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Temperature and salinity influence the chemistry in the pre-hatch otolith region of capelin, *Mallotus villosus*, during lab and field egg incubation experiments

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ABSTRACT

Identifying the natal origin of marine fish is important to understand connectivity and productivity among populations residing in different habitats. Capelin, *Mallotus villosus*, is a key marine forage fish species that spawns at both beach (warmer, less saline) and deep-water (15–40 m; cooler, more saline) habitats along the northeast Newfoundland coast. Currently, the contribution from each habitat to the spawning population is unknown. Previous research, where capelin eggs from known families were lab-reared, identified family-based otolith chemical signatures in the pre-hatch (embryonic) region of larvae. In this study, we investigated whether temperature and salinity influenced embryonic otolith chemistry to determine whether variation in environmental conditions would result in habitat-specific signatures, thereby overwhelming family-based signatures and allowing the identification of natal origin. Capelin eggs from many families were incubated together under controlled temperature (4, 8, 10 °C) and salinity (10, 20, 30 psu) treatments in the lab and uncontrolled conditions within each habitat in the field. Elemental concentrations (i.e., Sr, Ba, Mg, Mn) in the pre-hatch region of 1-day old larvae were quantified via LA ICP-MS. Elemental concentrations varied among individuals reared under identical conditions, likely due to family-based chemical signatures. Despite this variation, mean elemental concentrations differed when mean temperatures varied by ≥4 °C between treatments (lab) and rearing habitats (field), resulting in high treatment- and habitat-specific classification success (~73–88%) of individuals. In contrast, embryonic otolith chemistry did not vary consistently with salinity. Temperature differs consistently between rearing habitats and, thus, these findings suggest that when capelin rearing habitats differ by ≥4 °C within a year, chemical signatures in the embryonic otolith may be used to determine the natal origin of individuals.

1. Introduction

Connectivity within a species refers to the degree of exchange of individuals, or 'mixing', among geographically separated regions (Cowen et al., 2000; Cowen and Sponaugle, 2009). Most marine fish and invertebrate species experience a dispersive period during the larval stage and, thus, were traditionally considered to be 'open' populations (Cowen and Sponaugle, 2009). Indeed, it is possible for larvae to be transported potentially long distances away from the natal site through both active behavior and passive drift via ocean currents (Cowen et al., 2000; Di Franco et al., 2012). Despite this high potential for dispersal, there is growing evidence that larvae of many marine species may be retained nearby the natal site (e.g., Swearer et al., 1999; Jones et al., 1999; Jones et al., 2005) or return to the natal site (e.g., Thorrold et al., 2001), resulting in populations that act as relatively isolated geographic units. Understanding population connectivity is essential to estimate the stability and resilience of local populations and to design effective marine protected area networks (Thorrold et al., 2001). Quantifying connectivity, however, is logistically challenging and costly due to the difficulty of conducting mark-recapture studies on small larvae with high mortality (Thorrold et al., 2001; Starrs et al., 2016).

Natural tags, such as chemical signatures in the otoliths of teleost fish, have proven useful to identify natal origins of marine fish (e.g., Thorrold et al., 2001; Starrs et al., 2016). As a fish grows, elements from the surrounding seawater accumulate on the calcium carbonate surface of otoliths, thereby recording and preserving the timing of elemental deposition from egg incubation until death (Campana, 1999; Elsdon and Gillanders, 2003). If larvae disperse away from natal sites immediately upon hatch, natal origin can only be determined from

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chemical signatures in the pre-hatch, or embryonic, region of the otolith. Little is known, however, about elemental pathways from the environment into the developing egg and then into the developing otolith. Evidence suggests that embryonic otolith chemistry may not reflect environmental conditions (e.g., water chemistry, temperature) during egg incubation, but rather reflect maternally-derived trace elements in the yolk (Volk et al., 2000; Limburg et al., 2001; Zimmerman and Reeves, 2002), or genetically controlled uptake dynamics (Chittaro et al., 2006). Despite evidence of maternal investment, otoliths of embryonic larvae have been successfully marked during egg incubation using enriched concentrations of rare stable isotopes (e.g., ^{86}Sr, ^{137}Ba; Warren-Myers et al., 2015; Loeppky et al., 2018) and fluorochrome dyes (e.g., tetracycline hydrochloride, alizarin red) in a number of species (e.g., damselfish, Jones et al., 1999; peled, Dabrowski and Tsukamoto, 1986; Ayu, Tsukamoto, 1988; Atlantic cod, Blom et al., 1994; masu salmon, Nagata et al., 1995; whitefish, Eckmann, 2003). These findings indicate that inorganic materials are transported across the egg membrane of fertilized teleost fish eggs during incubation.

In the post-hatch region of larval otoliths, essential elements, such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and manganese (Mn^{2+}), may not reflect ambient environmental concentrations as they are physiologically regulated (Wood, 2012; Loewen et al., 2016). For instance, temperature influences fish growth rates and, thus, triggers an influx of these essential elements required for growth and organ development (Campana, 1999; Brophy et al., 2004; Loewen et al., 2016). In contrast, concentrations of nonessential elements, such as strontium (Sr^{2+}) and barium (Ba^{2+}), tend to reflect ambient water chemistry in post-hatch regions of larval otoliths (e.g., Bath et al., 2000) because they are inadvertently transported across biological membranes via calcium channels owing to their chemical similarities (Loewen et al., 2016). Otolith incorporation rates of nonessential elements in larvae can also be influenced by environmental conditions, such as temperature, salinity, pH, and dissolved oxygen (Campana, 1999; Elsdon and Gillanders, 2003; Loewen et al., 2016). For instance, otolith Sr and Ba concentrations in juveniles of some species vary across salinity gradients (e.g., black bream, Elsdon and Gillanders, 2002; Chinook salmon, Miller, 2009). It is unclear, however, whether these trends are similar in the embryonic otolith.

