

INTRINSIC AND EXTRINSIC FACTORS INFLUENCING THE
TIMING OF ARRIVAL OF CAPELIN (*MALLOTUS VILLOSUS*) TO
SPAWNING GROUNDS IN COASTAL NEWFOUNDLAND

by

Emily Maxner

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Department of Biological Sciences
University of Manitoba
Winnipeg

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Abstract

Capelin (*Mallotus villosus*) is an important forage fish species in the Northwest Atlantic and, thus, the primary prey species of many top predators. Capelin undergo extensive inshore migrations (> 350 km) from offshore wintering areas to coastal spawning grounds in the spring where the timing of inshore arrival is highly variable. I investigated the influence of intrinsic factors (i.e., length, age, spawning experience) and proxies for extrinsic factors (i.e., otolith microchemistry, stable isotope ratios) on the timing of arrival of capelin collected regularly at spawning sites during 2012 and 2013 on the northeast coast of Newfoundland. Despite high inter-annual variation in almost all factors examined, intrinsic factors, specifically length and age, consistently varied with timing of arrival at spawning sites for both males and females. In contrast, proxies of extrinsic factors did not consistently vary with timing of arrival in both years. These results suggest that extrinsic factors may be less important than intrinsic factors in determining individual-level variability in the timing of arrival of spawning capelin in coastal Newfoundland. Overall, these results are important for the management of this critical forage fish species, as selective harvesting by the inshore capelin fishery of early-arriving larger and older fish may impact the age/size structure of the population, recruitment, and result in increased variability in the timing of spawning.

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Chapter 1: General Introduction

Global climate change poses a threat to many species, resulting in changes to population size and life history, timing of breeding, range expansions, and structure/function of ecosystems (e.g. McCarty 2001). Specifically, migratory species are affected by changes in the temperature of their wintering grounds, migratory routes and breeding grounds (Ahola et al. 2004). Changes in temperature have evidently led to the variation in timing of breeding in plants, birds, insects (e.g. Sparks and Menzel 2002), amphibians (e.g. Reading 1998), and fish (e.g. Carscadden and Nakashima 1997). Environmental instability in marine ecosystems can change the timing of breeding of marine fish and may alter the temporal and spatial overlap of offspring (i.e. larvae) with high food availability (Sims et al. 2001), thereby leading to altered breeding success and population size (e.g. Cushing 1990, Quinn et al. 2000, Simmonds and Isaac 2007). Hjort (1914) hypothesized that when larvae hatch into favorable conditions (e.g. high food abundance), high survival and recruitment result; however, when larvae hatch into unfavorable conditions (e.g. low food abundance), low survival and recruitment result. The dynamic interaction between fish and their environment (Hjort 1914) has further led to the ‘match-mismatch hypothesis’ that recruitment (i.e. number of offspring ‘recruiting’ in to the spawning population) is linked to overlap in the timing of offspring production and the timing/quantity of production cycles in the ocean (Cushing 1990). As such, timing of spawning events of temperate marine fish are adaptively linked to annual spring production, with high temporal overlap leading to faster growth and higher survival rates of larvae prior to winter, resulting in enhanced recruitment (Cushing 1990).

Qasim (1973) suggested that both intrinsic (e.g. size, age) and extrinsic factors (e.g. temperature, food availability) influence the timing of spawning at the individual- and population-level in fish. For instance, older, larger marine teleosts typically spawn before younger, smaller individuals within a population (Wright and Trippel 2009). Owing to this general pattern, the timing of spawning is linked to the age structure of the population, which is correlated with size and spawning experience (i.e. larger individuals tend to be older and more experienced; Murawski et al. 2001). Varying age and size structure of the population, either natural or anthropogenic (e.g. selective harvesting of older, larger fish), may lead to a shift in the timing of spawning, which in turn, may influence the strength of future year classes (Wright and Trippel 2009). In addition, temperature has been related to delayed spawning migrations in many fish species (e.g. rainbow trout *Salmo gairdneri*, MacLean and Evans 1981; capelin *Mallotus villosus*, Carscadden et al. 1997, Regular et al. 2008, Davoren et al. 2012; flounder *Platichthys flesus*, Sims et al. 2004; herring *Clupea harengus*, Oskarsson and Taggart 2010), with lower temperature presumably resulting in slower migration speeds (see Comeau et al. 2002) and gonadal development (e.g. Hay 1985; Kjesbu 1994; Ware and Tanasichuk 1989). Other associated physical factors that regulate the timing of primary production, such as prolonged sea ice cover, can limit food availability to marine fish during energetically demanding periods and process, such as gonadal development or spawning migration, resulting in variable timing of spawning (e.g. Buren et al. 2014).

Fish Otoliths

Determining intrinsic and extrinsic factors for individual fish can be difficult, especially for highly mobile and dispersive marine fish. Otoliths, or ‘earstones’, however, provide an

opportunity to overcome this challenge. Otoliths are paired calcified structures in fish that are important for maintaining balance and aid in hearing (Barton 2007). Fisheries scientists often use otoliths to reconstruct the life history of individual fish (Campana 2005). Regardless of a fish's indeterminate growth patterns, otolith growth is continual, allowing for specific life history characteristics to be assessed, such as growth rate, age and spawning experience (Campana and Thorrold 2001). Otoliths have alternating wide opaque summer bands and narrow translucent (or hyaline) winter bands (Winters 1971) and annual deposits of these winter-hyaline bands allow age determination (Bailey et al. 1977). In addition, 'spawning checks' provide evidence of previous spawning and are typically recognized by reduced summer/opaque growth during a year when a fish has spawned (e.g. McKern et al. 1974). Although spawning checks have been observed in many fish species (e.g. Atlantic cod *Gadus morhua*, Pannella 1971; capelin, Winters 1971; rhomboid mojarra *Diapterus rhombeus*, Austin 1971; shad *Alosa sapidissima*, Leggett & Carscadden 1978), they are rarely quantified due to high individual variation in species-specific band widths.

Otoliths also store information, in the form of trace elements, which can be used to reconstruct fish movement and habitat use (Campana 1999, Elsdon and Gillanders 2003, Elsdon et al. 2008). Otoliths form daily-accreted layers on the surface, which include trace elements (Campana 1999) that can supply a chemical chronology over the lifetime of a fish (Elsdon et al. 2008). Trace elements are incorporated into otoliths in proportion to their concentration in the environment and, thus, can serve as proxies for different environmental factors (Miller 2011). For instance, barium (Ba) and strontium (Sr) will substitute for calcium (Ca) into the aragonite of otoliths in proportion to their concentrations (Campana 1999), owing to their chemical similarity to Ca (Phillis et al. 2011). Sr concentration and temperature tend to have an inverse relationship

in marine systems (Campana 1999) and are of particular interest because most fish are ectothermic animals, their behaviour and biology being heavily influenced by environmental temperatures. Ba concentrations are also of importance due to an inverse relationship of Ba and salinity in marine and anadromous fish (Walther and Limburg 2012). Therefore, concentrations of otolith Sr and Ba can aid in reconstructing the thermohaline habitats occupied by individual fish. Recent studies, however, have shown that physiological factors, such as growth rates, can influence the incorporation rates of trace elements into otoliths (e.g. Sadovy and Severin 1992, 1994; Kalish 1989; Walther et al. 2010), suggesting that these factors can confound the use of otolith chemistry to reconstruct habitat use by individual fish.

Stable Isotopes

Stable isotope ratios of carbon and nitrogen can provide an indication of diet, over a variety of temporal scales depending on the tissue sampled, all of which aid in determining the trophic ecology (e.g. niche width) of individual fish (e.g. Madigan et al. 2012). For instance, $\delta^{13}\text{C}$ values are typically associated with the primary carbon source, whereas $\delta^{15}\text{N}$ values can be used to determine trophic position (Post 2002). Together $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can indicate niche width and niche overlap between groups (e.g. Rubenstein and Hobson 2004). There are advantages to using stable isotope ratios, as longer-term (i.e. months) variation in the diet can be determined (Pinnegar and Polunin 2000), in contrast to stomach contents, which only provide a snapshot of diet at one point in time. As such, stable isotope ratios have the ability to provide a better understanding of the temporal and spatial foraging ecology of an individual (Estrada et al. 2005). This is important when studying migratory species that can only be sampled over a brief period post-migration (e.g. Estrada et al. 2005). For fish species, white muscle tends to be less variable

in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in comparison to blood, which has a faster turnover rate (e.g. Pinnegar and Polunin 1999). Lipids are typically depleted in $\delta^{13}\text{C}$ and are typically removed from tissue samples to minimize $\delta^{13}\text{C}$ variability (DeNiro and Epstein 1977).

Capelin (*Mallotus villosus*) of Northwest Atlantic

Capelin is a small, cold-water, migratory pelagic fish that inhabits most northern marine ecosystems, including Newfoundland and Labrador coasts, Barents Sea, Iceland and Greenland, and the Bering Sea (Carscadden and Vilhjálmsón 2002). Capelin and other forage fish species play a crucial role in marine food webs, providing an important link between lower and higher trophic levels (Pikitch et al. 2012). Capelin are the primary prey species of top predators, including seabirds, mammals and large predatory fish (e.g. Atlantic cod; Carscadden and Vilhjálmsón 2002) and, in turn, prey on zooplankton (e.g., copepods, hyperiid amphipods, euphausiids, larvaceans and chaetognaths; O'Driscoll et al. 2001). Stable isotope values of capelin muscle previously reported included $\delta^{13}\text{C}$ values ranging between -19.9 ± 0.6 (length: 149 ± 9 mm, timing of capture: July 2000; Davoren et al. 2002) and -21.0 ± 0.1 (length: 70 - 180 mm, timing of capture: January and June 2002; Sherwood and Rose 2005), and $\delta^{15}\text{N}$ values of 10.2 ± 1.9 (Davoren et al. 2002) and 12.2 ± 0.09 (Sherwood and Rose 2005). Isotopic signatures of prey are similar, with amphipods and euphausiids having $\delta^{13}\text{C}$ values of -22.5 to -20.6 and $\delta^{15}\text{N}$ of 9.3 to 10.3 (Sherwood and Rose 2005). Copepods have similar $\delta^{13}\text{C}$ values ranging from -22.2 to -21.4, but lower $\delta^{15}\text{N}$ values of 7.0 to 7.3 than euphausiids and amphipods (Estrada et al. 2005), possibly due to the smaller size (and feeding on a lower trophic level) of copepods (O'Driscoll et al. 2001).

Inhabiting offshore waters for most of their lives (Carscadden et al. 2013), Newfoundland capelin undergo extensive (> 350 km) spawning migrations from offshore wintering areas to coastal spawning grounds in the spring (Nakashima 1992) once they have reached sexual maturity (2-3 years old; Carscadden et al. 2013). During migration, capelin are primarily found close to the seabed (Mowbray 2002), but might divide into size-specific groups in different depth layers (Friis-Rodel and Kanneworff 2002). They also undergo diel vertical migration while migrating (Mowbray 2002) and within pre-spawning coastal staging areas (Davoren et al. 2008). The timing of inshore arrival and spawning of this sub-arctic species is highly variable (Carscadden et al. 1997, Davoren et al. 2012), similar to other forage fish species with short-lived ('r-selected') life history strategies (e.g. Atlantic herring; Oskarsson and Taggart 2010). There is also some indication that larger capelin arrive at spawning grounds before smaller individuals (Carscadden et al. 1997; Vandeperre and Methven 2007).

Capelin is a demersal spawner, producing eggs that adhere to sediment at spawning sites once fertilized, where they remain until hatching (Carscadden and Vilhjálmsson 2002). In coastal Newfoundland, capelin spawn both inter-tidally at beach sites and farther from the coast (2.5-17.7 km) at deep-water ('demersal'; Nakashima and Wheeler 2002, Davoren et al. 2008) spawning sites. Evidently, temperature acts as an important environmental cue for habitat selection by capelin (Davoren 2013). Newfoundland capelin are considered one stock (Carscadden et al. 2013) as they do not differ between spawning habitats in early life history traits (Penton and Davoren 2013), morphometrics (Penton 2013) or genetics (Penton et al. 2014).

Capelin are sexually dimorphic, with males being larger than females and developing enlarged pectoral and anal fins, as well as an enlargement of scales along the lateral line during spawning ('spawning ridges'). Sex-specific behaviour of capelin during spawning results in

males arriving first and remaining at the spawning sites (Templeman 1948; Friis-Rodel and Kanneworff 2002). Female capelin remain in pelagic schools at staging areas close to nearby spawning sites as maturation continues (Davoren et al. 2006, Davoren 2013), then proceed to spawning sites when gonadal development is complete (Friis-Rodel and Kanneworff 2002). Sex-specific differences in spawning behaviour may influence post-spawning mortality of male and female capelin (Shackell et al. 1994; Huse 1998). During a spawning event, females deposit all their eggs once and then leave the site (Friis-Rodel and Kanneworff 2002), resulting in higher post-spawning survival than males (Shackell et al. 1994), which remain at the spawning site to mate with multiple females. Laboratory experiments have revealed that females are iteroparous, spawning more than once during their lifetime, whereas males are semelparous, losing secondary sexual characteristics and dying shortly after spawning (Burton and Flynn 1998); however, Christiansen et al. (2008) showed that females and males spawned in the lab two years in a row.

A limited number of studies have used otoliths to investigate the intrinsic and extrinsic characteristics of individual capelin. Similar to other fish species, capelin otoliths have alternating bands of opaque (summer growth) and translucent (or hyaline winter growth; Winters 1971), which aid in determining age (Bailey et al. 1977) and growth rates (Winters 1982, Hedeholm et al. 2010). The otolith microstructure also provides information about spawning experience, as spawning checks have been previously described in capelin (Winters 1971), with the mean annual band width of otoliths identified as having spawning checks being significantly smaller than those identified as not having spawning checks (G. Davoren unpubl. data). Davoren and Halden (2014) recently used otolith chemistry to investigate population complexity and connectivity of Newfoundland capelin. These authors showed that fish spawning at beaches versus deep-water sites had significantly different otolith Sr and Ba concentrations as larvae and

significant differences of otolith Sr in the marginal zone (final winter and growth until capture), suggesting that some parts of the population experienced different conditions during larval drift as well as during spawning migrations and while inshore prior to spawning.

The capelin population of the Northwest Atlantic collapsed during an extreme cold-water event in 1991, which was the start of a large-scale regime shift (Buren et al. 2014), and associated with the moratorium on Atlantic cod (1992). During the early 1990s, capelin underwent dramatic changes in their distribution, biology (size at maturity and spawning times; Frank et al. 1996; Nakashima 1996; Carscadden and Nakashima 1997), and population size, which has remained ~10% of historic levels (DFO 2010). Prior to the 1990's, capelin were centered off the mid-eastern coast of Newfoundland, but have since shifted east and southeast (Flemish Cap and Scotian Shelf; Carscadden et al. 2001). Mowbray (2002) highlighted that offshore capelin had undergone a distinct vertical habitat shift to deeper water, and were now found in areas that likely represent an ideal combination of temperature and prey availability. Therefore, the variability of temperature and prey may have a profound influence on the timing of arrival to spawning sites. Carscadden et al. (1997) showed that delayed spawning in the early 1990s was associated with smaller fish length. Colder temperatures appeared to result in delayed spawning by up to 4 weeks (Carscadden and Nakashima 1997), and although temperatures have since returned to the historical average, later spawning events, smaller fish sizes and lower age at maturity of capelin have persisted (DFO 2010). There are indications, however, that the pattern of larger individuals arriving to the spawning sites first (Carscadden et al. 1997; Vandeperre and Methven 2007) broke down after the occurrence of colder sea temperatures in the early 1990s (Nakashima 1996).

