

**LIFE HISTORY VARIATION IN CAPELIN (*MALLOTUS VILLOSUS*) – A FORAGE FISH
IN THE NORTH ATLANTIC**

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ABSTRACT

Global temperatures are rising and although there are general predictions about how organisms will respond (e.g. distributional shifts to higher latitudes), the specific response of individual species remains largely unknown. Studies examining the response of a species to environmental variability can provide valuable information for species-specific predictions about responses to climate change. This is especially important for forage fish species that play a large role in food webs because their response to change will influence ecosystem dynamics. Capelin (*Mallotus villosus*) is the key forage fish in Newfoundland and they are sensitive to environmental change, as illustrated by experiencing long-term shifts in their biology after a short-term anomalous event in the early 1990's. Among many changes, this included excursions into deeper water (subtidal) spawning habitats that have drastically different environmental conditions than the beach sites that they have been using since at least the 1940's. This thesis examines various aspects of the reproductive biology of capelin to understand the mechanisms underlying the use of two environmentally divergent spawning habitats.

Local adaptation in early life history stages was examined using a Common Garden Experiment that raised artificially fertilized capelin eggs across three temperatures that capelin are likely to encounter at beach and subtidal spawning sites. The absence of a genotype x environment interaction indicates that capelin are not locally adapted, providing the first support for a facultative spawning strategy. At cool to medium temperatures, larvae experienced higher hatching success, were larger at hatching and had more nutritional reserves relative to warm temperature conditions. The utilization of alternate habitats when they are within this thermal optimum may buffer the

influence of global warming temperatures. Given that there was no evidence of genetic differences at both the early life history and adult levels, an alternate hypothesis was proposed, whereby I conducted a comparative analysis of adult body shape and condition between the two habitats and provided further evidence that spawning habitat use in a given year is facultative.

An examination of fecundity in females revealed that this trait had changed drastically since it was last examined prior to the 1990's. Not only was the average population fecundity lower than in previous years, but a wide range of fecundities were observed across all length-classes. This illustrated that what was once a strong relationship between length and fecundity had broken down, making size an inappropriate proxy for the reproductive potential of this fish.

Higher within-female variation in offspring size and developmental duration compared to among-female variation supports diversified bet-hedging in capelin, a strategy that would allow capelin to spawn in thermally available habitats without experiencing reproductive failure. It is likely that the high variation in traits allows capelin to utilize alternate spawning habitats, ensuring at least some larvae survive.

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CHAPTER ONE: GENERAL INTRODUCTION

Faced with environmental change, plant and animal species will shift their distribution, adapt to their new environment, or go extinct (Holt 1990; Aitken et al. 2008). Shifting current distributional ranges to track the current ecological niche is thought to be the preferable response to increasing temperatures (Wake et al. 2009). Poleward shifts of terrestrial and marine species ranges have been occurring at a rate of 16 km per decade (Chen et al. 2011). Alternatively, species can alter phenology – the timing of seasonal activities such as timing of breeding (Walther et al. 2002) - or adapt to new local conditions in order to occupy the new niche in their current location. Spring phenology is advancing by up to 4.3 days per decade (Burrows et al. 2011) and rapid evolution on a time-scale relevant to climate change has recently been documented for a variety of species with short generation times (Hendry and Kinnison 1999; Freidenburg and Skelly 2004; Franks et al. 2007; Cameron et al. 2013).

Poikilotherms, whose internal temperature varies with that of their environment, are highly susceptible to climate change. In aquatic poikilotherms, water temperature influences almost all aspects of their ecology and biology (Fry 1971; Magnuson et al. 1979). The phenology of events leading to reproduction such as maturation (Ware and Tanasichuk 1989; Kuparinen et al. 2011), migration (Sims et al. 2005; Kovach et al. 2013), and spawning (Hutchings and Myers 1994; Warren et al. 2012) can be modified by varying temperature. Temperature also impacts egg production (Tanasichuk and Ware 1987; Pawson et al. 2000), egg development rates (Pauly and Pullin 1988) and larval mortality (Rankin and Sponaugle 2011), traits that can directly influence reproductive success in fish.