Capelin, *Mallotus villosus*, is an important forage fish species found in Arctic and sub-Arctic zones in the Atlantic and Pacific Oceans and is important prey for many marine predators (Carscadden et al., 2013). Capelin typically spawn intertidally on sandy beaches during June and July off the northeast coast of Newfoundland, but subtidal spawning at nearby (< 20 km) deep-water (15–40 m; 'demersal') sites was recently discovered in coastal embayments (Nakashima and Wheeler, 2002; Davoren et al., 2008; Fig. 1a). Fertilized capelin eggs adhere to the substrate at spawning sites (Fridgeirsson, 1976), which become egg-rearing habitats. The warmer beach habitat is characterized by varying salinity, temperature, and oxygen concentrations due to wave action and tidal inundation (Penton et al., 2012; Davoren et al., 2015; Crook et al., 2017). In contrast, the demersal habitat is on average 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; Crook et al., 2017). Although there is evidence that individual capelin may be connected to a particular spawning habitat (e.g., natal philopatry; Davoren, 2013; Davoren and Halden, 2014), genetic analyses and common garden experiments suggest beach and demersal spawners are undifferentiated (Penton and Davoren, 2013; Penton et al., 2014; Kenchington et al., 2015). Therefore, capelin on the east coast of Newfoundland and Labrador are currently treated as one management unit (NAFO Divisions 2 J + 3KL; DFO, 2015) despite limited information on connectivity between the two spawning habitats within this region.

To identify the natal rearing habitat of individual capelin using otolith chemistry, habitat-specific otolith chemical signatures in the pre-hatch (embryonic) region are needed, as recently hatched larvae from nearby beach and demersal sites presumably mix and experience similar environmental conditions as they disperse away from rearing sites. In a previous experiment, we lab-reared artificially fertilized capelin eggs from different families (1 male + 1 female pairs) separately under controlled environmental conditions to investigate whether family-based otolith chemical signatures may confound our ability to distinguish individuals reared in each habitat (Loeppky et al., 2018). Although we found family-based otolith chemical signatures in embryonic otoliths of 1-day old capelin larvae and classification success of offspring into families was high (~83%), classification success was similarly high into temperature treatments (~83%; Loeppky et al., 2018). Additionally, a water spiking experiment revealed that trace elements from the ambient water are incorporated into developing otoliths during egg incubation (Loeppky et al., 2018). As temperature is the main physical factor that differs between beach and demersal rearing sites (Penton et al., 2012; Crook et al., 2017), these previous findings suggested that chemical signatures in the embryonic otolith region could allow identification of natal rearing habitat, despite family-based otolith chemical signatures.

In this study, we investigated whether chemical signatures in the pre-hatch (or embryonic) region of otoliths could be used to identify the egg rearing habitat of 1-day old capelin larvae. We tested whether temperature and salinity influenced embryonic otolith chemistry when capelin eggs from many families are lab-reared together under controlled temperature and salinity conditions, similar to those experienced at beach and demersal habitats (Penton et al., 2012; Crook et al., 2017). We predicted that individual eggs reared in cool, high salinity water (i.e. demersal habitat) will result in larvae with higher otolith Sr and lower otolith Ba concentrations relative to those reared in warm, low salinity water (i.e. beach habitat), similar to previous findings for bulk samples (i.e. ~500 larvae per sample) of 1–3-day old 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, we quantified embryonic otolith chemistry when capelin eggs from many families were field-reared within each spawning habitat located ~20 km apart. If distinct temperature- and/or salinity-based chemical signatures are present in the embryonic region of an individual's otolith, this will determine whether otolith chemistry can be used as a tool to determine the natal habitat of individuals and, thus, elucidate the relative productivity of spawning/rearing habitats of capelin as well as connectivity between habitats.

2. Methods

2.1. Lab-rearing experiment

Naturally fertilized capelin eggs adhered to substrate were collected by hand from a beach spawning site (Site C, Fig. 1b) on the first day of spawning in 2014 (July 7). As capelin form large spawning aggregations consisting of thousands of individuals (Davoren et al., 2006), eggs collected for experiments likely represented the offspring on many male-female pairs (i.e. families). Similar to Davoren et al. (2015), 750–1000 eggs and associated sediment were placed in separate plastic canisters (20 mL) with holes punched into the sides and covered with 0.270 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 from each canister were removed and placed in glass jars (120 mL) filled with seawater. Rearing jars and associated equipment were cleaned by soaking in ~10% NaClO prior to the experiment and seawater was prepared by filtering purified distilled water (19 L) using a *Lifeguard Aqua Step* UV Filter. Large batches of experimental rearing water of each salinity treatment were created by adding *Seachem Marine Salt*™ until the desired salinity was met by measuring conductivity using a multi-parameter YSI Pro30 probe. Water from each salinity treatment was then divided into three separate containers and placed in the