Thesis Objectives

Overall, there is uncertainty in the factors that influence the timing of capelin arrival to coastal spawning grounds on the northeastern coast of Newfoundland. As capelin appear to have distinct spawning waves (Davoren et al. 2008), the goal of this thesis was to investigate the influence of intrinsic and extrinsic factors on the timing of arrival of capelin at spawning sites in coastal Newfoundland within a spawning season. I first investigated the influence of intrinsic factors, length, age and spawning experience, on timing of arrival. Second, I examine otolith Sr and Ba concentrations in the outer edge of the otolith (marginal zone) as proxies of the physical environment experienced during spawning migration. I also investigate the stable isotope ratios of muscle tissue, as proxies of diet during spawning migration. Further understanding of the factors that influence the timing of arrival is important, as breeding time determines whether larvae hatch into favourable or unfavourable conditions and, thus, is directly related to recruitment and population dynamics (Hjort 1914; Cushing 1990). As capelin is a key forage fish species in the Northwest Atlantic, population dynamics of capelin have the potential to mediate ecosystem-wide changes (Carscadden and Vilhjálmsson 2002; Davoren et al. 2008).

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Chapter 2. Intrinsic and extrinsic factors influencing the timing of arrival of capelin (*Mallotus villosus*) to spawning grounds in coastal Newfoundland

2.1 Introduction

It is critical to understand the factors influencing the timing of breeding of fish. Timing influences the environmental conditions experienced by offspring, leading to variation in reproductive success, and, ultimately to interannual differences in recruitment and year-class strength. The timing of spawning of marine teleost fish at the population level has been linked to environmental conditions favorable for offspring survival (Qasim 1973), such as temperature, food abundance, and predator abundance (Hjort 1914, Cushing 1990), with a ‘match’ or ‘mismatch’ of offspring production and favourable conditions leading to high and low survival and recruitment, respectively (Cushing 1990). The timing of spawning of migratory fish may also vary among individuals within a population, resulting in temporally separate, spawning waves, due to both intrinsic and extrinsic factors (Qasim 1973). Typically, older, larger marine teleosts spawn before younger, smaller individuals within a population (Wright and Trippel 2009). There are still uncertainties as to why this pattern occurs, but it may be due to 1) the size-dependent speeds of migration to breeding grounds (e.g. larger fish have faster swimming speeds, Bainbridge 1958; Beamish 1970; Webb et al. 1984; Videler and Wardle 1991), 2) advanced gametogenesis in larger fish (e.g. rate of maturation, Flynn and Burton 2003), or 3) size-dependent start of maturation (Slotte et al. 2000). Owing to this general pattern, the timing of spawning may be linked to the age structure of the population, which is correlated with size and spawning experience (i.e. larger individuals tend to be older and more experienced; Murawski et al. 2001). Therefore, varying age and size structure of the population, caused by

either natural or anthropogenic forces (e.g. selective harvesting of older, larger fish), may lead to a shift in the timing of spawning, which in turn, may influence the strength of future year classes (Wright and Trippel 2009).

Multiple, temporally distinct, spawning waves may also result from groups of fish experiencing different environmental conditions during migration to spawning grounds. Temperature has been linked to delayed spawning, spawning migrations or arrival at spawning grounds at the population-level in many fish species (e.g. rainbow trout *Salmo gairdneri*, MacLean and Evans 1981; capelin *Mallotus villosus*, Carscadden et al. 1997, Regular et al. 2008, Davoren et al. 2012; flounder *Platichthys flesus*, Sims et al. 2004; herring *Clupea harengus*, Oskarsson and Taggart 2010), with lower temperature presumably resulting in slower migration speeds (see Comeau et al. 2002) and slower gonadal development (e.g. Ware and Tanasichuk 1989). Other associated physical factors that regulate the timing of primary production, such as prolonged sea ice cover, can limit the prey available to marine fish during gonadal development or spawning migration, also leading to variable timing of spawning (e.g. Buren et al. 2014).

The environmental conditions experienced during spawning migrations by individual fish, however, are difficult to quantify. Scientists have overcome this challenge using ‘natural tags’, such as otolith chemistry (Campana 1999, 2005). Trace elements are typically incorporated into otoliths in proportion to their concentration in the environment (e.g. Bath et al. 2000, Elsdon and Gillanders 2003, Veinott et al. 2009) and, thus, have been used to reconstruct habitat use and movement of individual fish throughout their lifespan (Campana 1999, 2005). In particular, barium (Ba) and strontium (Sr) often substitute for calcium (Ca) in the aragonite of otoliths in proportion to their environmental concentrations (Campana 1999), owing to their chemical similarity to Ca (Phillis et al. 2011). Otolith Sr concentrations also are interpreted to be inversely

related to temperature in many marine fish (Campana 1999), particularly in cold-adapted species, including herring (Townsend et al. 1992), Atlantic cod (*Gadus morhua*; Townsend et al. 1995), and Pacific cod (*Gadus macrocephalus*; DiMaria et al. 2010). Therefore, Sr concentrations may provide a proxy for thermal habitats occupied by individual fish. Ba is incorporated into otoliths opposite to Sr across salinity gradients, with higher otolith Ba concentrations related to lower salinities, but seems less related to temperature in marine fish (see review in Walther and Limburg 2012). Therefore, otolith Ba concentrations may act as a proxy for movement across salinity gradients (Walther and Limburg 2012). Recent studies have highlighted the importance of physiological regulating factors, such as growth rates, on the incorporation rates of trace elements into otoliths (e.g. Walther et al. 2010). Therefore, there is growing evidence that the influence of environmental conditions (e.g., ambient trace element concentrations, temperature, salinity) on otolith chemistry is species-specific (see reviews in Campana 1999, Elsdon and Gillanders 2003).

Prey availability may also influence the timing of spawning. Many organisms, including marine fish, undergo energetically demanding activities, such as breeding during periods of high prey availability (Love 1970). Owing to the reliance of marine fish on stored energy reserves during reproduction (Anthony et al. 2000), variation in prey availability and nutritional quality of prey may influence growth (e.g. O'Driscoll et al. 2001) and energy reserves (e.g. Comeau et al. 2002) many months prior to reproduction (Anthony et al. 2000), and, in turn, could explain variation in the timing of spawning (Buren et al. 2014). As turnover rates of stable isotopes in muscle tissue are known to occur over months in forage fish (e.g. ~ 4.5-6.5 months in Pacific herring *Clupea pallasii*, Miller 2000; ~3-8 months in Pacific Bluefin Tuna *Thunnus orientalis*, Madigan et al. 2012), $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of fish sampled on spawning grounds act as proxies of

dietary composition or geographic origin prior to, and during spawning migrations, providing a method to investigate the influence of diet prior to spawning on timing of arrival at spawning grounds of individual fish.

Capelin (*Mallotus villosus*) is a small pelagic fish belonging to the Osmeridae family (Barton 2007) and is an important forage fish species in the Northwest Atlantic. These and other forage fish species play a crucial role in marine food webs, providing a important link between lower and higher trophic levels (Pikitch et al. 2012). Capelin eat zooplankton (e.g., copepods, hyperiid amphipods, euphausiids; O'Driscoll et al. 2001) and are in turn the primary prey species of top predators, including seabirds, mammals and large predatory fish (e.g. Atlantic cod; Carscadden and Vilhjálmsson 2002). Inhabiting offshore waters for most of their life (Carscadden et al. 2013), capelin undergo extensive (> 350 km) spawning migrations from offshore wintering areas near the shelf edge to coastal spawning grounds in the spring (Nakashima 1992). Capelin is a demersal spawner, releasing eggs that adhere to sediment at spawning sites once fertilized, where they remain until hatching (Carscadden and Vilhjálmsson 2002). In coastal Newfoundland, capelin spawn both inter-tidally at beach sites and farther from the coast (2.5-17.7 km) at deep-water (15-40 m), or 'demersal', sites (Nakashima and Wheeler 2002, Davoren et al. 2008). The timing of inshore arrival and spawning of this sub-arctic species is highly variable (e.g., Carscadden et al. 1997, Davoren et al. 2012), as observed with other forage fish species with similar short-lived ('r-selected') life history strategies (e.g. Atlantic herring; Oskarsson and Taggart 2010).

Sex-specific behaviour of capelin during spawning results in males arriving first and remaining at spawning sites (Templeman 1948; Friis-Rodel and Kannevorff 2002). Female capelin remain in pelagic schools at staging areas nearby spawning sites as maturation continues

(Davoren et al. 2006, Davoren 2013), then proceed to spawning sites when gonadal development is complete (Friis-Rodel and Kanneworff 2002). Sex-specific differences in spawning behaviour may influence post-spawning mortality of male and female capelin (Shackell et al. 1994; Huse 1998). During a spawning event, females deposit all their eggs once and then leave the spawning site (Friis-Rodel and Kanneworff 2002), whereas males remain at the spawning site to mate with multiple females throughout the season, resulting in higher post-spawning mortality than females (Shackell et al. 1994). Laboratory experiments have revealed that females are iteroparous, spawning more than once during their lifetime, whereas males are semelparous, losing secondary sexual characteristics and dying shortly after spawning (Burton and Flynn 1998).

Capelin in the Northwest Atlantic underwent dramatic changes in their distribution (Frank et al. 1996, Mowbray 2002), biology (e.g., size and age at maturity, timing of spawning; Nakashima 1996; Carscadden and Nakashima 1997) and abundance (i.e. population collapse; Buren et al. 2014) following a cold-water event in 1991. In particular, spawning was delayed up to four weeks during this cold-water event, and this delay was associated with smaller fish length (Carscadden et al. 1997). Although temperatures have since returned to the historical average, delayed spawning, smaller fish sizes and lower age at maturity of capelin have persisted (DFO 2010). As larger capelin appeared to arrive at spawning grounds before smaller individuals prior to the cold-water event in 1991 (Vandeperre and Methven 2007), delayed spawning might be explained by the lack of larger fish in the population. However, this pattern appeared to break down after 1991 (Nakashima 1996). Buren et al. (2014) suggested that delayed spawning was more related to the timing of seasonal sea ice coverage and primary production. In addition, Mowbray (2002) showed that capelin during spawning migration had undergone a distinct vertical habitat shift, and now occupied deeper waters, likely due to a trade-off between

temperature and prey availability. As such, this habitat shift may also have a profound influence on the timing of spawning.

Overall, there is uncertainty in the factors that influence the timing of capelin arrival to coastal spawning grounds on the northeastern coast of Newfoundland. With potentially distinct spawning waves of capelin (Davoren et al. 2008), the goal of this paper is to investigate the influence of intrinsic factors, along with proxies for extrinsic factors, on the timing of arrival of capelin at spawning sites in coastal Newfoundland. To investigate intrinsic factors, I hypothesize that capelin length influences the timing of arrival. I predict larger fish will arrive at spawning grounds earlier than smaller fish, possibly due to faster swimming speeds and maturation rates of larger fish. Second, I hypothesize that age influences the timing of arrival and predict that older individuals are larger, and in turn, arrive first to spawn. Third, I hypothesize that spawning experience influences timing of arrival and predict that individuals with more experience will arrive first to spawn. To investigate extrinsic factors, I hypothesize that otolith Sr and Ba concentrations in the year prior to capture (marginal zone) will influence the timing of arrival. Differences in these trace element concentrations among fish arriving throughout the spawning season would suggest that environmental conditions, including ambient water chemistry along with temperature and salinity, during the final winter and spawning migration, influence the timing of arrival. Finally, I hypothesize that diet, reflected in the stable isotope ratios of carbon and nitrogen are correlated with the timing of arrival. Differences in stable isotope ratios among fish arriving throughout the spawning season would suggest that dietary composition during the final winter and spawning migration influences the timing of arrival. Overall, this investigation will provide insight into the factors underlying the timing of arrival of capelin at spawning grounds in coastal Newfoundland.

2.2 Methods

Study Area and Sampling Design

This study focused on the exposed northeast coast of Newfoundland (49° - 50° N, 53° - 54° W; Fig. 1), where five beach spawning sites and 11 deep-water (18-40 m) spawning sites are known to co-occur (Davoren et al. 2008). In the study area, at least two distinct spawning runs are observed, with capelin arriving and spawning at beach sites (spawning duration: 2-4 days) 10-17 days prior to deep-water sites (spawning duration: 8-12 days; Davoren et al. 2008, Penton et al. 2012). Upon arrival inshore in the study area in the spring, maturing fish remain in a deep-water (≥ 200 m) trench prior to spawning (Davoren et al. 2006; 'trench', Fig. 1), presumably until maturation is complete, and then move north along the coast to beach and deep-water spawning sites in the study area (Davoren 2013).

Biological sampling

Of the known spawning sites in Notre Dame Bay, four persistently used beach sites (A, C, D, E; Fig. 1) were monitored each night at high tide in 2012 (June 29-July 26) and 2013 (June 29-July 22). Due to limited ship time, four persistently used deep-water spawning sites near Gull Island (1, 2, 6, 10; Fig. 1) were monitored every 1-4 days in 2012 and 2013 (July 9–August 9). Samples of capelin were collected at each spawning site whenever capelin were present to differentiate spawning runs during the short (2-4 week) capelin spawning season (Vanderperre and Methven 2007).

Capelin were sampled using dip nets (38 cm diameter, 0.32 cm mesh) at beaches and a purse seine (26 x 146 m, 0.21 cm capelin mesh) deployed from a 14 m commercial fishing vessel, the *Lady Easton*, at deep-water spawning sites. Each sample consisted of 220 capelin

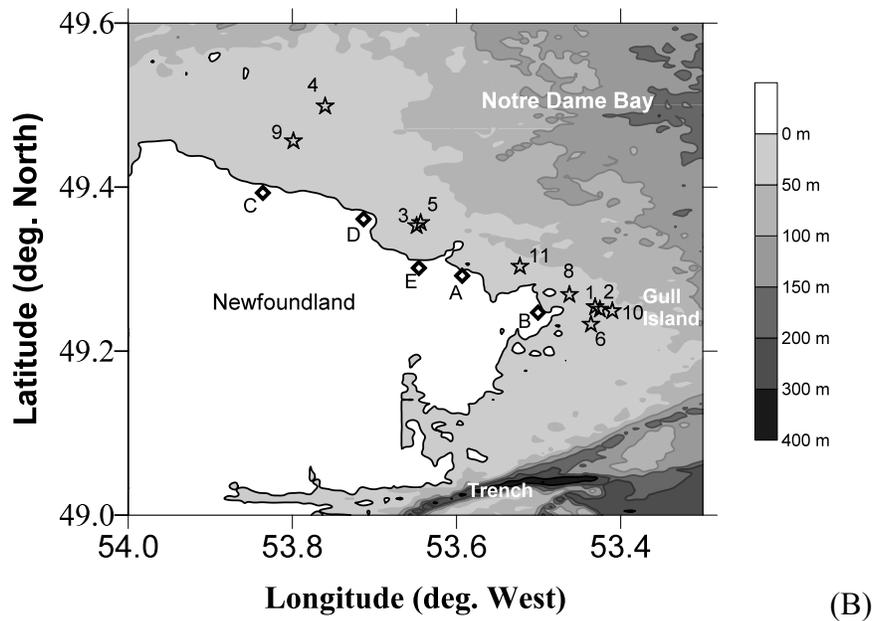
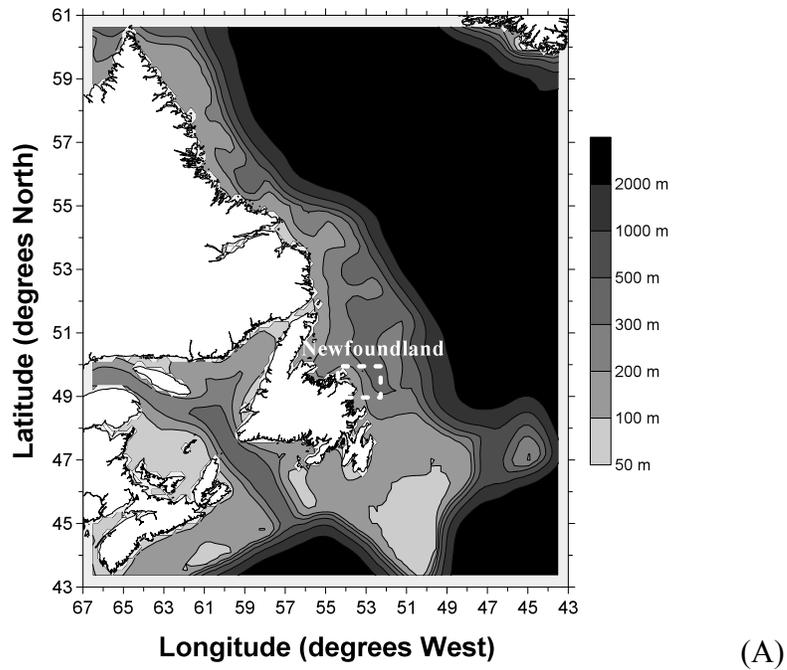


Figure 1. Map of the study area (Notre Dame Bay, square) on the northeast Newfoundland coast (A), indicating known deep-water, or ‘demersal’ (★), and beach spawning sites (◆) of capelin, along with the trench, a pre-spawning staging area, Gull Island, and depth contours (B).

