were important for classifying larvae into families. To allow comparison among more families, otolith chemistry was determined from seven families within a treatment with high hatching success (10°C, 20 psu) and the analysis was repeated. Similarly, otolith Ba concentrations of all trace elements differed significantly among families ($p \leq 0.0001$), with five of the seven families differing significantly in otolith Ba concentrations, while only one, two, and three families differed significantly for Sr, Mg, and Mn, respectively (Fig. 2.4). Linear DFA analyses revealed that larvae continued to be successfully classified into families based on otolith chemical signatures ($F_{24,189}$=0.02 $p \leq 0.0001$) with high success (81.3%).

Otolith concentration ratios of $^{138}Ba/^{137}Ba$ differed significantly among all spiking treatments ($F_{3,111}$=233.5 $p \leq 0.0001$; Fig. 2.5). Post-hoc Tukey HSD tests revealed that the otolith $^{138}Ba/^{137}Ba$ ratios decreased as the duration in the spiked water decreased (Fig. 2.5). Interestingly, when ratios in larval otoliths from all spiked water treatments (i.e. T1,2,3) were compared to the control (i.e. T4) $^{138}Ba/^{137}Ba$ differed significantly ($F_{1,111}$=622.4 $p \leq 0.0001$) and classification success was 100%.

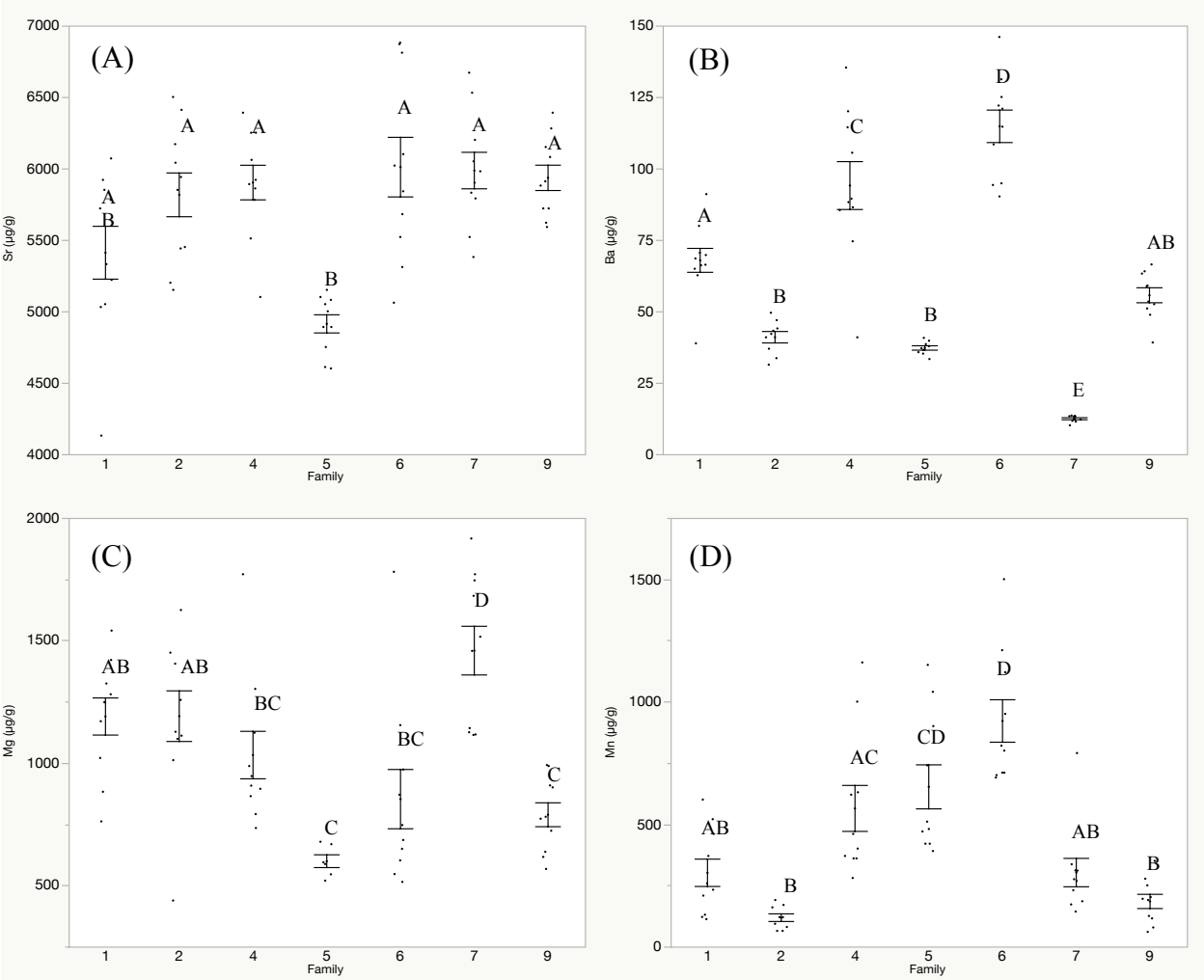

Figure 2. 4 Mean concentration (±SE) of otolith strontium (A), barium (B), magnesium (C), and manganese (D) of each family within the 10°C, 20 psu treatment. Points represent average elemental concentrations of individual otoliths. Families not sharing the same letter are significantly different ($p<0.05$).