Forage fish are small, short-lived, pelagic schooling fish that transfer large amounts of energy from lower trophic levels to large predators (Pikitch et al. 2012). Due to their rapid growth and a small number of age-classes in the population, marine forage fish are highly susceptible to changes in ocean climate (Alheit et al. 2009). As the key forage fish in the north Atlantic, capelin (*Mallotus villosus*) are a major component in the diets of large finfish predators such as Atlantic cod (*Gadus morhua*; Rose and O’Driscoll 2002; Link et al. 2009), humpback (*Megaptera noaeangliae*) and minke (*Balaenoptera acutorostrata*) whales (Whitehead and Carscadden 1985; Folkow et al. 2000) and seabirds such as common murre (*Uria aalge*; Davoren and Montevecchi 2003). Capelin spend most of their lifespan (3-6 years) feeding in offshore waters until they reach maturity and undergo extensive inshore migrations to spawning grounds (Carscadden and Nakashima 1997). Males spawn multiple times during the spawning season whereas in one spawning event, females express all of their eggs (Templeman 1948; Fridgeirsson 1976). Eggs adhere to the sediment until hatching and egg developmental times are inversely related to temperature (Fridgeirsson 1976; Frank and Leggett 1981). In the northeast Atlantic (Barents Sea and Iceland), capelin spawn in deep-water (demersal sites are up to 75 m deep; Sætre and Gjøsæter 1975) whereas capelin in Newfoundland spawn primarily on beaches along the eastern coast, exposed to the terrestrial environment (Templeman 1948). If beach temperatures become too warm late in the season, capelin in Newfoundland are hypothesized to spawn just off the beach in deeper water (Templeman 1948).

Capelin are considered a “sea canary” for climate change due to their quick response to changes in ocean temperature (Rose 2005). In support of this statement, a

cold-water event in the northwest Atlantic in the 1990's (Colborne et al. 1994; Drinkwater 1996) coincided with many changes in the biology of this important forage fish in Newfoundland. After this event, the offshore distribution of capelin shifted southwards (Frank et al. 1996), spawning capelin were younger and smaller (Carscadden and Frank 2002), and spawning was delayed by approximately four weeks (Shackell et al. 1994; Therriault et al. 1996) due to late maturation (Carscadden et al. 1997). These changes had an impact on predator diet and reproductive success (e.g., seabirds; Carscadden et al. 2002) and were hypothesized to have led to the use of off-beach (deep-water) spawning habitats in the 1990's due to warm beach temperatures when spawning capelin arrived inshore late in the spawning season (Nakashima and Wheeler 2002). Despite warming conditions, the changes in capelin biology in coastal Newfoundland have persisted throughout the 2000's, resulting in unknown spawning biomass and uncertain stock assessment (DFO 2011).

The oceans are warming (Levitus et al. 2000; Cheung et al. 2010), with sea surface temperatures in the north Atlantic predicted to increase up to 4°C over the next century (Hulme et al. 2002). Capelin in Newfoundland are expected to be the first throughout their north Atlantic distribution to respond to climate change (Carscadden et al. 2013) and with stock status being uncertain, understanding present-day capelin biology is imperative. The purpose of my doctoral research was to elucidate the current reproductive biology of the main forage fish in the north Atlantic. I addressed three aspects of capelin reproductive biology that have the potential to influence individual reproductive success:

1) Factors surrounding capelin spawning in two habitats. Although the predominant spawning habitat of capelin in Newfoundland is thought to be beaches, the recent occupation of deep-water spawning in two coastal embayments (Nakashima and Wheeler 2002; Davoren et al. 2007) has been attributed to delayed spawning that began in the 1990's. However, the conspicuous nature of beach spawning and the lack of systematic studies documenting presence/absence of spawning at deep-water sites suggests that the regular use of deep-water spawning sites is not a recent phenomenon.

Spawning site selection and timing of reproduction have been shown to affect offspring fitness in a wide variety of taxa (Einum and Fleming 2000). Differential fitness in alternate habitats can lead to local adaptation, creating reproductively isolated sub-populations (Hendry and Day 2005). Beach and deep-water (sub-tidal and demersal) spawning sites of capelin differ dramatically in environmental features that act on the spawning adult and early life history traits to influence reproductive success and ultimately, evolution. The beach is a highly variable environment, with incubating eggs experiencing unpredictable magnitudes of wave action, inundation with water, solar radiation and oxygen concentrations (Frank and Leggett 1981). Adults that spawn on beaches are often left stranded at high tide (Jangaard 1974). Conversely, the demersal spawning environment is less variable. Adults are not exposed to the terrestrial environment, embryos experience colder but less variable temperature regimes and currents are sufficient to replenish oxygen to the egg layer (Stergiou 1989; Penton 2007).

I addressed questions surrounding whether spawning habitat use by capelin is obligate or facultative and whether there is evidence of adaptation to divergent environmental conditions at beach and demersal spawning habitats in early life history

stages (Chapter 2). Is there evidence of genetic divergence in adults (Appendix I)? Alternatively, is the use of deep-water spawning sites facultative? Do capelin that spawn at the beach have to be in better condition to meet the high energetic demands of spawning in that habitat (Chapter 3)? Are some capelin better equipped to use a particular habitat based on their body shape (Chapter 3)?