(males and females combined), which were collected haphazardly from the larger netted sample and frozen immediately, following Fisheries and Oceans Canada monitoring protocol (DFO 2010). One sample of 220 fish was collected at each site upon each visit when capelin were present.

Laboratory processing

In the lab, each fish was measured for total length (snout to tip of tail) using a 36 cm Wildco fish measuring board, and sex and stage of maturity (1=immature, 2=maturing, 3=ripe, 4=partially spent, 5=spent; see Flynn et al. 2001 for details) were macroscopically assessed and recorded for 200 fish, following standard protocol for capelin (DFO 2010). On a subsample of up to 10 fish per sex per 5 mm length category (range: 100-195 mm), fish were weighed on a Denver MAXX-123 electronic precision scale to determine total body mass (g) and then gonads were dissected out and weighed to record gonad mass (g). A ~1 g sample of muscle tissue was collected from directly behind the head on each subsampled fish (2013 samples only) and frozen in a 2 mL micro-centrifuge vial. Finally, both otoliths were removed from the subsample and placed in 10% bleach solution to remove any remaining tissue.

Both sagittal otoliths from one fish were photographed at 2x and 5x magnification using a Nikon Polarizing Microscope ECLIPSE 50i POL. Photographs were taken under reflected light (fiber lights), with otoliths immersed in ethanol (Hedeholm et al. 2010). To estimate age (years), two independent observers counted the number of alternating winter (translucent) and summer (opaque) bands from photographs, with a pair of translucent and opaque bands representing one year of growth (Fig. 2A). Each observer assigned a confidence level to the estimated age of each otolith, including 'very poor', 'poor', 'fair' and 'good'. If ageing discrepancies occurred between

the two readers, otoliths were discarded from further analysis (Hedeholm et al. 2010), resulting in only otoliths classified as ‘good’ included in analyses.

To determine spawning experience, or whether the fish had spawned in a previous year (‘repeat spawner’) or not (‘recruit spawner’; Fig. 2), otolith photographs were examined for the presence of a ‘spawning check’. While ageing, the two independent observers determined the presence of spawning checks by identifying pronounced winter translucent bands followed by thin, irregular summer growth bands, as described by Winters (1971). This is the only published description of spawning checks in capelin; however, experienced age readers of capelin report that similar irregular banding patterns are observed often (F. Dawson pers. comm.). Mean annual band width of otoliths identified as having spawning checks was significantly smaller than those identified as not having spawning checks ($n = 400$; G. Davoren unpubl. data), corroborating this visual interpretation of a spawning check in capelin. Observers recorded whether they were confident or not about the presence of a spawning check and otoliths were discarded from future analyses if discrepancies occurred between observers. Therefore, the final data set only included individuals confidently aged and classified as having a spawning check or not.

Each subsample was further subsampled to obtain a representative sample of all length categories, age classes and spawning experience for males and females within each year to quantify otolith trace elements, as a proxy of environmental conditions experienced, and stable isotopes (muscle), as a proxy of dietary composition. First, I classified each sample into ‘early’, ‘mid’ and ‘late’ periods during each spawning season using a temporal separation of at least four days between samples. Due to variability in the timing of spawning between years, sampling dates were normalized to the first date capelin were sampled, representing the date of first arrival at any spawning site in the study area in each year (e.g. day 1 = first day of arrival), which

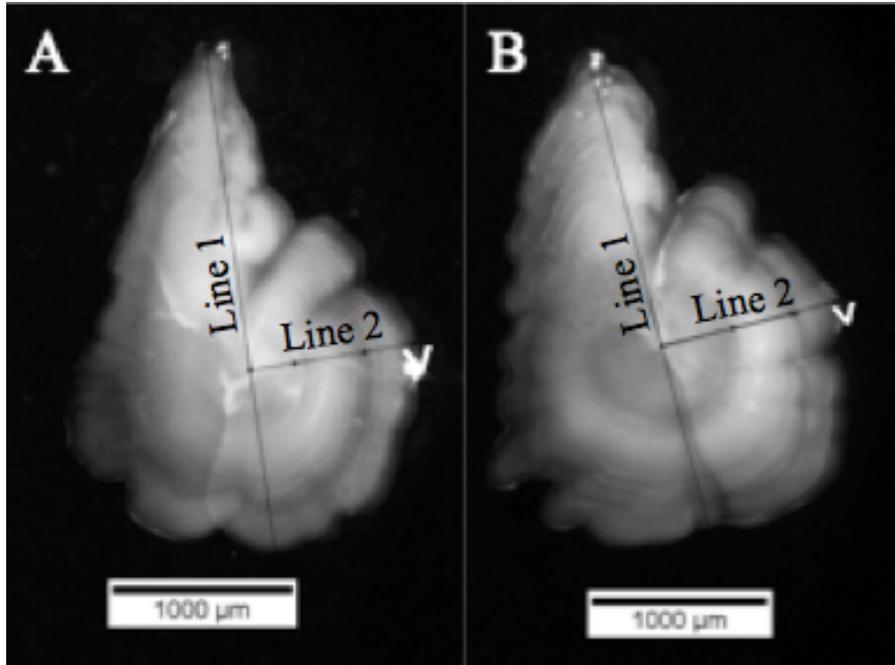


Figure 2. The otolith of a three-year-old first time spawning capelin ('recruit'; A) and a three-year-old repeat spawning capelin ('repeat'; B). The 'core' is represented by the dot in the center of the otolith at the start of Line 2, while the black arrow heads along Line 2 indicate each growth year. White brackets indicate the marginal zone (final winter and growth prior to capture).

allowed comparison of samples between years, during which the timing of arrival varied by calendar date. For each period within a year, a maximum of five two-year-olds, five three-year-old recruit spawners, and 5 three-year-old repeat spawners were arbitrarily selected over five 10 mm length categories (females range: 120-179 mm; males range: 150-199 mm). Only confidently aged, mature (stage 3-4) males and females with at least one undamaged otolith were considered for the subsample. The subsample resulted in $n = 15$ for each early-, mid- and late-sampled individuals for each length and age category when available. The subsample consisted of a total of 184 individuals (2012: $n = 84$, 2013: $n = 100$).

Chemical Analyses

One otolith from each subsampled fish was used for chemical analysis. The whole otolith was embedded in an epoxy resin (5 parts EpoxiCure Epoxy Resin to 1 part Hardener, Buehler) and a 2 mm transverse section near the otolith core region was cut (Precision Saw IsoMet 1000 with 15LC Wafering Blade, Buehler), sanded (Roll Grinder Handimet 2, Buehler) and polished (Grinder-Polisher, Buehler) with aluminum polishing solution to ensure a smooth surface. Prior to chemical analysis, otolith sections were sonicated in Milli-Q water for 30 min to remove any surface contaminants and then air-dried.

A laser ablation system (New Wave UP213) coupled to a sector field ICP-MS (Thermo Finnigan Element 2) at the Department of Geological Sciences, University of Manitoba was used to quantify Sr, Ba, Mg and Zn (2012 samples) or Sr, Ba, Mg and Mn (2013 samples). A transect line over the entire transverse section of the otolith was performed, running perpendicular to the annuli. Laser ablation was conducted with a small beam (8 μm -diameter), with 0.003/mJ of output energy, travelling at a speed of 2 $\mu\text{m}/\text{s}$ and repetition rate of 10 Hz. Ion concentration of

the analyzed isotopes were converted to $\mu\text{g/g}$ (or ppm) by determining the background with the external standard, NIST SRM 610 and internal standard Ca (40.04 wt % in otoliths) ratio to obtain a data reduced line profile in Program Iolite (Paton et al. 2011). The final dataset included elemental concentrations every 1.53 μm along the transect.

Photographs of subsampled otoliths taken for ageing were used to classify each laser pit associated with the ‘marginal zone’, or section of the otolith representing the final winter along with the spawning migration and period inshore prior to spawning. I drew a straight line extending from the rostrum tip through the center of the otolith (i.e. ‘core’, Line 1) and a second line starting at the core and running perpendicular to the growth rings (Line 2; Fig. 2) using ImageJ (version 1.43, National Institutes of Health, USA). Along Line 2, I measured the otolith increment width from the beginning of the final winter/translucent annulus to the outer edge of the winter/translucent zone (Winters 1982, Hedeholm et al. 2010). This measurement allowed the trace element concentrations in the marginal zone to be isolated from the rest of the otolith. Mean elemental concentrations were calculated within the marginal zone of subsampled individuals as a proxy of the environmental conditions experienced by each fish during their final winter, as well as during spawning migration and inshore prior to spawning. I focused on Sr and Ba concentrations, as Mn, Mg and Zn concentrations were highly variable and frequently dropped below detection limits.

Stable Isotope Analyses

Muscle samples in the subsample were freeze-dried for 48 h and then homogenized with a mortar and pestle. Samples were placed in a Soxhlet apparatus with petroleum ether as the solvent for at least 8 h to remove lipids and dried at 60 °C for 48 h. Subsamples were weighed

(range: 0.4 - 0.6 mg) and sent to the Department of Earth and Environment Sciences, University of Windsor and processed with an Elemental Analyzer (Costech 4010) – Isotope Ratio Mass Spectrometer (Thermo Delta V) (EA-IRMS). Stable isotope ratios (^{13}C : ^{12}C and ^{15}N : ^{14}N) are reported in delta (δ) notation in parts per thousand (‰) relative to standard material (PeeDee belemnite (PDB) and atmospheric nitrogen).

Data analysis

To investigate whether intrinsic factors (i.e. length, age, spawning experience) and proxies of extrinsic factors (i.e. otolith chemistry, stable isotope ratios) differed among potential spawning waves of capelin, mean values among samples classified into ‘early’, ‘mid’ and ‘late’ sampling periods during the spawning seasons of 2012 and 2013 were compared. Due to sex-specific life history and spawning behaviour (Shackell et al. 1994; Huse 1998), I first pooled years to look for differences between males and females for all intrinsic and extrinsic factors, except stable isotope ratios, which were only collected in 2013. Only mature individuals (stage 3-4) were used in analyses because it could not be determined when maturing individuals (stage 2) would be ready to spawn, or how long spent individuals (stage 5) had been at spawning sites. Partly spent (stage 4) were grouped with ripe (stage 3) individuals due to eggs or milt being lost when handling, sampling and/or processing fish, appearing partly spent.

Statistical procedures were completed in SYSTAT 13 or JMP10. Prior to performing parametric statistical analyses, I tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene’s test). Length data (unsubsamped) were not normally distributed and had unequal variances, but multiple data transformations did not rectify normality. Owing to the robustness of parametric tests (Quinn and Keough 2002), raw length data were used throughout

the analyses. Mean otolith Sr concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ met the assumptions of parametric statistics; however, mean Ba was \log_{10} transformed to meet these assumptions. Analysis of Variance (ANOVAs) with timing as a fixed variable and year as a random variable was done to determine if each intrinsic and extrinsic factor differed significantly among timing categories (i.e. early, mid, late) when controlling for variation between years (i.e. 2012, 2013). If interannual variation in factor values differed significantly from zero (i.e. reject $H_0: \sigma^2 = 0$), suggesting that variation between years influenced the ability to detect differences among timing categories, then years were examined separately. An ANOVA also was performed with year as a fixed variable to determine whether intrinsic and extrinsic factors differed between years. Tukey honestly significant difference (HSD) *post-hoc* tests were used to further determine which timing categories differed from each other. Chi-square tests were used to compare the frequency of age classes and the frequency of age 3 recruit and repeat spawners among early-, mid- and late-sampled capelin. Age 2 and 4 capelin were not included in the analysis of spawning experience to avoid biasing results, as two-year-old capelin were always recruit spawners and 4 year-olds were almost always repeat spawners. A value of $\alpha = 0.05$ was used to determine statistical significance.

2.3 Results

During 2012, capelin were first observed in the study area at beach spawning sites on July 9 and were present in the study area until July 23. Early arriving capelin were sampled at one beach spawning site on two consecutive nights (July 9-10, $n=2$; Table 1). After this early beach sampling, spawning only occurred at deep-water sites, resulting in mid-season samples (July 14-18, $n=7$) and late samples (July 23, $n=2$; Table 1). During 2013, capelin were first observed in

the study area at beach spawning sites on July 2 and were no longer present by July 22. The early arriving capelin were sampled at different beach spawning sites (July 2-3, n=2), and mid-samples were collected at both deep-water (July 9-13, n=3) and beach spawning sites (July 11-13, n=3; Table 1). Late samples (July 17-22, n=3) were collected at deep-water sites only. In 2012, the proportion of female capelin in samples increased over the spawning season, whereas females were primarily collected during the mid-season and were in low proportions in early and late samples in 2013 (Table 1). Interestingly, males collected late in the season in 2013 were difficult to obtain, with the seine collecting 200-400 males compared to several thousand earlier in the season. For both years, 21% of otoliths were discarded due to aging and spawning check discrepancies between observers.

Sex differences

The majority of all sampled male and female capelin at spawning sites, in both years combined were mature (i.e. maturity index 3-4; males: 80 - 81%; females: 85 - 95%). Males were larger than females ($F_{1,3379} = 5405.672$, $p < 0.0001$), which supports reported sexual dimorphism. Males were also older than females ($\chi^2 = 150.633$, $p < 0.0001$), as a higher proportion of females were age 2 (50-68%), whereas a higher proportion of males were age 3 (57-77%; Table 1). The length of males and females increased with age ($r^2 = 0.293$, $p < 0.0001$). There also was a higher proportion of female repeat spawners in comparison to males ($\chi^2 = 7.796$, $p = 0.0052$).

Differences between sexes were also observed in the proxies of extrinsic factors. Females had higher otolith Sr concentrations than males ($F_{1,184} = 19.011$, $p < 0.0001$), but there were no sex-differences in otolith Ba concentrations ($F_{1,184} = 3.238$, $p = 0.0736$). Males had higher $\delta^{13}\text{C}$ values than females ($F_{1,99} = 3.899$, $p = 0.0511$), whereas females had higher $\delta^{15}\text{N}$ values than

Table 1. The year, date and location (i.e. spawning site) of all subsampled capelin in 2012 and 2013 in Notre Dame Bay, along with percentage of male fish in each sample, as well as the number of fish, mean (\pm S.E.) total length (mm), percentage of each age class and percentage of repeat and recruit spawners for males and females in each subsample. See Fig. 1 for the locations of each spawning site ('location') in the study area.