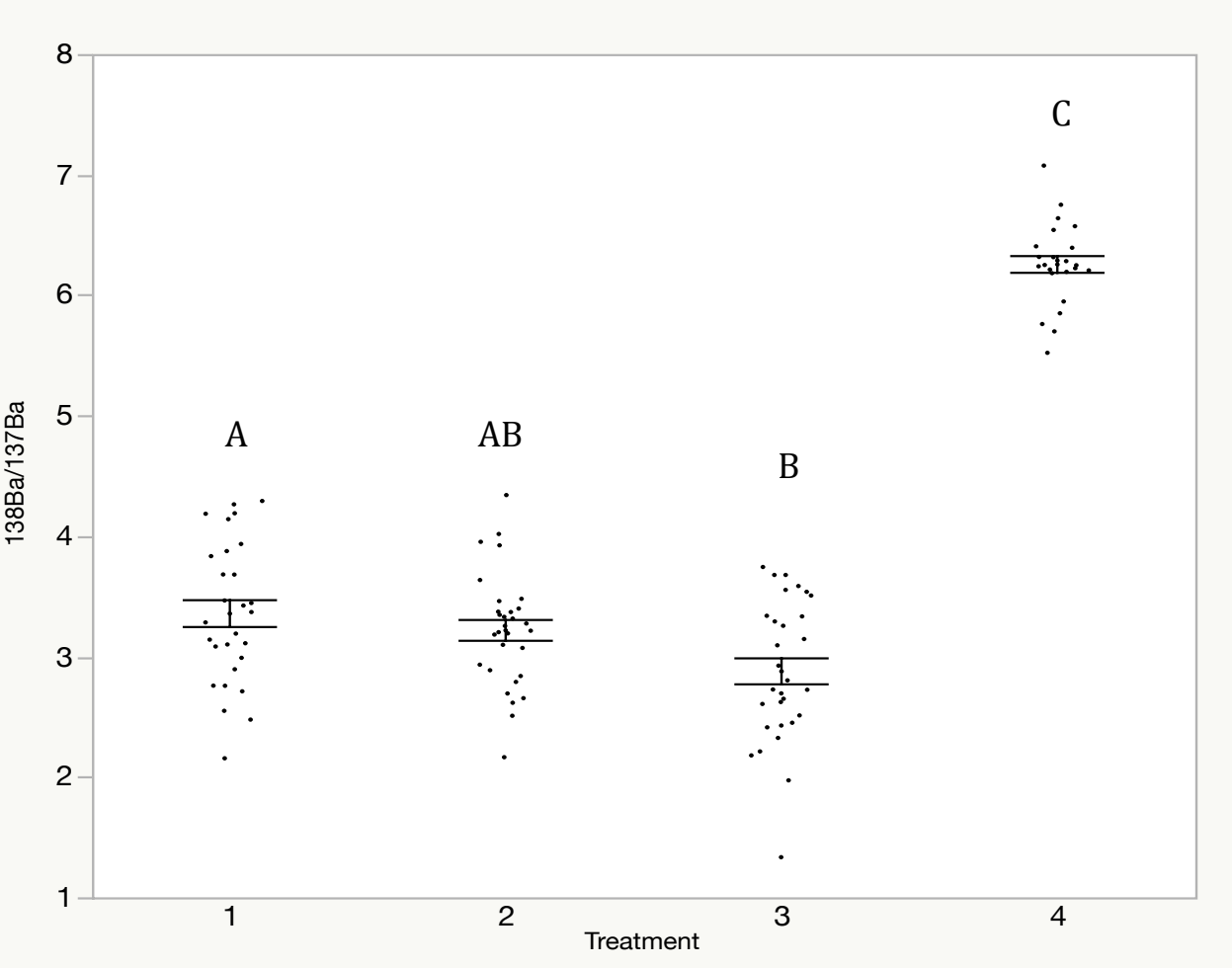

Figure 2. 5 Mean ratio (±SE) of otolith ^{138}Ba:^{137}Ba of each treatment of the spiking experiment. Treatments were based on the timing of exposure to enriched ^{138}Ba water and included exposure immediately after fertilization (T1), three days later (T2), six days later (T3) and no exposure. Points represent average ratio of otolith ^{138}Ba:^{137}Ba of individuals. The natural ratio ^{138}Ba/^{137}Ba in seawater is 6.3 (T4) and, thus, any deviation from this ratio indicates the rare isotope is being absorbed and precipitated into the larval otolith.

Discussion

In comparison to naturally fertilized eggs (Chapter 1), otolith trace element concentrations of larvae from artificially fertilized eggs showed markedly less variability within a family when reared under identical, controlled conditions. Additionally, all otolith trace element concentrations (Sr, Ba, Mg, Mn) differed significantly among families and classification success within families based on otolith chemical signatures was high (~83%). These findings suggest that maternal investment may influence chemical signatures in the primordial otolith region of offspring. Otolith trace element concentrations, however, differed among temperature and salinity treatments, similar to a previous study (Chapter 1). Additionally, the spiking experiment indicated that trace elements are incorporated into the primordial otolith region during egg incubation. Altogether, these results suggest that maternal investment has an important influence on chemical signatures in the otolith primordium, but that ambient water chemistry as well as temperature and salinity also influence otolith trace elemental incorporation rates.

Maternal investment may be defined as the degree to which the chemical environment the mother experiences during gonadal development is reflected in the chemistry of the primordial region of the otoliths of her offspring. Maternal investment has been illustrated in a number of species, whereby typically Sr concentrations in the primordial region of larval and juvenile salmonids otoliths indicated whether they were the progeny of anadromous versus freshwater resident mothers (e.g., sockeye salmon, *Oncorhynchus nerka*, coho salmon, *O. kisutch*, chum salmon, *O. keta*, Volk et al., 2000). Previous focus on maternal investment of Sr into the otoliths of offspring is likely due to large differences in ambient Sr concentrations between marine and freshwater habitats (Elsdon and Gillanders, 2004), resulting in divergent concentrations being