In 2009, I successfully raised eggs and larvae from one demersal and two beach sites in environmental chambers representative of temperatures experienced at both spawning habitats in the wild. The absence of a genotype x environment interaction indicated no adaptation in early life history stages. Furthermore, the incubation of eggs at the highest temperature in the experiment was not conducive to the success of early life history stages. This was due to lower hatching success and the production of smaller larvae with lower nutritional reserves. This investigation (Chapter 2) was published in *Journal of Experimental Marine Biology and Ecology* (Penton and Davoren 2013a). In adults, no genetic divergence between habitats was detected using eight microsatellite loci. This study, using fin clips of adult capelin that were collected in 2008 and 2009, was a collaborative effort between myself, Dr. Gail Davoren, Craig McFarlane and Dr. Margaret Docker and is prepared for submission to a journal for publication (Appendix I). Together, the results of these investigations into early life history stages and adults indicated that the use of a particular spawning habitat by capelin in coastal Newfoundland is not obligate.

Based on the rejection of the first hypothesis of obligate spawning habitat use in capelin, I examined a second, alternate hypothesis that fish shape and condition play a role in spawning habitat use by capelin in coastal Newfoundland. Over two years, I

collected spawning adults (females in 2008 and both sexes in 2009) at a beach and deep-water spawning site in a coastal embayment of Newfoundland. I processed these fish for a morphometric and condition-based analysis that revealed sex-based differences in traits between adults using the two spawning habitats. Males differed in body shape, as did female condition, depending on the habitat in which they were collected. I suggest that spawning habitat use may be a trade-off between predation risk and the energy available to maximize individual reproductive success (Chapter 3).

2) *Factors influencing reproductive potential.* Since 1979, capelin in Newfoundland have been managed according to an exploitation rate of no higher than 10% of the estimated spawning stock biomass (SSB) (DFO 2011). Incorporating life history characteristics such as fecundity into management regimes has been shown to be beneficial to the management of cod, a commercially important fish in the north Atlantic (Stares et al. 2007). For capelin, incorporating life history characteristics of spawning adults may become increasingly important because estimates of SSB have been uncertain since 2000 (DFO 2011). At present, capelin are smaller than before the 1990's and some capelin continue to spawn at sub-tidal spawning sites. The impact of this is unknown but can have major implications for stock reproductive potential, as deep-water spawning capelin in the Barents Sea have lower fecundity than beach spawning capelin (Johansdottir and Vilhjalmsen 1999; Christiansen et al. 2008). The last investigation of capelin fecundity in Newfoundland was in the 1980's (Nakashima 1987).

I addressed the following questions about capelin fecundity. Does fecundity differ based on spawning habitat utilized? Have patterns in fecundity changed since it was last

examined in the 1980's, prior to the major oceanographic changes in the 1990's? What factors influence the number of eggs produced by female capelin in coastal Newfoundland? Do simple proxies that are indicative of the reproductive potential of capelin exist for incorporating into the decision-making process made by fisheries managers (Chapter 4)?

In 2008 and 2009, females from beach and deep-water spawning sites were collected from two coastal embayments. Capelin were processed for fecundity, life history traits and condition indices. Capelin fecundity did not differ between spawning habitats. Potential fecundity was not related to life history traits or condition indices. In contrast to studies conducted prior to the 1990's, I report a lower maximum potential fecundity and a weak relationship between fecundity and body size. This investigation (Chapter 4) was published in *Marine Biology* (Penton and Davoren 2013b).

3) *Variability in maternal and early life history traits.* The interannual variability in prevailing oceanic conditions determines the availability of spawning habitats for capelin in coastal Newfoundland. This results in high levels of uncertainty with respect to the thermal conditions encountered by spawning adults and their developing progeny in a given year. During maturation and oocyte development in offshore waters, female capelin cannot account for this uncertainty and must have a strategy for maximizing reproductive success, especially as a short-lived species with up to two spawning years (Shackell et al. 1994; Flynn et al. 2001; Christiansen et al. 2008). In an unpredictable environment, high individual variation in early life history traits can increase fitness (Kassen 2002; Donaldson-Matasci et al. 2008, Marshall et al. 2008). Investigating

individual variability in addition to population means in support of this idea has been gaining attention (Bradford and Roff 1993; Koops et al. 2003; Crean and Marshall 2009; Bolnick et al. 2011) but has not been examined for capelin.