Year	Sampling Date	Timing Category	Location	% Male	Females							Males						
					No.	Length (mm)	Age 2	Age 3	Age 4	Recruit	Repeat	No.	Length (mm)	Age 2	Age 3	Age 4	Recruit	Repeat
2012	09-Jul	Early	Site A	87%	12	147 \pm 10.1	67%	33%	0%	83%	17%	39	172 \pm 9.9	21%	69%	10%	79%	21%
	10-Jul		Site A	86%	14	158 \pm 14.1	29%	57%	14%	79%	21%	38	172 \pm 7.1	8%	89%	3%	92%	8%
	14-Jul		Site 6	79%	29	143 \pm 9.1	45%	48%	7%	86%	14%	41	164 \pm 11.3	46%	49%	5%	85%	15%
	14-Jul		Site 1-2	66%	28	135 \pm 12.3	75%	25%	0%	93%	7%	44	165 \pm 10.1	45%	48%	7%	89%	11%
	15-Jul		Site 6	86%	18	132 \pm 5.6	44%	50%	6%	72%	3%	35	166 \pm 12.6	37%	51%	11%	83%	17%
	16-Jul	Mid	Site 6	96%	5	135 \pm 10.0	60%	20%	20%	80%	20%	32	166 \pm 11.1	34%	63%	3%	91%	9%
	16-Jul		Site 1	35%	45	132 \pm 11.4	73%	27%	0%	91%	9%	7	155 \pm 4.0	43%	57%	0%	71%	29%
	16-Jul		Site 2	85%	14	146 \pm 11.9	79%	14%	7%	100%	0%	46	167 \pm 10.5	35%	59%	7%	80%	20%
	18-Jul		Site 1	32%	46	138 \pm 12.0	72%	26%	2%	96%	4%	30	164 \pm 10.3	47%	50%	3%	77%	23%
	23-Jul	Late	Site 10	82%	33	133 \pm 9.1	88%	9%	3%	94%	6%	29	165 \pm 10.1	45%	45%	10%	69%	31%
	23-Jul		Site 10	84%	28	139 \pm 11.8	79%	18%	4%	96%	4%	35	166 \pm 10.9	46%	49%	6%	86%	14%
2013	02-Jul	Early	Site C	96%	7	153 \pm 10.5	57%	43%	0%	86%	14%	52	172 \pm 11.5	15%	83%	2%	73%	27%
	03-Jul		Site D	76%	30	157 \pm 12.1	37%	60%	3%	60%	40%	45	174 \pm 11.2	13%	82%	7%	90%	10%
	09-Jul		Site 1	29%	69	148 \pm 12.8	46%	54%	0%	74%	26%	44	170 \pm 9.7	16%	80%	4%	86%	16%
	09-Jul		Site 2	16%	65	145 \pm 13.6	52%	48%	0%	85%	15%	24	172 \pm 7.3	12%	88%	0%	67%	33%
	11-Jul	Mid	Site D	85%	26	147 \pm 14.6	65%	27%	8%	88%	12%	67	171 \pm 11.9	18%	79%	3%	81%	19%
	12-Jul		Site D	92%	13	154 \pm 11.9	69%	31%	0%	92%	8%	62	171 \pm 11.4	13%	83%	5%	79%	21%
	13-Jul		Site D	96%	6	153 \pm 11.4	33%	67%	0%	83%	17%	57	170 \pm 12.4	33%	65%	2%	72%	28%
	13-Jul		Site 2	47%	73	146 \pm 13.2	51%	44%	5%	88%	12%	49	171 \pm 9.4	20%	76%	4%	92%	8%
	17-Jul		Site 2	99%	1	165	100%	0%	0%	100%	0%	47	176 \pm 11.5	23%	75%	2%	77%	23%
	19-Jul	Late	Site 2	93%	12	144 \pm 12.1	42%	58%	0%	90%	10%	33	172 \pm 11.8	24%	76%	0%	88%	12%
22-Jul	Site 2		99%	1	127	100%	0%	0%	100%	0%	30	173 \pm 11.4	30%	67%	3%	93%	7%	

males ($F_{1,99} = 18.934$, $p < 0.0001$). Due to differences of most intrinsic and extrinsic factors between males and females, sexes were kept separated when testing hypotheses in subsequent analyses.

Length

Overall, the common trend was for the largest individuals of each sex to arrive first (see Appendix A, Fig. A1). The mean total length of mature females differed significantly throughout the spawning season, but there was significant interannual variation in length (Table 2). Mature females were larger in 2013 (148.9 ± 13.4 mm) and heavier (15.1 ± 4.1 g) compared to 2012 (139.6 ± 13.0 mm; length: $F_{1,727} = 91.58$, $p < 0.0001$; 11.8 ± 3.6 g; mass: $F_{1,727} = 23.79$, $p < 0.0001$). *Post-hoc* tests revealed that females sampled early in both years were significantly larger than those collected mid- (2012 & 2013: $p < 0.0001$) and late-season (2012: $p < 0.0001$; 2013: $p = 0.0035$), whereas there was no significant difference in length between mid- and late-season females in either year (2012: $p = 0.3349$; 2013: $p = 0.9353$). Overall, large females were present early in the spawning season, whereas more small and medium-sized individuals were present later in the spawning season (Fig. 3).

Similar to females, mean total length of mature males also differed significantly throughout the spawning season and there was significant interannual variation in length (Table 2). Mature males in 2013 were larger (172.0 ± 11.3 mm) and heavier (33.8 ± 7.1 g) compared to 2012 (166.8 ± 10.56 mm; length: $F_{1,1142} = 64.63$, $p < 0.0001$; 29.7 ± 5.8 g; mass: $F_{1,1142} = 98.35$, $p < 0.0001$). *Post-hoc* tests revealed that males sampled early in 2012 were significantly larger than those collected during the mid- ($p < 0.0001$) and late-season ($p < 0.0001$), with no significant difference between mid- and late-season samples ($p = 0.4871$). In

Table 2. Results of ANOVAs and Chi-Square tests examining intrinsic and extrinsic factors of mature male and female capelin sampled throughout the spawning period, over two consecutive years (2012, 2013) in Notre Dame Bay, Newfoundland. Stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were performed in 2013. The effect of timing as a fixed effect and year as a random effect were evaluated when conducting ANOVAs.

	Males			Females		
	df	f, t, χ^2	p	df	f, t, χ^2	p
Length						
Timing	2	f = 61.803	<0.0001	2	f = 44.697	<0.0001
Year	1	t = 17.43	<0.0001	1	t = 12.36	<0.0001
Error	2465			944		
Age						
Timing	2	$\chi^2 = 18.106$	0.0012	2	$\chi^2 = 17.906$	0.0013
Year	1	$\chi^2 = 17.341$	<0.0001	1	$\chi^2 = 15.868$	<0.0001
Experience						
Timing	2	$\chi^2 = 1.906$	0.3856	2	$\chi^2 = 5.411$	0.0668
Year	1	$\chi^2 = 0.237$	0.6268	1	$\chi^2 = 3.609$	0.0575
Sr						
Timing	2	f = 2.0824	0.1303	2	f = 2.639	0.0775
Year	1	t = 3.19	0.0023	1	t = 2.40	0.0236
Error	94			83		
Ba						
Timing	2	f = 1.436	0.2504	2	f = 0.273	0.7618
Year	1	t = 2.23	0.0371	1	t = 2.36	0.0265
Error	94			83		
$\delta^{13}\text{C}$						
Timing	2	f = 0.718	0.4924	2	f = 1.449	0.2459
Error	50			43		
$\delta^{15}\text{N}$						
Timing	2	f = 4.442	0.0168	2	f = 0.025	0.9750
Error	50			43		

Significant effects ($p > 0.05$) are in bold.

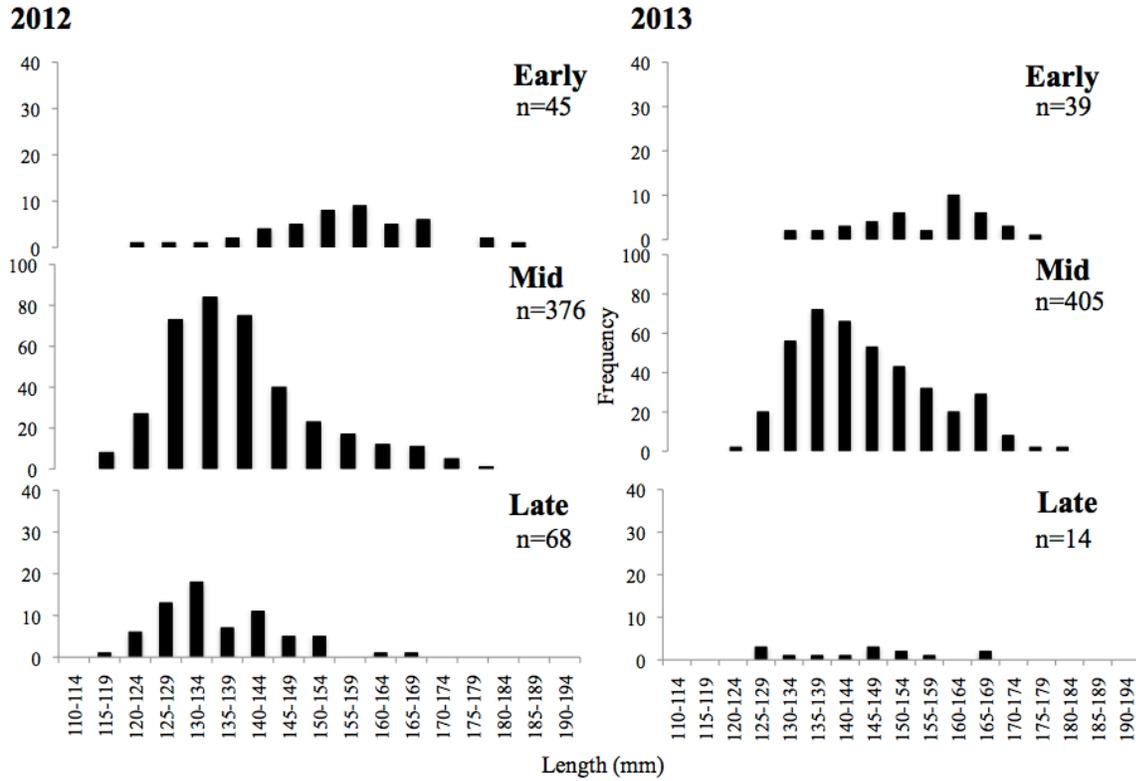


Figure 3. Length frequencies of mature female capelin (maturity index = 3-4) during the spawning seasons in 2012 and 2013 divided into early-, mid- and late-season samples. The number of female fish sampled per period is indicated by n. Note that the y-axis scale for mid-season fish differs from early and late.

2013, however, male length did not differ significantly between early- and late-season samples ($p = 0.8514$), but mid-season fish were significantly smaller than those sampled early ($p < 0.0001$) and late ($p < 0.0001$). Therefore, more large males were present early and late, but a mix of small, medium and large individuals were present mid-season in 2013 (Fig. 4).

Age and spawning experience

For both males and females, differences in age class frequencies were observed between years and timing of sampling and arrival when years were combined (Table 2). As it was not possible to control for year as a random variable because age and spawning experience are categorical variables, timing in each year was examined separately (Table 2). In 2012, the age class frequencies differed significantly throughout early-, mid- and late-season samples for both female ($\chi^2 = 12.48$, $p = 0.0141$) and male ($\chi^2 = 21.78$, $p = 0.0002$) capelin. More older individuals (ages 3 and 4) were observed in samples early in the spawning season, while more younger individuals (age 2) were present as the spawning season progressed (Fig. 5). In 2013, age class frequencies did not differ significantly throughout the spawning season for females ($\chi^2 = 2.06$, $p = 0.725$) or males ($\chi^2 = 4.46$, $p = 0.347$), but rather relatively similar proportions of each age class were observed throughout the spawning season (Fig. 5). The age class frequencies differed significantly between years for females and males separately (Table 2). For females, there were more age 2 (68%) in 2012 than 2013 (50%) and consequently fewer age 3 in 2012 (28%) relative to 2013 (47%). For males, there were more age 2 (36%) in 2012 than 2013 (20%) and consequently less age 3 in 2012 (57%) than 2013 (77%).

For both males and females, the frequencies of recruit and repeat spawners did not differ between years or among timing of sampling/arrival periods when years were combined (Table

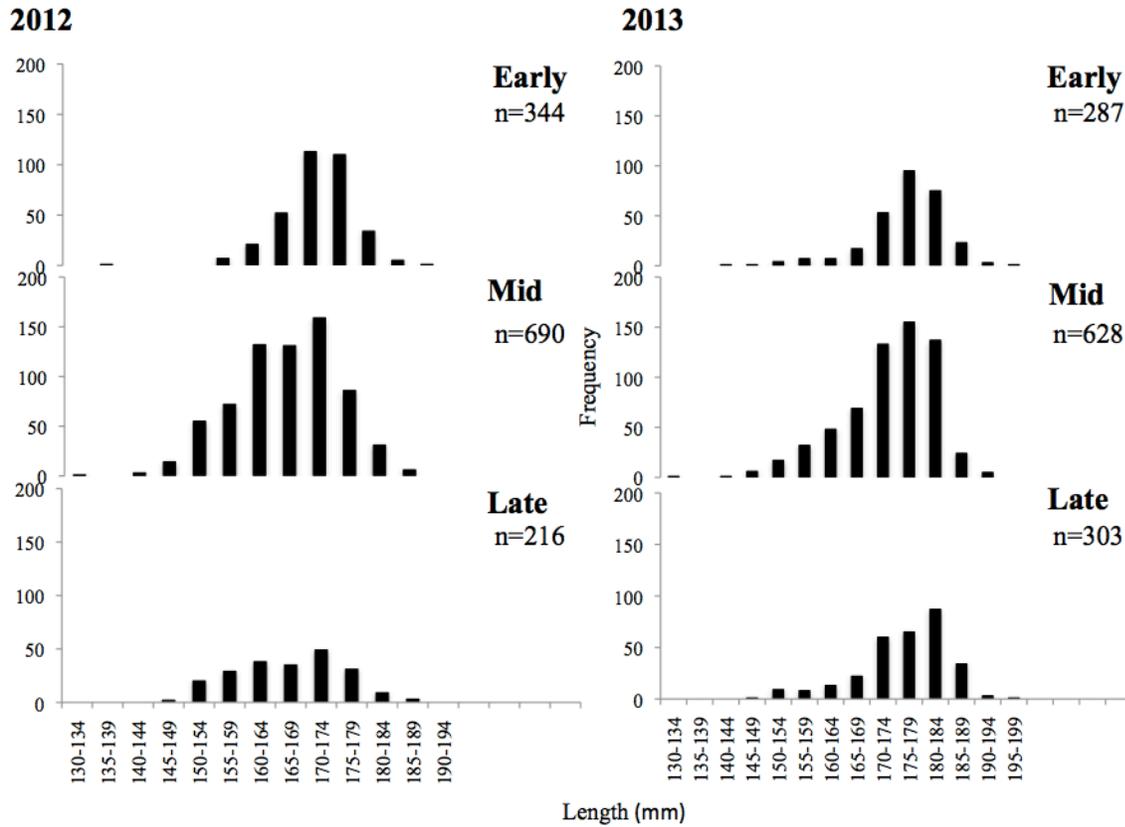


Figure 4. Length frequencies of mature male capelin (maturity index = 3-4) during the spawning seasons in 2012 and 2013 divided into early-, mid- and late-season samples. The number of male fish sampled per period is indicated by n.