incorporated in the primordial otolith region during gonadal development (Volk et al., 200; Zimmerman and Reeves, 2002; Zimmerman and Reeves, 2009). For capelin, however, otolith Ba appeared to be important for successfully distinguishing capelin progeny from different families. This suggests that maternal investment of Ba may be high in developing capelin otoliths relative to other trace elements (e.g., Sr, Mg, Mn), thereby possibly explaining the high variation and lack of significant differences among temperature and salinity treatments in otolith Ba concentrations among naturally fertilized individuals in a previous lab-rearing experiment (Chapter 1). In support, transgenerational marking studies, whereby enriched Ba isotopes are injected into gravid females, have illustrated that Ba ions are passed from the mother to the otoliths of her offspring (Kuroki et al., 2010) in several marine fish species (e.g., clownfish, *Amphiprion melanopus*, Thorrold et al., 2006; black sea bass, *Centropristis striata*, Almany et al., 2007; golden perch, *Macquaria ambigua*, Munro et al., 2009). As ambient Sr and Ba concentrations are relatively homogeneous throughout marine systems (Elsdon and Gillanders, 2003), it is unclear why otolith Ba concentrations vary enough to distinguish among offspring from different families of this purely marine fish. Regardless of the mechanism, these results suggest that identifying natal origins of capelin using otolith Ba concentrations will be highly confounded by maternal investment.

In contrast to otolith Ba concentrations, otolith Mn and Mg concentrations seemed to be less important for distinguishing among families and, thus, may not be maternally invested to a high degree. As an essential element, Mg is involved in regulating the activity of enzymes and Ca transport channels (Loewen et al., in press) and, thus, Mg incorporation rates may be more directly linked to Ca uptake and growth (Loewen et al., in press). Elevated concentrations of Mn have been found in the primordial region relative to post-hatch regions of capelin otoliths

(Lazartigues et al., 2014; Davoren et al., 2015) as well as other species (e.g., Atlantic hering, *Clupea harengus*; spot, *Sprattus sprattus*, Brophy et al., 2004; painted greenling, *Oxylebius pictus*, Ruttenberg et al., 2005). As an essential element, Mn is actively absorbed for enzyme activation during the embryonic development of the respiratory system (Brophy et al., 2004), Mn is likely influenced more by regulatory pathways than maternal investment.