I explored the variability in capelin early life history traits, addressing the following questions. Do individual capelin produce offspring that is consistent with bet-hedging in an uncertain environment (i.e. is variability within individual females greater than among all females; Morrongiello et al. 2012)? How does the mean and variation in egg development time compare to commonly reported metrics (i.e. day of first hatch) used to describe temperature-dependent developmental rates? How do the levels of variability in egg size and larval size at hatch compare to other commercially important marine fish (Chapter 5)?

I examined individual- and population-level variability in capelin early life history traits from the common garden experiment (Penton and Davoren 2013a (Chapter 2)). Within-female variability in traits was higher than among-female variability, consistent with a bet-hedging strategy that increases maternal fitness in an unpredictable environment. When compared with other marine fish species, capelin and other temperate forage fish demonstrated high variability in offspring size but tropical forage fish were not as variable. As environmental conditions are expected to be more variable at higher latitudes (Koops et al. 2003), these findings are consistent with the hypothesis that bet-hedging is common in an uncertain environment.

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CHAPTER TWO: MANUSCRIPT SUMMARY

Penton, P.M., and Davoren, G.K. 2013. A common garden experiment on capelin (*Mallotus villosus*) early life history stages to examine use of beach and deep-water spawning habitats. *Journal of Experimental Biology and Ecology* **439**: 54-60. doi: 10.1016/j.jembe.2012.10.009

For this manuscript, I formulated the question, designed the experiment and sampling protocol and ran the experiment at the Ocean Sciences Centre. Further, I analyzed the data and wrote the manuscript. Dr. Gail Davoren provided financial and logistical support and provided comments on all versions of the manuscript prior to submission.

**A COMMON GARDEN EXPERIMENT ON CAPELIN (*MALLOTUS VILLOSUS*) EARLY
LIFE HISTORY STAGES TO EXAMINE USE OF BEACH AND DEEP-WATER SPAWNING
HABITATS**

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ABSTRACT

Capelin (*Mallotus villosus*) in coastal Newfoundland are known to spawn on beaches but deep-water (demersal) spawning sites in close proximity (<4 km) have recently been documented. Environmental features differ dramatically between the two spawning habitats, creating divergent selection pressures on early life history stages. We test for evidence of local adaptation or facultative spawning using a common garden experiment (CGE) design. Artificially fertilized eggs from two beach sites and one demersal site were incubated across a temperature gradient: 4°C (cold; representative of demersal sites), 7°C (medium) and 12°C (warm; representative of beach sites). Reaction norms did not differ between beach and demersal sites, providing support that capelin are facultative spawners. Irrespective of the habitat adults were collected in, temperatures from 4-7 °C produced larvae with a higher probability of survival due to higher hatching success, length at hatch and nutritional reserves. The ability to behaviourally adjust spawning habitat based on prevailing temperature may be critical to maximize recruitment and minimize population-level impacts of climate change on this key forage fish.

Keywords: capelin, *Mallotus villosus*, reproduction, habitat, facultative spawning, local adaptation

INTRODUCTION

Breeding habitat is critical due to the direct impact local environmental conditions have on the development and growth of young, especially those that remain in the natal area. Climate change and habitat fragmentation are influencing the availability of habitat for many organisms, resulting in the increasing importance of behavioural flexibility, phenotypic plasticity or local adaptation to reduce the risk of extinction (Chevin et al. 2010; Oberle and Schaal 2011). Recent studies report that organisms with short generation times experiencing divergent selection pressures in different habitats can undergo rapid evolution on ecological timescales (e.g. 30-60 generations in guppies, Reznick et al. 1990; <13 generations in salmon, Hendry 2001; 6 generations in finches, Grant and Grant 2006).

Capelin (*Mallotus villosus*) are a small marine schooling fish that are native to the northern hemisphere where they are important as both a commercial and forage species (Carscadden and Nakashima 1997). Most of their short (3-6 yr) life span is spent feeding in offshore waters and when mature, capelin undergo extensive inshore migrations to spawning grounds (Carscadden and Nakashima 1997). Throughout their circumpolar distribution, capelin spawn adhesive eggs at beach and demersal (deep-water) sites where environmental features drastically differ. The beach is highly variable due to unpredictable levels of wave action, inundation with water, solar radiation, salinity, temperature and oxygen concentrations (Davenport et al. 1979; Frank and Leggett 1981; Stergiou 1989; Christiansen et al. 2008; Præbel et al. 2009). Conversely, the demersal spawning environment is less variable (Christiansen et al. 2008) due to colder but less

variable temperature regimes (Penton et al. 2012) and currents that are sufficient to replenish oxygen to the egg layer (Stergiou 1989; Penton and Davoren 2012).