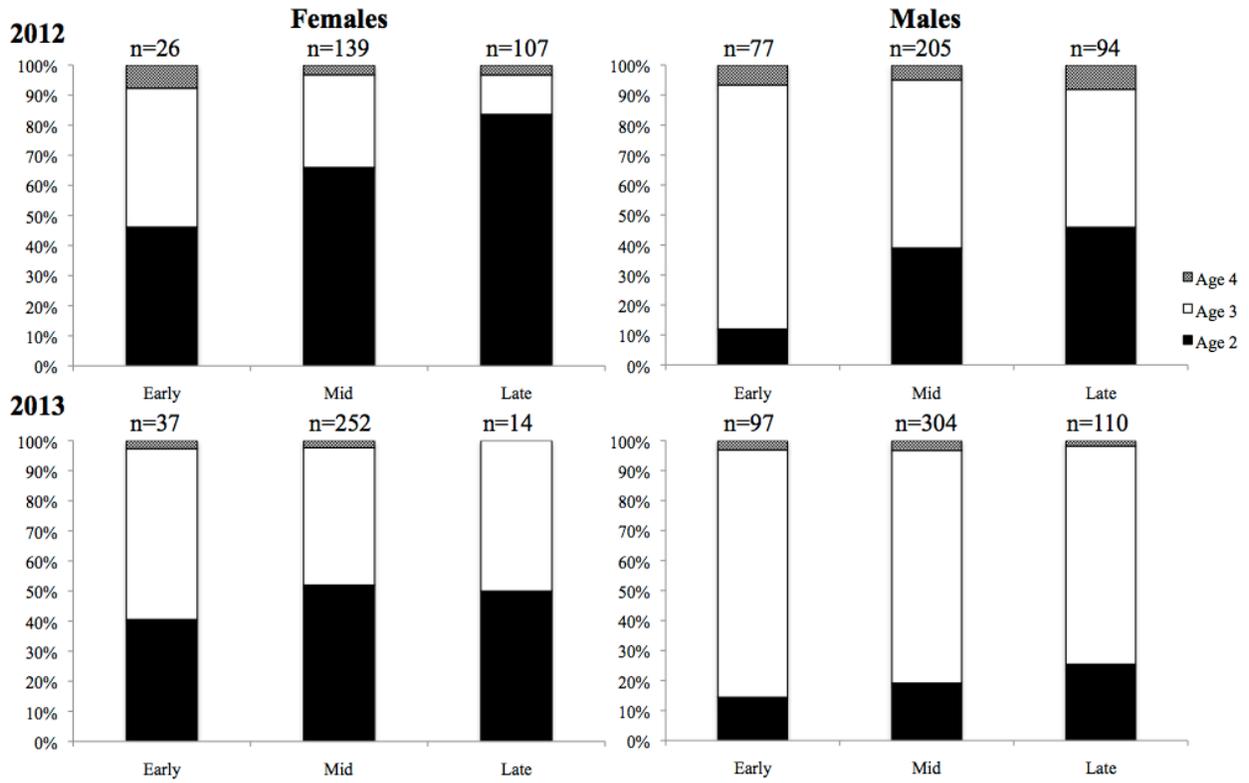


Figure 5. Percentage of different age classes of female and male capelin subsampled throughout the spawning seasons of 2012 and 2013, divided into early-, mid- and late-sampled fish. The number of fish sampled per period is indicated by n.

2). Further investigation revealed that the frequency of three-year-old female repeat spawners did not differ significantly throughout the spawning season in 2012 ($\chi^2 = 0.279$, $p = 0.8698$), but did in 2013 ($\chi^2 = 5.961$, $p = 0.0508$), with more repeat spawners sampled early in the spawning season (Fig. 6). The frequencies of male repeat spawners displayed opposite trends, with no significant difference in 2013 ($\chi^2 = 0.386$, $p = 0.8202$), but significantly higher frequencies of repeat spawners later in the spawning season of 2012 ($\chi^2 = 10.50$, $p = 0.0052$, Fig. 6).

Otolith microchemistry

There was no relationship between growth during the final winter, as indicated by the width of the marginal zone of the otolith (i.e. year prior to capture), and fish length (Appendix B, Fig. B1) or between otolith Sr and Ba concentrations and the width of the marginal zone (Appendix B, Fig. B2), suggesting that trace element concentrations were not related to growth rate differences among subsampled fish. Otolith Sr and Ba concentrations in the marginal zone did not differ between early-, mid- and late-sampled capelin, but there was significant interannual variation in otolith Sr and Ba (Table 2). In 2013, males and females had higher Ba concentrations in the marginal zone in comparison to 2012 ($F_{1,180} = 13.008$, $p = 0.0004$), whereas Sr concentrations were higher in 2012 than 2013 ($F_{1,180} = 15.415$, $p = 0.0001$). Owing to inter-annual differences, trends in otolith chemistry were examined with respect to timing of sampling and arrival within each year. In 2012, Sr concentrations differed significantly throughout the spawning season for females ($F_{2,36} = 3.696$, $p = 0.0347$) and males ($F_{2,42} = 5.197$, $p = 0.0096$). *Post-hoc* tests revealed that early-season individuals had significantly higher Sr concentrations than mid-season samples (females: $p = 0.0371$; males: $p = 0.0068$); however, Sr concentrations did not differ between early- and late-season (females: $p = 0.1185$; males: $p = 0.2555$) or mid- and late-season samples

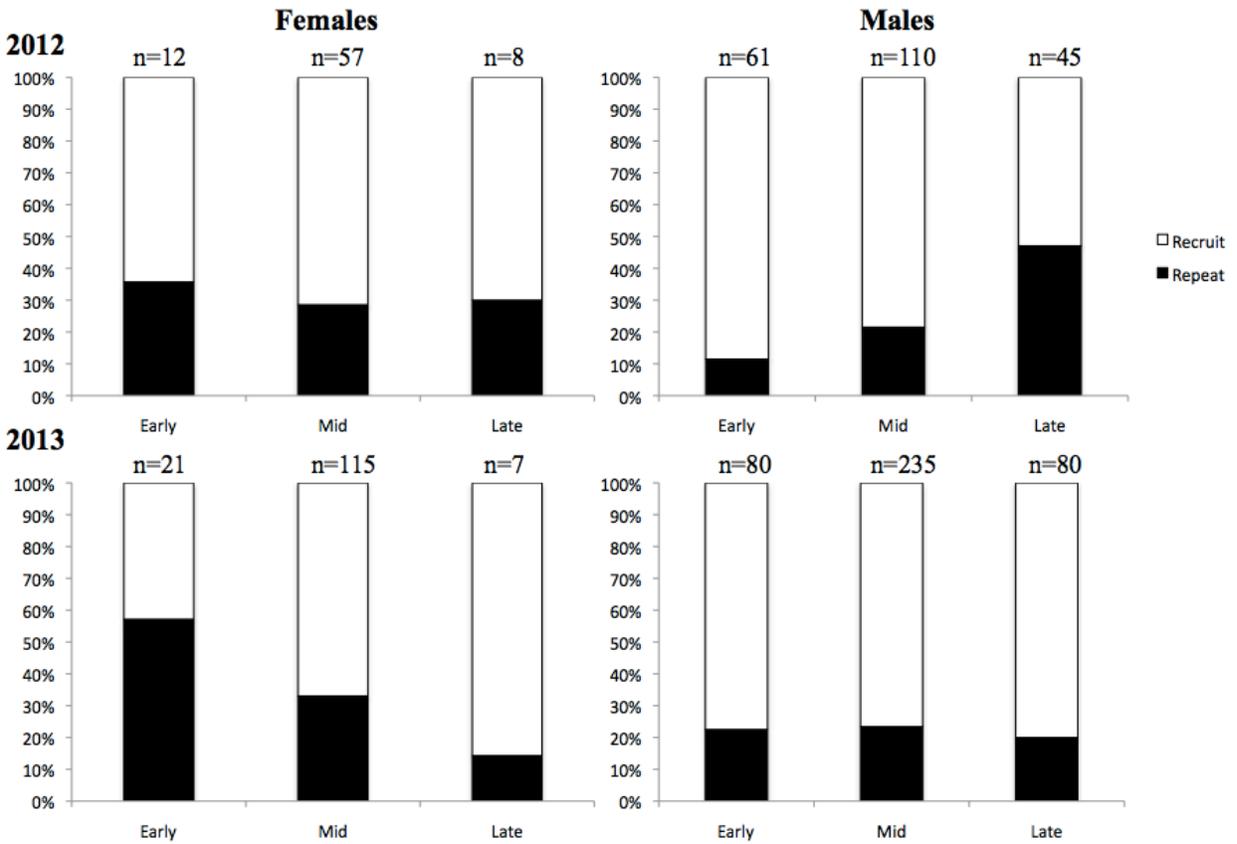


Figure 6. Percentage of three year old recruit (i.e. first-time) relative to repeat spawning male and female capelin throughout the spawning seasons of 2012 and 2013 divided into early-, mid- and late-sampled fish, with n representing the number of fish sampled per period.

(females: $p = 0.9318$; males: $p = 0.2482$; Fig. 7). In contrast, there were no significant differences between early-, mid- and late-season samples in 2013 (females and males: $p > 0.05$; Fig. 7). Otolith Ba concentrations did not differ throughout the spawning season for females or males (Table 2), with the exception of 2012 males ($F_{2,42} = 4.066$, $p = 0.0243$). A *post-hoc* test revealed that Ba concentrations of early-season males differed from mid-season samples ($p = 0.0375$); however, Ba concentrations did not differ between early- and late-season ($p = 0.563$) or mid- and late-sampled fish ($p = 0.9830$; Fig. 8).

Stable isotope analysis

As muscle tissue for stable isotope analyses were only sampled in 2013, interannual comparisons were not possible. An ANOVA revealed that $\delta^{15}\text{N}$ values differed throughout the spawning season for males (Table 2), with a *post-hoc* test revealing that early-season fish had lower $\delta^{15}\text{N}$ values compared to late-season samples ($p = 0.0150$); however, $\delta^{15}\text{N}$ values did not differ between early- and mid-season samples ($p = 0.5909$) or mid- and late-season samples ($p = 0.0687$; Fig. 9). In contrast, $\delta^{13}\text{C}$ values did not differ among early-, mid- and late-season sampled female and male capelin (Table 2, Fig. 9). Interestingly, $\delta^{13}\text{C}$ values increased with length for both males and females, whereas there was no relationship between $\delta^{15}\text{N}$ values and length for males or females (Appendix B, Fig. B3).

2.4 Discussion

Despite high inter-annual variation in almost all factors examined, intrinsic factors, specifically length and age, consistently varied with timing of arrival of both males and females to spawning sites in coastal Newfoundland. In particular, the pattern of larger fish arriving at spawning sites

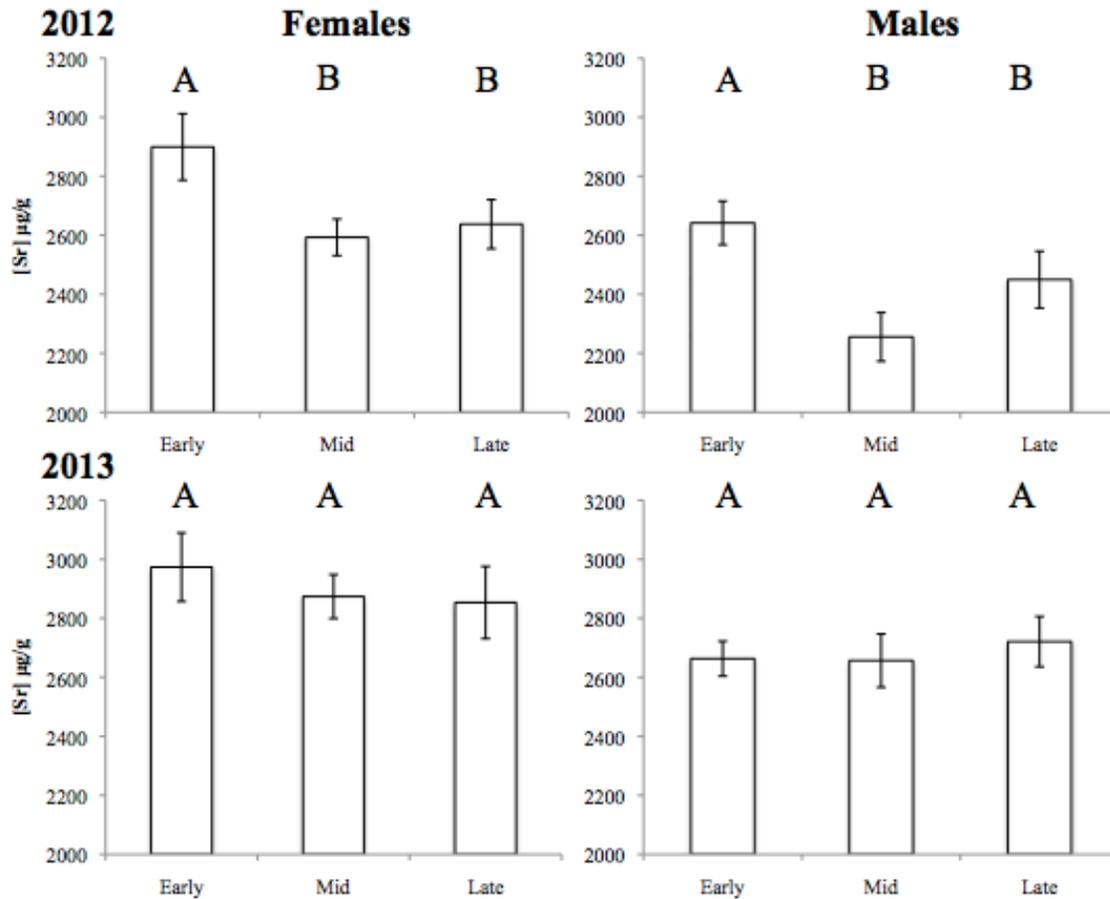


Figure 7. Mean \pm SE otolith Sr concentrations ($\mu\text{g/g}$) during the final year of male and female capelin collected throughout the 2012 and 2013 spawning seasons. Note that the y-axis scale starts at 2000 $\mu\text{g/g}$ to enhance differences/similarities. Differences throughout the spawning season, within each year are indicated by ‘A’ and ‘B’ notation.

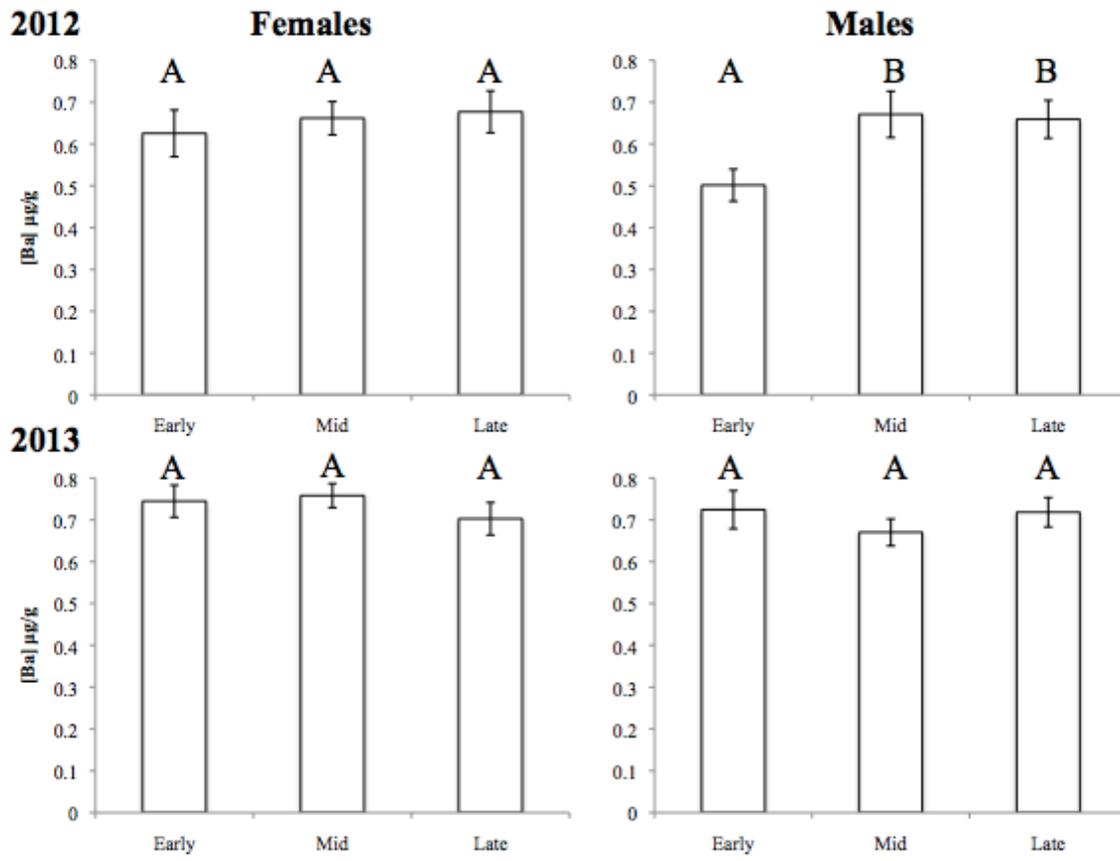


Figure 8. Mean Ba concentrations ($\mu\text{g/g}$) with $\pm\text{SE}$ bars throughout the spawning season for male and female capelin in 2012 and 2013. Differences throughout the spawning season, within each year are indicated by 'A' and 'B' notation.