Our ability to identify the natal habitat of larval capelin relies on the capacity of trace elements to pass through the chorion and be incorporated into the developing otoliths of embryonic capelin during egg incubation. Despite evidence of maternal investment in salmonids, the chorion of salmonid eggs are permeable to several small molecules (e.g., water, inorganic ions) and the rate at which these particles are transported across the membrane is controlled by factors such as concentration gradients inside and outside of the egg and the ionic mobility of the membrane (Shephard et al., 1989). While these processes are likely species-specific and, thus, currently unknown for capelin, the spiking experiment clearly indicates that Ba ions from the ambient water are transported and incorporated into the primordial region of capelin otoliths. Regardless of the timing of exposure to the enriched stable isotope, concentrations of ^{138}Ba incorporated into the embryonic otolith were enough to significantly decrease the natural ratio of ^{138}Ba/^{137}Ba in seawater. In fact, when eggs were exposed to ^{138}Ba later during incubation (e.g., T3 versus T1), the incorporation rate of Ba was higher (i.e. lower ratio ^{138}Ba/^{137}Ba), possibly due to the deterioration of the chorion as the inner layer of the chorion is dissolved by enzymes as larvae approach hatching (e.g., Medaka, *Oryzias latipes*, Suga, 1963).

Otolith chemistry in the primordial region was also influenced by environmental factors (i.e. temperature, salinity) as seen in previous studies on larval capelin (e.g., Davoren et al., 2015; chapter 1). Interestingly, otolith Ba concentrations did not differ significantly among

temperature and salinity treatments in Chapter 1, but differed significantly among similar treatments when controlling for maternal investment (this study). This suggests that controlling for maternal investment may result in more clear effects of temperature and salinity on the rate at which Ba is incorporated into the developing otoliths. For other trace elements, trends among treatments became nonsignificant (e.g., otolith Sr with temperature, otolith Mn with salinity), again opposing results in Chapter 1. Similarly, mean otolith Mg concentrations showed opposite trends with temperature relative to Chapter 1, being higher at lower temperatures in this study. While otolith Mg concentrations likely increase with Ca intake and, thus, growth rate at higher temperatures, the warmer treatment (15°C) may have surpassed optimal incubation temperatures (4-7°C, Penton and Davoren, 2013). This is supported by poor hatching success of capelin at 15°C (this study) and above 12°C in other studies (C. Purchase unpublished data; Penton and Davoren, 2013). Therefore, differing trends along environmental gradients in this study relative to Chapter 1, may result from different temperature treatments among studies (i.e. 4, 8, 12°C in Chapter 1; 10, 15°C in this chapter) as well as controlling for the variation in otolith chemistry among families, particularly in the case of otolith Ba concentrations.

In conclusion, the influence of maternal investment on chemical signatures in the primordial region of capelin otoliths may limit our ability to determine otolith chemical signatures associated with divergent temperature and salinity conditions. Trace elements (e.g., Ba) from the ambient water, however, are being incorporated into the embryonic otolith during egg incubation, indicating that ambient water chemistry also has an influence on otolith chemical signatures, as do varying temperature and salinity. Future studies focusing on the relative influence of maternal investment and ambient water chemistry on otolith chemical signatures of recently hatched larvae will aid in deciphering the amount of variation in ambient water

chemistry and environmental conditions needed to distinguish the natal habitat of individuals. These studies will determine whether the natal origin of capelin larvae can be identified using otolith chemistry, thereby providing insight into population structure, connectivity and the contribution to recruitment from different spawning habitats and regions where ambient chemistry and environmental conditions differ.

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Synthesis Discussion

Quantifying otolith trace element concentrations of recently hatched capelin larvae reared under both controlled conditions in the lab and uncontrolled conditions in the field revealed that the influence of maternal investment on chemical signatures in the primordial region of capelin otoliths may limit our ability to determine the natal origin of capelin spawning on the northeast Newfoundland coast if eggs are reared in close proximity (<20 km; i.e. beach versus demersal). Trace elements (e.g., Ba) from the ambient water, however, are being incorporated into the embryonic otolith during egg incubation, indicating that ambient water chemistry also has an influence on otolith chemical signatures in the primordial otolith region, as do varying temperature and salinity. Therefore, the ability to identify the natal habitat of capelin using otolith chemical signatures in the future will rely on whether differences in environmental conditions (i.e. temperature, salinity, ambient water chemistry) are large enough to overcome differences due to maternal investment.