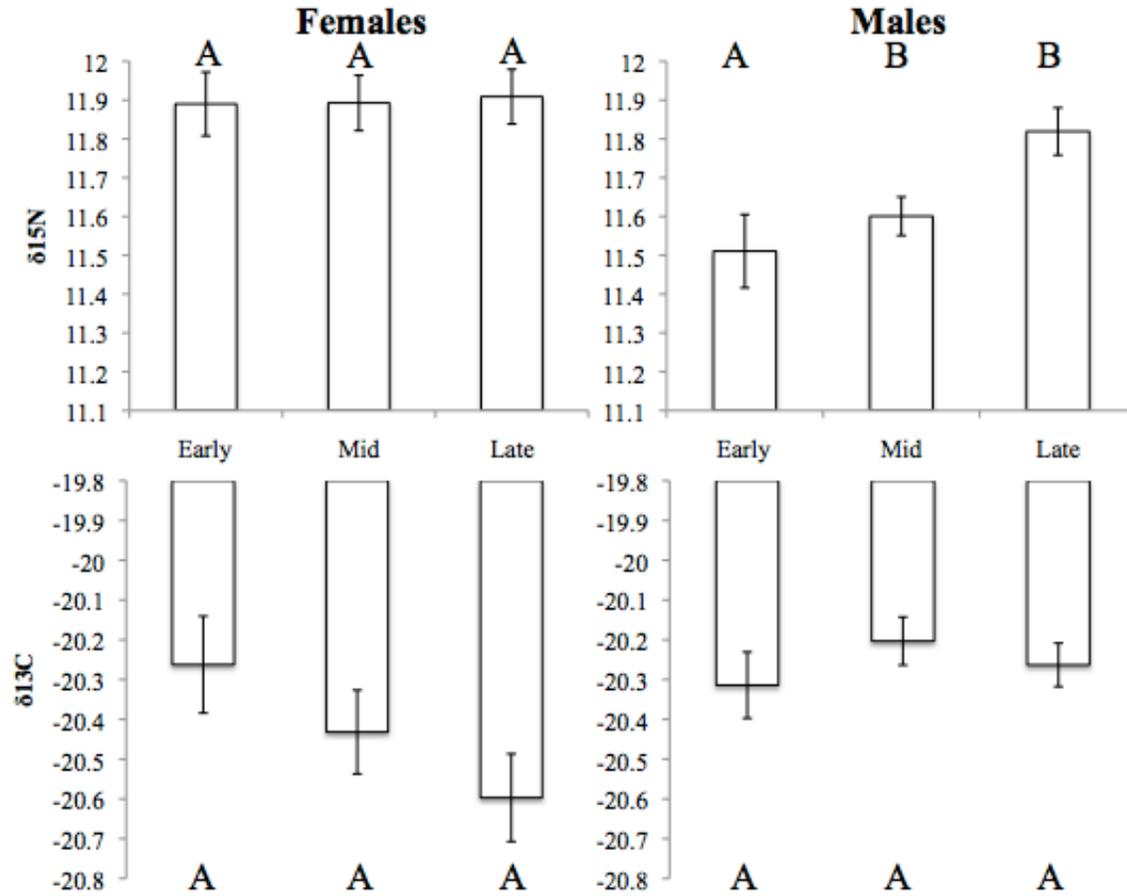


Figure 9. Mean \pm SE $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of females and males sampled throughout the spawning season in 2013 divided into early-, mid- and late-season sampled fish. Differences throughout the spawning season, within each year are indicated by ‘A’ and ‘B’ notation.

prior to smaller individuals in this study is similar to that shown by Vandeperre and Methven (2007) before the cold-water event in the early 1990's, suggesting that this trend still holds. In contrast, proxies of extrinsic factors did not consistently vary with timing of arrival in both years. For instance, females and males with higher otolith Sr concentrations arrived first in 2012, but not in 2013. In addition, males with lower Ba concentrations (in 2012) and $\delta^{15}\text{N}$ values (in 2013) arrived first, but again these trends were not observed in both years, which suggests that extrinsic factors may be less important than intrinsic factors in determining individual-level variability in the timing of arrival of spawning capelin in coastal Newfoundland.

Timing of arrival

As predicted, the intrinsic factors, including length, age and spawning experience were important in explaining the variation in the timing of arrival. Similar trends in length and age with timing of arrival across males and females, especially in 2012, suggest that similar mechanisms influence the timing of arrival of all individuals in coastal Newfoundland. Size of capelin was correlated with age and experience, similar to many species (e.g. Hunter and Macewicz 1985; Ridgway et al. 1991; Murawski et al. 2001), thereby suggesting why not only larger but older and more experienced fish arrive first at spawning grounds. For instance, older Chum salmon (*Oncorhynchus keta*) are present earlier in the spawning season than younger fish in British Columbia (Beacham and Murray 1987) and older female Atlantic cod spawn prior to younger individuals in Newfoundland (Lawson and Rose 2000).

Many mechanisms have been proposed to explain this common trend. First, experimental studies have shown that larger fish have faster swim speeds than smaller fish (e.g., Bainbridge 1958; Beamish 1970; Webb et al. 1984; Videler and Wardle 1991). Owing to size-dependent

swim speeds, smaller fish may simply take longer to reach spawning grounds from overwintering areas and, thus, arrive and spawn later in the season than larger individuals (Carscadden et al. 1997). Second, larger, older fish may start maturing earlier than smaller fish and, thus, are ready to spawn earlier. For instance, larger herring appear to mature and spawn first relative to smaller individuals due to delayed onset of maturation (Ware and Tanasichuk 1998; Slotte et al. 2000). Similarly, larger, older smallmouth bass (*Micropterus dolomieu*; Ridgway et al. 1991) and pumpkinseed sunfish (*Lepomis gibbosus*; Danylchuk and Fox 1996) allocate more energy to reproduction, thereby start to mature earlier, whereas smaller, younger males allocate more energy to growth, resulting in older, larger mature males being ready to spawn before small males. As larger fish tend to have lower body mass-specific metabolic rates and higher energy reserves (Shuter and Post 1990), they may simply have more energy to begin gonadal development earlier. Third, larger fish may have higher rates of maturation. Indeed, large capelin had more advanced rate of gametogenesis than smaller fish and, thus, are able to spawn earlier (Flynn and Burton 2003). It is possible that all of these size-dependent factors are important in explaining the trends observed in this study.

Otolith Sr concentrations differed between early- and late-arriving male and female capelin in 2012 and otolith Ba concentrations differed between early relative to mid- and late-arriving males in 2012. Otolith Sr and Ba concentrations did not vary with growth in the final year (i.e. final increment width), suggesting that differences in otolith chemistry reflect variation in environmental conditions experienced by individuals rather than variation in growth rates. Higher Sr concentrations in early-arriving males and females may indicate that these individuals experienced cooler temperatures during their final winter and spawning migration relative to fish arriving mid- and late-season, owing to the negative relationship between Sr concentration and

temperature of other cold-adapted marine fish (e.g. Townsend et al. 1992, 1995; DiMaria et al. 2010). Higher otolith Sr in early-arriving capelin could also result from experiencing higher salinity (see review in Elsdon and Gillanders 2003, Elsdon et al. 2008), suggesting that early-arriving fish may have occupied regions farther from the coast relative to those arriving later (D’Avingon and Rose 2013). Interestingly, lower otolith Ba concentrations in early-arriving males in 2012 may support that these fish experienced higher salinities and colder temperatures (see review in Walther and Limburg 2012). The interpretation of early-arriving fish experiencing colder conditions, however, contradicts delayed spawning associated to colder temperatures at a population-level (e.g. Carscadden et al. 1997; Regular et al. 2008; Oskarsson and Taggart 2010; Davoren et al. 2012). In contrast, Davoren and Halden (2014) recently showed that otolith Sr concentrations were significantly higher in the marginal zone of otoliths of capelin spawning at deep-water sites (typically late-arriving) relative to beach sites (typically early-arriving) in a particularly cold year (2009). Unfortunately, the influence of different environmental conditions on incorporation rates of trace elements into capelin otoliths has yet to be determined and, thus, reconstructing the environmental conditions that resulted in these differences in otolith chemistry is speculative.

Differences in otolith Sr and Ba between early- and later-arriving fish suggest that these fish experienced different environmental conditions, which could have arisen from occupying different locations or different depths, or similar locations at different times of the year. As there is low variability in temperature and salinity across the Newfoundland shelf within years (Petrie et al. 1988), environmental conditions might not have differed much between different locations or months throughout the winter as well as during spawning migration (Davoren and Halden (2014). In contrast, temperature and salinity differ widely with depth, as warm surface and

bottom layers are separated by a cold intermediate layer (CIL) of sub-zero water on the Newfoundland and Labrador shelf (Petrie et al. 1988), as well as within coastal pre-spawning staging areas (Davoren et al. 2006). Occupying different depths could have resulted in observed differences in otolith chemistry. It is important to note as well that the lack of consistent differences in otolith chemistry among fish varying in their timing of arrival does not necessarily mean that fish migrated together, as they could have occupied different areas with similar environmental conditions (Campana et al. 2000).

Stable isotope ratios did not differ among early-, mid- and later-arriving individuals, suggesting that the dietary composition did not differ among capelin arriving throughout the spawning season. One exception was lower $\delta^{15}\text{N}$ in early relative to mid- and late-arriving males. As $\delta^{15}\text{N}$ tends to increase with trophic level of marine organisms (Davenport and Bax 2002), which suggests that males arriving early may have been feeding at a lower trophic level relative to males arriving later in the season. This is surprising as early arriving males were larger and larger capelin tend to feed on larger prey (e.g. amphipods, euphausiids), presumably at a higher trophic level (Stowasser et al. 2012) relative to smaller prey (e.g. copepods) eaten by smaller fish (O'Driscoll et al. 2001, Dalpadado and Mowbray 2013). I, however, found no relationship between fish length and $\delta^{15}\text{N}$ values, suggesting that stable isotope differences were not related to differences in length. Therefore, lower nitrogen in early-arriving males may be associated with spatially variable $\delta^{15}\text{N}$ values within the marine environment (Chikaraishi et al. 2009), suggesting that these individuals could have been feeding on similar prey sources, but in different locations.

Sex-based differences

Although not a primary goal of this study, many differences were found between male and female capelin throughout the spawning season for both intrinsic and extrinsic factors. More experienced female capelin arrived at spawning sites first in 2013, with the first wave of females consisting of the largest, oldest and most experienced individuals, whereas the next wave(s) consisted of more smaller, younger, recruit individuals. Although larger and older male capelin similarly arrived at spawning sites first, there was an increasing proportion of more experienced males later in the season. Opposite trends in spawning experience may be explained by sex-based differences in life history and spawning behaviour (Shackell et al. 1994, Huse 1998). Females typically move onto spawning sites, deposit their eggs all at once and then leave (Friis-Rodel and Kannevorff 2002); therefore, females sampled in different periods of each season likely represent different spawning waves. In contrast, males typically arrive at spawning sites first and are responsible for spawning site selection, similar to other species (e.g. Atlantic cod; Lawson and Rose 2000), and remain at sites to mate with multiple females (Templeman 1948). Therefore, the number of older, repeat spawners may simply build up at spawning sites over time (Shackell et al. 1994), as shown for other species (e.g. cod, Morgan et al. 2013). These sex-based differences may also explain why mostly males were collected at the spawning sites and why the proportion of females in samples peaked during mid-season in both years, but was low during the early and late periods. In addition, these differences may also account for only small numbers of males observed late in 2013, possibly awaiting another wave of females. Similarly, Lawson and Rose (2000) showed that male cod dominated early and late spawning aggregations, whereas females were most abundant mid-season.

Most spawning females were two years old, whereas most spawning males were three years old. This result supports a growing literature suggesting that male and female capelin use different strategies to maximize their lifetime reproductive success (e.g. Nakashima 1992; Huse 1998; Davoren 2013). Females may spawn earlier in life than males because they have a higher likelihood of surviving spawning than males (Shackell et al. 1994; Friis-Rodel and Kannevorff 2002), as remaining at spawning sites results in high physical damage to the body (Templeman 1948). Although male capelin can survive spawning, as evidenced in lab (e.g. Christiansen et al. 2008) and field studies (e.g. Nakashima 1992), estimated survival rates are low (Shackell et al. 1994; Huse 1998). Observations in this study show significantly higher proportions of repeat spawning females than males; however, estimates of repeat spawning were higher in this study (35-59 %) than reported previously (2.5-50%; Shackell et al. 1994).

Otolith chemistry differed between males and females, with females having higher Sr concentrations than males, suggesting that males and females experience different environmental conditions during the final winter, spawning migration and inshore prior to spawning. During migration, capelin primarily occupy the warm (~0-4°C) bottom layer underneath the CIL, but also undergo diel vertical migration (Mowbray 2002). If otolith Sr concentration is negatively related to temperature in capelin, higher Sr in females may indicate that they remain within the CIL for longer periods during vertical migration than males. Higher Sr concentrations may also be indicative of the cooler staging environment where females remain prior to spawning (deeper waters, Friis-Rodel and Kannevorff 2002; Davoren et al. 2006; Davoren 2013), relative to the warmer, inshore water nearby spawning sites occupied by males during the same period.

Females also had higher $\delta^{15}\text{N}$ values than males, possibly reflecting differences in diet and pre-spawning behaviour. Females, however, tend to eat smaller prey relative to males

(Dalpadado and Mowbray 2013), likely owing to their smaller size, suggesting that I should have observed the opposite trend in $\delta^{15}\text{N}$ values. It is possible that the observed trend may be explained by catabolic breakdown of protein reserves as a result of females fasting prior to spawning (Estrada et al. 2005), as energy allocation is directed primarily toward reproductive behaviour (i.e. spawning migrations; Anthony et al. 2000). Unlike $\delta^{15}\text{N}$ values, $\delta^{13}\text{C}$ values increased with length for both male and female capelin, and males had higher $\delta^{13}\text{C}$ values than females. Larger prey, namely hyperiid amphipods and euphausiids, occur in epibenthic habitats (O'Driscoll et al. 2001) whereas smaller prey, namely copepods, tend to be mostly pelagic (e.g. Pepin and Dower 2007). This suggests that larger capelin, particularly males, are feeding on larger, more benthic prey ($\delta^{13}\text{C}$ enriched), whereas females are feeding on smaller, more pelagic prey ($\delta^{13}\text{C}$ depleted; Pinnergar and Polunin 2000, O'Driscoll et al. 2001; Davenport and Bax 2002, Sherwood and Rose 2005). Although all capelin undergo diel vertical migration (e.g. Mowbray 2002, Davoren et al. 2006), large and small capelin do so differently, with larger individuals remaining deeper (higher $\delta^{13}\text{C}$) and smaller individuals remaining closer to the surface (Davoren et al. 2008). Therefore, variation in $\delta^{15}\text{N}$ values suggests that capelin may aggregate and forage in size- and sex-specific schools.

Variability in intrinsic and extrinsic factors

Despite trends in intrinsic and extrinsic factors with timing of capelin arrival, there was high variability throughout the spawning season. Notably, stronger trends in length and age throughout the spawning season were observed in 2012 relative to 2013, suggesting that distinct multiple spawning waves did occur in 2012, but not in 2013 (e.g. one spawning wave or multiple waves all at once). Ware and Tanasichuk (1989) suggested that the higher the diversity in

size/age classes of herring stocks, the greater the ability to distinguish spawning runs of different size and age. In this study, two age classes (2, 3) dominated the capelin spawning population in both years, likely obscuring the ability to distinguish spawning runs. In addition, variation in year class strength among years may have resulted in difficulties distinguishing spawning waves. Capelin populations are known to be highly variable, similar to other forage fish species (+/- 40 %; Pikitch et al. 2012), and spawning stock biomass is driven by strong year classes (i.e. years in which conditions are favourable for recruitment; Carscadden et al. 2013). In support, capelin in 2013 appeared to be dominated by a particularly strong year class in 2013 (i.e. age 3 fish). Overall, the high variability in intrinsic and extrinsic factors associated with timing of arrival may be characteristic of fish with a short spawning season (2-3 weeks) and lifespan (3-6 years), such as capelin. Indeed, less variable trends are typically observed in long-lived fish species (i.e. 20+ year-old herring, Lambert 1990; 12+ year-old cod, Morgan et al. 2013) and species with longer spawning seasons (i.e. months) and, thus, more distinct spawning waves (e.g. herring, Hay 1985; anchovy *Engraulis mordax*, Hunter and Macewicz 1985; cod and haddock *Melanogrammus aeglefinus*, Morgan et al. 2013).

In addition, there was high variability in intrinsic and extrinsic factors between years. In particular, differences in trace element concentrations within the final increment between years suggest that individuals encountered different environmental conditions, with Sr concentrations being higher in 2012 and Ba concentrations being higher in 2013. Differences in otolith chemistry between years have previously been found in this region (e.g. D'Avignon and Rose 2013) and others (e.g. Fowler et al. 2005). As physical factors, such as temperature and salinity, are known to vary considerably among years on the Newfoundland shelf (Petrie et al. 1988), it is

likely that differences in environmental conditions may confound comparisons being investigated.

Conclusion

Although proxies for extrinsic factors were inconsistently associated with timing of arrival in both years of this study, my results suggest that these factors may indeed be responsible for some variation in timing of spawning, as suggested from population-level studies (e.g. Carscadden et al. 1997). Owing to the inter-annual variability shown here, it will be critical to obtain environmental profiles (e.g. temperature, salinity, water chemistry) and prey distribution along migratory routes over multiple years to reconstruct habitat use and diet during spawning migration and, thus, to clarify the role of these factors in individual-level variability in timing of inshore arrival at coastal Newfoundland spawning grounds. In contrast to extrinsic factors, length and age had a consistent influence on the timing of arrival of capelin in the two years of this study. Larger, older, and more experienced (for females) capelin arrived at spawning sites first, similar to other marine teleosts (Wright and Trippel 2009) and similar to capelin in coastal Newfoundland prior to the cold-water event in 1991 (Vandepierre and Methven 2007). Therefore, knowledge of the age structure of the population is critical to understanding the inter-annual variability in the timing of Newfoundland spawning capelin. Larger, older and more experienced females typically have higher fecundity (Winters 1971, Nakashima 1987; cf Penton and Davoren 2013) and, thus, have the potential to contribute proportionately more to recruitment. Therefore, if the capelin fishery is opened early in the season when only larger, older individuals are present inshore to spawn, harvesting a large proportion of these individuals could negatively impact the age/size structure of the population, as well as recruitment (Wright and Trippel 2009). As such,

fisheries managers should carefully consider the results of this study when developing future harvest strategies of Newfoundland capelin.

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Chapter 3. General Discussion

Determining both intrinsic and extrinsic factors that influence the timing of arrival of capelin to the spawning grounds in coastal Newfoundland has provided insight into the factors underlying the variation in timing of spawning observed in coastal Newfoundland (Carscadden et al. 1997; Regular et al. 2008; Davoren et al. 2012). For instance, intrinsic factors, specifically length and age, consistently varied with the timing of arrival of both males and females to spawning sites in coastal Newfoundland. The pattern of larger fish arriving at spawning sites prior to smaller individuals in this study is similar to that shown by Vandeperre and Methven (2007) before the cold-water event in the early 1990s, suggesting that this trend still holds. In contrast, proxies of extrinsic factors did not consistently vary with timing of arrival in both years. For instance, females and males with higher otolith Sr concentrations arrived first in 2012, but this was not the case in 2013. In addition, males with lower Ba concentrations (in 2012) and $\delta^{15}\text{N}$ values (in 2013) arrived first, but these trends were not observed in both years, suggesting that extrinsic factors, such as temperature and availability of different prey types, may be less important than intrinsic factors in determining individual-level variability in the timing of arrival of capelin and, thus, timing of spawning in coastal Newfoundland. Therefore, changes in size/age structure of the population will be more important factors influencing timing of spawning and, thus, possibly recruitment (Wright and Trippel 2009).

Although proxies for environmental conditions and diet were inconsistently associated with timing of arrival in both years, results suggest that these may also be involved in influencing the timing of arrival, as suggested from population-level timing studies (e.g. Carscadden et al. 1997). Obtaining environmental profiles (e.g. temperature, salinity, water chemistry) along migratory routes over multiple years will be required to accurately reconstruct

habitat use during spawning migration, owing to interannual variability shown here, as well as to clarify the influence of incorporation rates of Sr and Ba concentrations into capelin otoliths under varying environmental conditions. To further investigate capelin migration, additional markers, such as $\delta^{18}\text{O}$ should also be taken into consideration (Miller 2011).

To quantitatively analyze diet and to better understand habitat use in capelin through stable isotope analysis, relationships between the metabolic turnover and environmental factors need to be experimentally determined (Maruyama et al. 2001). Sherwood and Rose (2005) suggested that $\delta^{15}\text{N}$ signatures would be useful to understand long distance migration of capelin from shelf edge to the shore and back, as $\delta^{15}\text{N}$ varies significantly at large scales in marine consumers. Therefore, sampling capelin at different locations (offshore and near shore) and different times of the year will aid in understanding the influence of diet of this pelagic fish. In addition, because of the spatial differences in $\delta^{15}\text{N}$ values, these signatures could be used to determine if all capelin migrate, which has previously been used in determining non-migratory herring individuals (Hansson et al. 1997).

Timing of arrival was associated with spawning habitat, as early-arriving fish were found at beach sites, whereas late-arriving fish were found at deep-water sites. Beach temperatures are typically ideal for early-arriving individuals to spawn, but are likely too warm when the later individuals arrive (Nakashima and Wheeler 2002, Davoren 2013) and, thus, beach temperatures are no longer suitable for incubation of eggs or successful hatching (Penton et al. 2012). Spawning events at deep-water sites were previously thought to contribute very little to the overall reproductive success of the population (Nakashima and Wheeler 2002); however, these deep-water sites have since been suggested to have high potential for recruitment (Penton and Davoren 2008). Penton et al. (2012) have recently suggested that the use of both beach and deep-

water habitats provide two distinct development strategies to achieve recruitment (beach: rapid development, low to high mortality; deep-water: slow development, low mortality), which both require different management approaches, as each will respond differently to environmental changes. Therefore, the results of this study together with these data suggest that differences in the timing of arrival as well as differences in habitat might work together to influence recruitment.

The differences in extrinsic and intrinsic factors found in this study between early-beach fish and late-deep-water fish may indicate that beach versus deep-water spawning individuals represent different groups within the larger population, as suggested by Davoren and Halden (2014). Regardless of whether this is the case, the early-arriving, larger fish may contribute more to recruitment, as larger females typically have higher fecundity (Winters 1971, Nakashima 1987; cf Penton and Davoren 2013). Therefore, it is important to allow these early fish to spawn first before opening the fishery, to maintain recruitment and the size/age dynamics of the population. This management practice could be easily established through volunteer networks checking beaches for spawning, after which the fishery could be opened.

Overall, the importance of maintaining diversity in the timing of spawning and spawning habitat use within the population will be critical for the persistence of capelin population under future environmental changes (e.g. climate change) and fishing pressures (e.g. exploitation, lack of marine protected areas; Hilborn et al. 2003, Schindler et al. 2010). By a further understanding of the structure of the population, the long-term stability of the population can be predicted and managed appropriately. Identifying the factors that influence the timing of arrival of capelin to the spawning sites in coast Newfoundland further enhances our knowledge of capelin population

dynamics, thereby allowing us to predict effects on higher trophic levels, as well as provide appropriate fisheries management for this critical forage fish species.

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Appendix A

Introduction

There is some indication that larger capelin arrive at coastal spawning grounds in Newfoundland before smaller individuals (Carscadden et al. 1997; Vandeperre and Methven 2007). For instance, Carscadden et al. (1997) showed that delayed spawning at the population-level in the early 1990s was associated with smaller fish length. In addition, a study in the 1980s revealed a linear relationship between timing of arrival and capelin size, with larger capelin arriving inshore first in coastal Newfoundland (Vandeperre and Methven 2007). There are indications, however, that this pattern broke down after the colder sea temperatures in the early 1990's (Nakashima 1996), which was associated with dramatic changes in capelin biology (Carscadden et al. 1997). Owing to these changes, I investigate here whether capelin size decreases linearly as the spawning season progresses, with the goal of comparing these to linear relationships observed pre-1990's (Vandeperre and Methven 2007). Male capelin arrive first at spawning sites in coastal Newfoundland, where they remain throughout spawning to mate with multiple females (Templeman 1948; Friis-Rodel and Kannevorff 2002). Therefore, I predict that larger males will be present earlier in the spawning season, but as the season progresses there will be a mix of small and large individuals, or more variation in length. In contrast, females arrive at sites, spawn and then depart (Friis-Rodel and Kannevorff 2002) and, thus, I predict a stronger linear relationship between female size and timing of arrival.

Methods

Capelin were collected throughout the spawning season (July-August) in 2012 and 2013 on the northeast Newfoundland coast and length, sex, maturity and somatic mass of each fish were

recorded (see Chapter 2 methods). Condition was estimated by calculating Fulton's K, a good condition index for capelin (Carscadden and Frank 2002), using the following equation:

$$\{[\text{total body weight (g)} - \text{gonad weight (g)}] / L^3 \text{ (cm)}\} \times 10^3.$$

Due to variability in the timing of spawning between years, sampling dates were standardized from the first date capelin were sampled, representing the date of first arrival at any spawning site in the study area in each year (e.g. day 1 = first day of arrival), allowing comparison of samples between years, during which the timing of arrival varied by calendar date. Data analyses were restricted to mature (maturity index: 3-4) female and male capelin. Prior to data analysis, I tested length, somatic mass and condition for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). To investigate relationships, I performed linear regressions, with the timing of arrival at spawning sites as the predictor variable and the response variables being length, body mass (somatic), and condition. I then examined the relationship between the residuals of length against timing of arrival to test whether variation in length increased throughout the spawning season.

Results

Length, somatic mass and condition data were non-normally distributed with unequal variances, but multiple data transformations did not rectify this. Owing to the robustness of parametric tests (Quinn and Keough 2002), I used the raw data in regression analyses. Fish length declined significantly as the spawning season progressed in both years for females (2012: $r^2 = 0.05$, $p < 0.0001$; 2013: $r^2 = 0.03$, $p = 0.0005$) and for males in 2012 ($r^2 = 0.08$, $p < 0.0001$) but not in 2013 ($p = 0.2261$; Fig. A1). Similarly, somatic mass decreased as the season progressed in both years for females (2012: $r^2 = 0.14$, $p < 0.0001$; 2013: $r^2 = 0.03$, $p = 0.0009$) and for males in 2012 ($r^2 = 0.03$, $p < 0.0001$) but not in 2013 ($p = 0.7319$). In contrast, no significant trends were observed

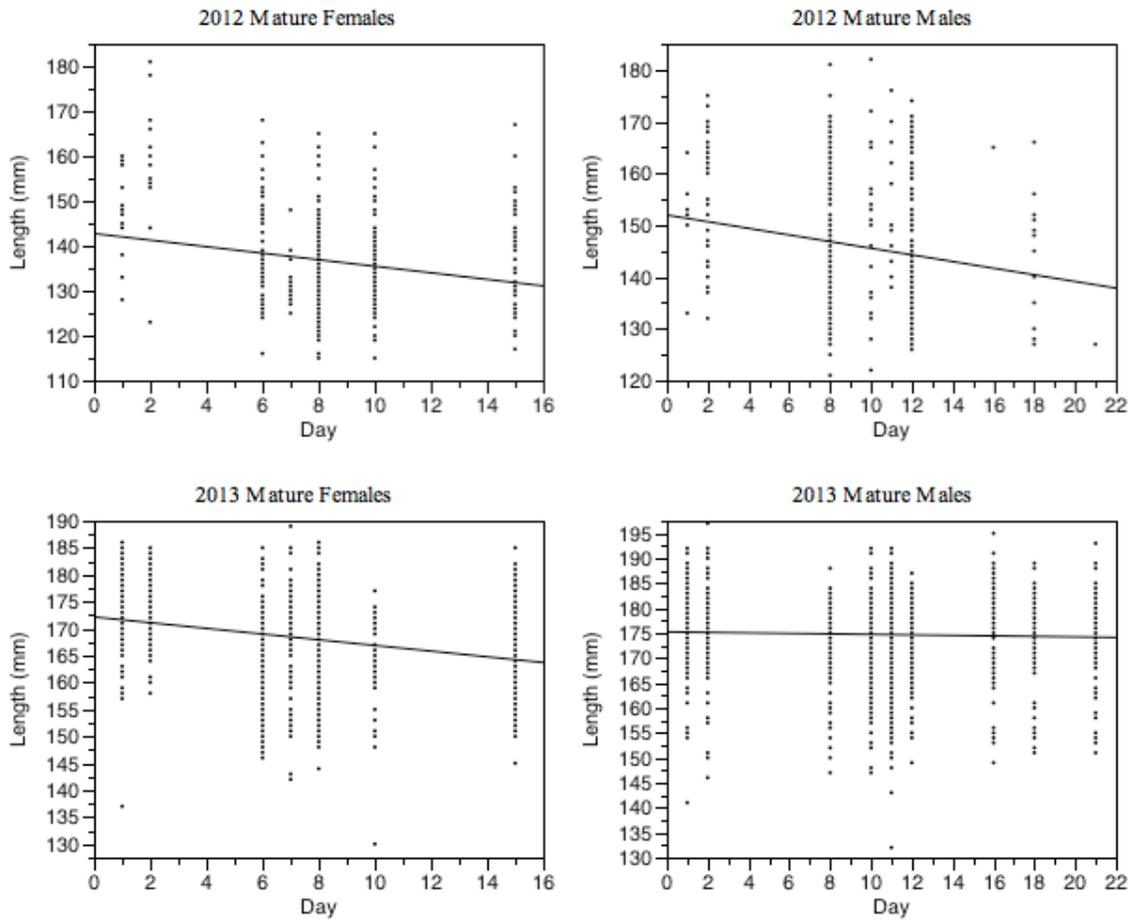


Figure A1. Linear relationships between total length of mature (maturity index: 3-4) female and male capelin collected during spawning in 2012 and 2013 on the northeast Newfoundland coast and the timing of arrival of capelin at spawning sites.

for condition over the spawning season in both years for males (2012: $p = 0.8657$; 2013: $p = 0.1430$) and for females in 2013 ($p = 0.3285$), but condition declined as the spawning season progressed for females in 2012 ($r^2 = 0.02$, $p = 0.0162$). As somatic mass was highly correlated with total length in 2012 (females: $r_p = 0.354$, $p < 0.0001$; males: $r_p = 0.658$, $p < 0.0001$) and 2013 (females: $r_p = 0.952$, $p < 0.001$; males: $r_p = 0.938$, $p < 0.001$), I restricted subsequent analyses of capelin size to length. The residuals of length did not vary significantly with timing of arrival for males and females in either year ($p > 0.05$), as predicted for males.