Although otolith chemistry of naturally fertilized and lab-reared larvae differed significantly among some temperature and salinity treatments in Chapter 1, similar to other otolith chemistry studies (e.g., Kalish, 1989; Elsdon and Gillanders, 2002; Elsdon and Gillanders, 2004; Martin and Thorrold, 2005), the high variability among individuals reared under identical conditions resulted in poor classification success (42-55%), suggesting a low predictive capacity. Our inability to successfully classify individuals into temperature and salinity treatments (lab-rearing experiment) and rearing habitat (field-rearing experiment) may have been confounded by maternal investment, as evidenced by the high classification success of artificially fertilized larvae (83%) into families (Chapter 2). Additionally, otolith trace element concentrations, especially Ba, differed among temperature and salinity treatments to a higher degree when variation among families was controlled in Chapter 2. This suggests that maternal

investment has an important influence on chemical signatures in the otolith primordial region of capelin and may explain the high variation and lack of significant differences among naturally fertilized capelin larvae in Chapter 1.

Despite evidence of maternal investment in the primordial region of capelin otoliths, the spiking experiment (Chapter 2) clearly indicated that Ba ions from the ambient water are transported across the chorion and incorporated into the embryonic otolith during egg incubation. Therefore, if ambient water chemistry varies to a large degree, then the maternally invested signature may be overwhelmed. These findings suggest that distinguishing larvae reared in habitats with different temperature and salinity but similar ambient water chemistry (i.e. beach and demersal habitats in close proximity) may only be possible in some years when moderate-high differences in temperature (i.e. 4-8 °C) and salinity (10-20 psu) occur. This is supported by our ability to successfully distinguish capelin larvae from the field-rearing experiment (Chapter 1) into rearing habitat in one of the two years when temperatures at the beach site were ~4°C higher relative to the demersal site. In support, otolith chemical signatures also differed among capelin larvae when temperature differed by ~3-7°C between beach and demersal rearing sites (Davoren et al., 2015) and among capelin larvae from spawning regions 100-200 km apart with presumably different water chemistry (Lazartigues et al., 2016).

In conclusion, analyzing otolith chemical signatures may not provide a useful tool to determine the natal origin of capelin larvae spawned at habitats in such close proximity where water chemistry is relatively homogeneous, despite differing temperature and salinity. It may be possible, however, to distinguish rearing habitats of individuals spawned in different bays or regions on a larger spatial scale (100-200 km), where both environmental conditions and ambient water chemistry would vary to a presumably larger degree. Future studies investigating the

relative influence of maternal investment and ambient water chemistry on otolith chemical signatures of recently hatched larvae will aid in determining whether otolith chemical signatures in the primordial region will be able to distinguish the natal habitat of capelin. By quantifying trace element concentrations of unfertilized eggs collected from gravid females, we may be able to determine baseline concentrations of maternally invested trace elements. Additionally, using these same eggs to artificially fertilize families and rearing them in water where the ambient water chemistry has been altered, we may be able to both quantify the effect of ambient chemistry on otolith chemical signatures and the relative contribution from the environment and the mother on chemical signatures in the otolith primordial region of capelin.

Although some studies have suggested that capelin may be connected to a particular spawning habitat (e.g., natal philopatry; Davoren, 2013; Davoren and Halden, 2014), genetic analyses combined with common garden experiments suggested undifferentiated populations (Penton and Davoren, 2013; Penton et al., 2014). Understanding the relative influence of maternal investment relative to temperature, salinity, and ambient water chemistry on capelin otolith chemistry will allow us to interpret chemical signatures from wild caught larvae and adults, and possibly estimate their natal habitat. The ability to quantify the percentage of wild-caught larvae originating from different spawning habitats each year will allow us to determine whether individuals from different natal origins mix along their offshore dispersal routes. Quantifying the percentage of wild-caught adults originating from different spawning habitats each year will allow us to determine the relative contribution of each spawning habitat to recruitment, thereby providing an indication of critical habitats, as well as shifts between critical habitats, with ocean climate change. Additionally, connectivity estimates derived from these findings can be used to build metapopulation models of capelin dynamics and, thus, estimate the

stability and resilience of local populations (Thorrold et al. 2001). This in turn, may be used to determine more appropriate fisheries management regimes and aid in the conservation of this important forage fish species and the top predators that are reliant on them as a key food source.

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