Discussion

Similar to the pre-1990's study by Vandeperre and Methven (2007), the common trend in fish length was for the largest individuals to arrive first. The largest males and females arrived first, but a mix of medium and small individuals of both sex arrived throughout the remainder of the spawning season, following my prediction for males. This was corroborated by significantly larger capelin arriving early in the season relative to mid- and late-season, but the lack of difference in length of fish arriving mid- and late-season (see Chapter 2). In contrast, the variation in length (i.e. residuals) did not increase significantly as the season progressed for males or females, likely owing to the high variation in fish length within each sample. Trends differed between years for males. A positive linear trend was observed for males in 2012, but not in 2013, corroborating multiple spawning waves in 2012 but possibly only one spawning wave in 2013 (i.e. no progression from larger to smaller fish over the season; see Chapter 2). In contrast, positive linear trends were observed in both years for females.

Similar to Vandeperre and Methven (2007), little of the variation in timing of arrival was explained by fish length (male r^2 : 0.08, female r^2 : 0.03 - 0.05), despite statistically significant trends, likely again due to the high variability in fish length observed throughout the season. This

high variability may be explained by the short spawning season (2-3 weeks) and lifespan (3-6 years) of capelin. For instance, less variable trends are typically observed in long-lived fish species (i.e. 20+ year-old herring, Lambert 1990; 12+ year-old cod, Morgan et al. 2013) and species with longer spawning seasons (i.e. months) and, thus, more distinct spawning waves (e.g. herring, Hay 1985; anchovy, Hunter and Macewicz 1985; cod and haddock, Morgan et al. 2013). Overall, the pattern of larger fish arriving at spawning sites prior to smaller individuals in this study is similar to that shown by Vandeperre and Methven (2007) before the cold-water event in the early 1990's, suggesting that this trend still holds.

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Appendix B

Introduction

Growth rates are known to influence trace element incorporation rates into fish otoliths (e.g. Kalish 1989, Sadovy and Severin 1992, Walther et al. 2010). As larger fish arrived earlier in the season relative to smaller fish (see Appendix A, Chapter 2), differences in otolith trace element concentrations between early and late-arriving fish may be due to divergent growth rates, rather than individuals experiencing different environmental conditions during their final winter and during spawning migration. Owing to this, I explored relationships between the width of the final increment in the otolith, a proxy of fish growth rate during the final year, and otolith Sr and Ba concentrations.

In addition, as individual fish grow, they can eat larger prey items and, thus, their dietary composition can change (Werner and Gilliam 1984). In support, larger fish tend to have higher $\delta^{15}\text{N}$ values, suggesting they are able to select and consume larger prey as they grow (see review in Hobson 1999). Pepin and Dower (2007) found a decrease in $\delta^{13}\text{C}$ with increasing size of larval fishes (including capelin), indicating that fish shift foraging habitat from demersal to pelagic as they grow. Owing to the potential of length-based differences in diet, I examined relationships between fish length and stable isotope signatures to determine if different stable isotope values between early and later arriving fish could be simply due to differences in fish length.

Methods

Prior to regression analyses, I tested length, otolith chemistry in the final increment width and stable isotope values for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). For length-otolith chemistry relationships, I performed an Analysis of Covariance

(ANCOVA) with both years combined to examine if the width of the marginal zone (i.e. final increment width, IW) was positively linearly related to total fish length, while examining differences between males and females, to determine whether larger fish grew more during this period than smaller fish. Second, I performed an ANCOVA to examine if the final IW was positively linearly related to otolith Sr and Ba concentrations, while examining differences between males and females, to determine whether larger fish had higher Sr and Ba concentrations. If there was a significant interaction between males and females (i.e. unequal slopes), then a linear regression was performed on each sex separately. If the interaction was not significant, the ANCOVA was re-run without the interaction term to test whether the y-intercepts differed between males and females. I conducted similar analyses to examine relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and fish length.

Results

Mean otolith Sr concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ met the assumptions of parametric statistics; however, mean Ba was \log_{10} transformed and length was non-normally distributed with unequal variances, but multiple data transformations did not rectify this to meet the assumptions. Owing to the robustness of parametric tests (Quinn and Keough 2002), I used the raw data in regression analyses. The ANCOVA examining the relationship between the width of the marginal zone (i.e. final IW) and total fish length (both years combined) revealed a significant interaction between males and females ($F_{1,180} = 87.940$, $p < 0.0001$; Fig. B1), indicating that slopes were different. When each sex was examined separately, there was no significant relationship between total length and final IW for males ($r^2 = 0.001$, $F_{1,96} = 0.066$, $p = 0.978$) and females ($r^2 = 0.029$, $F_{1,84} = 2.552$, $p = 0.1139$). This indicates that small and large fish had similar growth rates during the final winter and spawning migration, during which I quantified otolith chemistry.

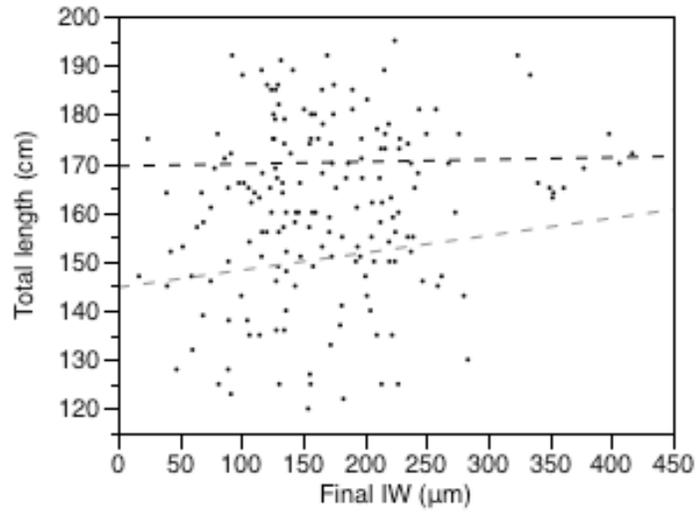


Figure B1. The relationship between total fish length and the final otolith increment width (IW) for male (black) and female (grey) capelin in 2012 and 2013. Dashed lines represent relationships that are not statistically significant.

Second, I examined whether the final IW was related to otolith Sr and Ba concentrations during this period. For otolith Sr concentration, the ANCOVA revealed a significant interaction between males and females ($F_{1,180} = 17.078$, $p < 0.0001$; Fig. B2A) again indicating that the slopes differed. The relationship between Sr concentration and final IW was not significant for either males ($r^2 = 0.005$, $F_{1,96} = 0.530$, $p = 0.46823$) and females ($r^2 = 0.015$, $F_{1,84} = 1.283$, $p = 0.2605$). Finally, the ANCOVA revealed that the relationship between Ba concentrations and final IW was similar for males and females (i.e. slopes did not differ significantly; $F_{1,180} = 2.809$, $p = 0.0955$; Fig. B2B). When the ANCOVA was re-run without an interaction, however, there was a significant difference in y-intercepts, with females being higher than males ($t_{1,181} = 22.41$, $p < 0.0001$). Linear regressions determined that there were no significant relationships between Ba concentrations and the final IW for males ($F_{1,96} = 0.044$, $p = 0.8345$) or females ($F_{1,84} = 1.585$, $p = 0.2116$). Overall, this suggests that growth during the final winter did not differ among fish of different length and that otolith chemistry was not influenced by the minimal differences in growth among fish during this period.

A similar analysis on $\delta^{13}\text{C}$ values revealed a significant interaction between males and females ($F_{1,95} = 20.274$, $p < 0.0001$; Fig. B3A) indicating slopes differed. Linear regressions revealed that there was a significant relationship between $\delta^{13}\text{C}$ and length for both males ($r^2 = 0.573$, $F_{1,51} = 68.496$, $p < 0.0001$) and females ($r^2 = 0.667$, $F_{1,44} = 88.236$, $p < 0.0001$). When length was controlled for, differences in $\delta^{13}\text{C}$ remained between sex ($F_{1,95} = 9.147$, $p = 0.0032$). Therefore, larger individuals might use different habitats relative to smaller individuals. Similarly, an ANCOVA revealed a significant interaction between male and female $\delta^{15}\text{N}$ values ($F_{1,95} = 9.5439$, $p = 0.0026$; Fig. B3B) indicating slopes differed. The relationship between $\delta^{15}\text{N}$ and total length, however, was not significant for males ($r^2 = 0.001$, $F_{1,51} = 0.063$, $p = 0.8037$) or

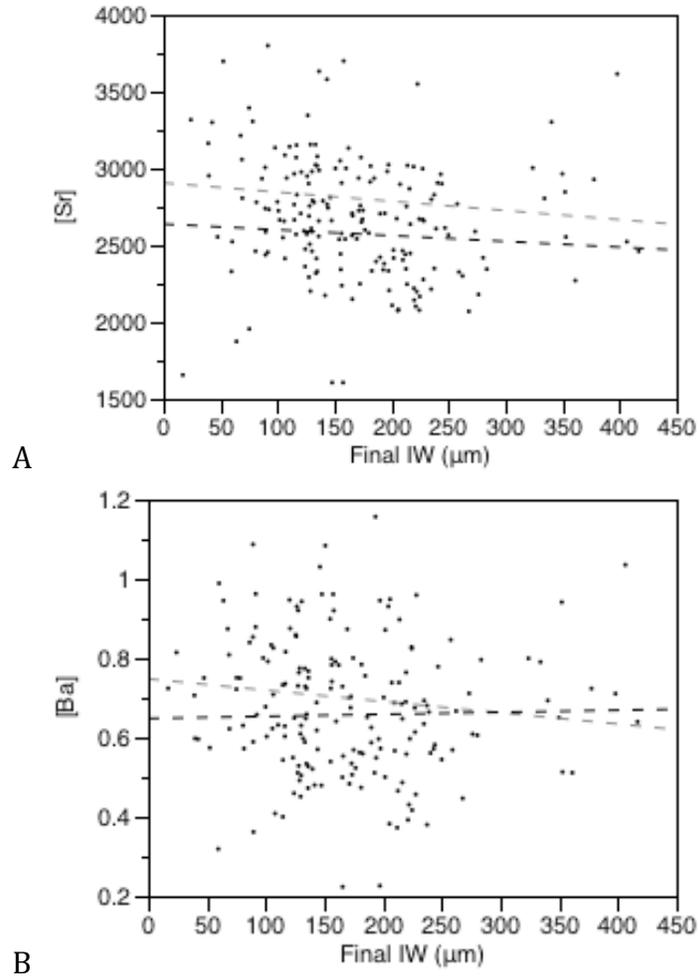


Figure B2. The relationship between the final otolith increment width (IW) and otolith Sr concentrations (A) and otolith Ba concentrations (B) for male (black) and female (grey) capelin in 2012 and 2013. Dashed lines represent relationships that are not statistically significant.

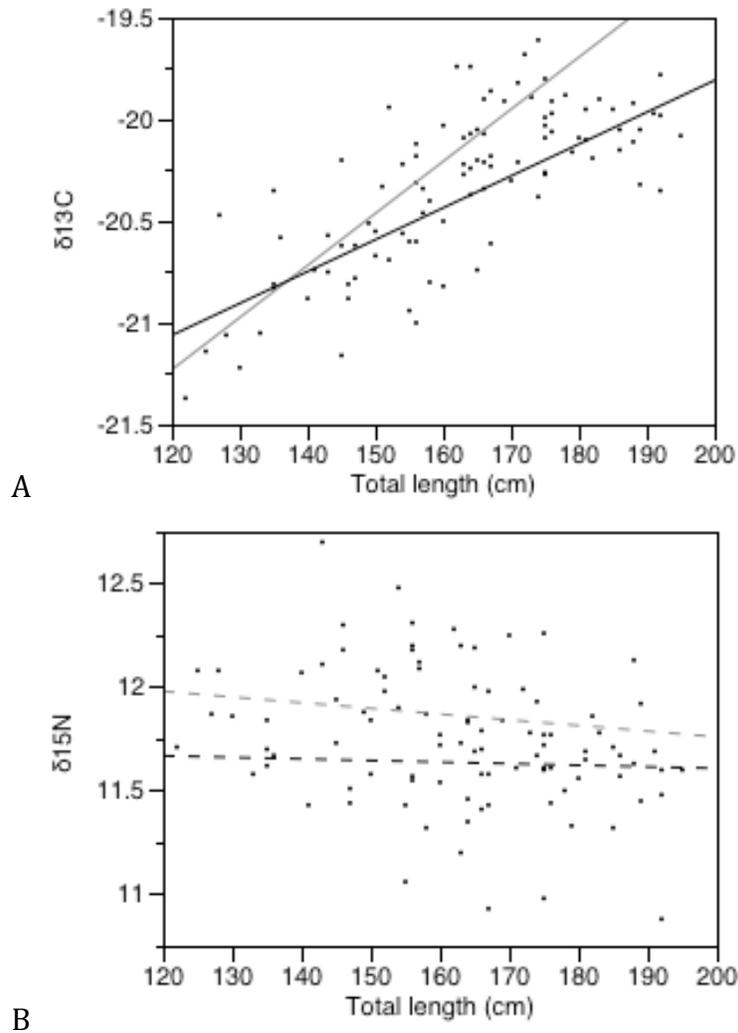


Figure B3. The relationship between $\delta^{13}\text{C}$ values (A) and $\delta^{15}\text{N}$ values (B) and total capelin length of 2012 and 2013 samples combined. The solid lines (A) represent a significant increase in $\delta^{13}\text{C}$ with length. The dashed lines (B) represent relationships that are not significantly different for males (black) and females (grey).

females ($r^2 = 0.019$, $F_{1,44} = 0.871$, $p = 0.3557$).

Discussion

Recent studies have highlighted the importance of physiological regulating factors on the incorporation rates of trace elements into otoliths (e.g. Walther et al. 2010). The otolith increment width in the final winter and spawning migration was similar among all fish, suggesting that there was little variation in growth of maturing fish during this period regardless of size. In addition, the lack of relationships between final IW and otolith Sr and Ba concentrations suggested that otolith chemistry was not influenced by minimal differences in growth during this period for capelin, suggesting that any differences in trace element concentrations within the final IW were likely due to individuals experiencing different environmental conditions.

$\delta^{13}\text{C}$ increased with increasing length for both male and female capelin. This suggests that larger individuals, particularly males, may feed on more benthic prey (C-enriched), whereas smaller individuals feed on more pelagic prey (C-depleted; Pinnergar and Polunin 2000, Sherwood and Rose 2005). Friis-Rodel and Kannevorff (2002) found that Greenland capelin split into two pelagic layers within the water column, with older, larger capelin below (i.e. closer to the seabed) and younger, smaller fish and larvae above, which would explain the trend shown here. Although capelin are more associated with the seabed in Newfoundland (Mowbray 2002, Davoren et al. 2006), both capelin and their prey undergo diel vertical migration (DVM, Davoren et al. 2008), possibly resulting in the use of a mix of pelagic and epibenthic prey resources (Pepin and Dower 2007). Interestingly, there are size-based differences in capelin DVM, with smaller fish remaining closer to the surface and not migrating as deep as larger fish (Davoren et al. 2008), which also may explain the increase in $\delta^{13}\text{C}$ with increasing length shown here.

$\delta^{15}\text{N}$ values did not increase with total fish length in this study, as is commonly observed in other fish species (e.g. Jennings et al. 2001). Indeed, smaller male capelin (<120 mm) are known to feed on copepods (*Calanus finmarchicus*), whereas larger males (>120 mm) fed on larger prey, including euphausiids (*Thysanoessa inermis*) and/or amphipods (*Themisto libellula*; O'Driscoll et al. 2001, Dalpadado and Mowbray 2013). The lack of a relationship between $\delta^{15}\text{N}$ and fish length may be due to the narrow length range sampled in this study, as only mature capelin were sampled (>120 mm). Although amphipods and euphausiids have higher $\delta^{15}\text{N}$ values in comparison to copepods (Stowasser et al. 2012), both copepods and euphausiids can occur in a wide range of development stages and, thus, sizes in capelin diet (O'Driscoll et al. 2001). A combination of these other factors, likely resulted in the lack of variation in $\delta^{15}\text{N}$ values with fish length.

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