Capelin populations in the northeast Atlantic (Barents Sea and Iceland) occupy demersal spawning beds (Bakke and Bjørke 1973; Sætre and Gjøsæter 1975; Davenport 1989) with the exception of beach spawning capelin in Norwegian fjords (Davenport and Stene 1986; Vilhjálmsson 1994; Christiansen et al. 2008). Beach spawning is thought to be the primary reproductive mode for the northwest Atlantic (Newfoundland and Gulf of St. Lawrence) populations with the exception of the Southeast Shoal; a demersal spawning area located 350 km from shore that is thought to be an ancestral beach (Thors 1981; Carscadden et al. 1989; Stergiou 1989). Demersal spawning in coastal areas also was reported anecdotally (Templeman 1948). It was hypothesized that demersal spawning occurs late in the season in coastal areas when beach temperatures become too warm for spawning (i.e. facultative spawning habitat use; Templeman 1948; Stergiou 1989; Dodson et al. 1991; Nakashima and Wheeler 2002) however, this hypothesis has not been directly tested until recently. Davoren (2012) found that although some mature male capelin (17 %) visited both beach and demersal habitats, the majority (76 %) visited one spawning habitat.

Persistently used beach and demersal sites in close proximity (<4 km) have been discovered in two coastal embayments on the east coast of Newfoundland (Bellevue, Trinity Bay, Nakashima and Wheeler 2002; Lumsden, Notre Dame Bay, Davoren et al. 2007). Egg mortality, developmental rates, hatching success and larval emergence mechanisms differ between these beach and demersal sites (Nakashima and Wheeler 2002; Penton and Davoren 2008; Penton et al. 2012). Adult life history characteristics

(e.g. vertebral number, fecundity and egg size) differ between demersally spawning capelin in the northeast Atlantic and beach spawning capelin in the northwest (Stergiou 1989). Recent investigations reveal that within the northeast Atlantic, beach spawning adults in local fjords have smaller eggs, higher fecundity and are iteroparous relative to demersal spawners (Christiansen et al. 2008). This information together with different selection pressures in the two spawning habitats instead suggests that capelin may be locally adapted to spawning habitat (i.e. obligate spawning habitat use).

To investigate the mechanisms underlying the use of environmentally divergent spawning habitats by capelin, we examine early life history stages of capelin from two coastal regions in Newfoundland in a common garden experiment (CGE). Common garden experiments expose groups of organisms to a gradient of controlled environmental variables, thereby distinguishing between genetic variability and phenotypic plasticity in traits of interest (Beacham and Murray 1986; Hendry et al. 1998; Haugen and Vøllestad 2000; Conover et al. 2006; Hutchings 2011). If spawning habitat use by capelin is obligate, we predict that egg and larval traits will be optimal at temperatures representative of their natal habitat due to local adaptation, manifested as a genotype by environment interaction. Alternatively, if spawning habitat use is facultative, we predict that egg and larval traits from both habitats should be similar at all temperatures but will be optimal at a specific temperature.

MATERIALS AND METHODS

Study area and Sample collection

Capelin were collected in 2009 from Bellevue, Trinity Bay and Lumsden, Notre Dame Bay (Fig. 2.1), the only two regions in coastal Newfoundland where both beach and demersal sites have been documented in close proximity (Nakashima and Wheeler 2002; Davoren et al. 2006). Spawning at Bellevue Beach begins approximately one week earlier than at Lumsden but annual variations in timing of spawning are correlated between the two regions (Davoren et al. 2012). In Trinity Bay, demersal spawning begins after beach spawning with the possibility of overlapping spawning at the two habitats if temperatures are suitable (Nakashima and Wheeler 2002). In Notre Dame Bay, beach and demersal spawning is separated by approximately one week (Penton et al. 2012).

Bellevue Beach is located deep (100 km; Yao 1986) within the northeast oriented Trinity Bay and demersal sites (<20m deep) are located less than 1 km from shore (Nakashima and Wheeler 2002). A modified crab pot (a 1.3 m crab pot wrapped with 16 mm mesh) was deployed from the *Narry Face*, a Fisheries and Oceans Canada research vessel, to collect fish at the demersal site on 24-July. Due to the close proximity of the demersal site to Bellevue Beach, it was important to distinguish between capelin spawning demersally and those migrating to spawn at the beach. Pre-spawning capelin shoals are unlikely to be captured with the modified crab pot due to the dispersed nature of these shoals that are responsive to the presence of incoming gear (i.e. a Remote Operated Vehicle freely descending from the surface; Penton 2007). The ability to capture spawning capelin with the modified crab pot is due to the dense, tight to the

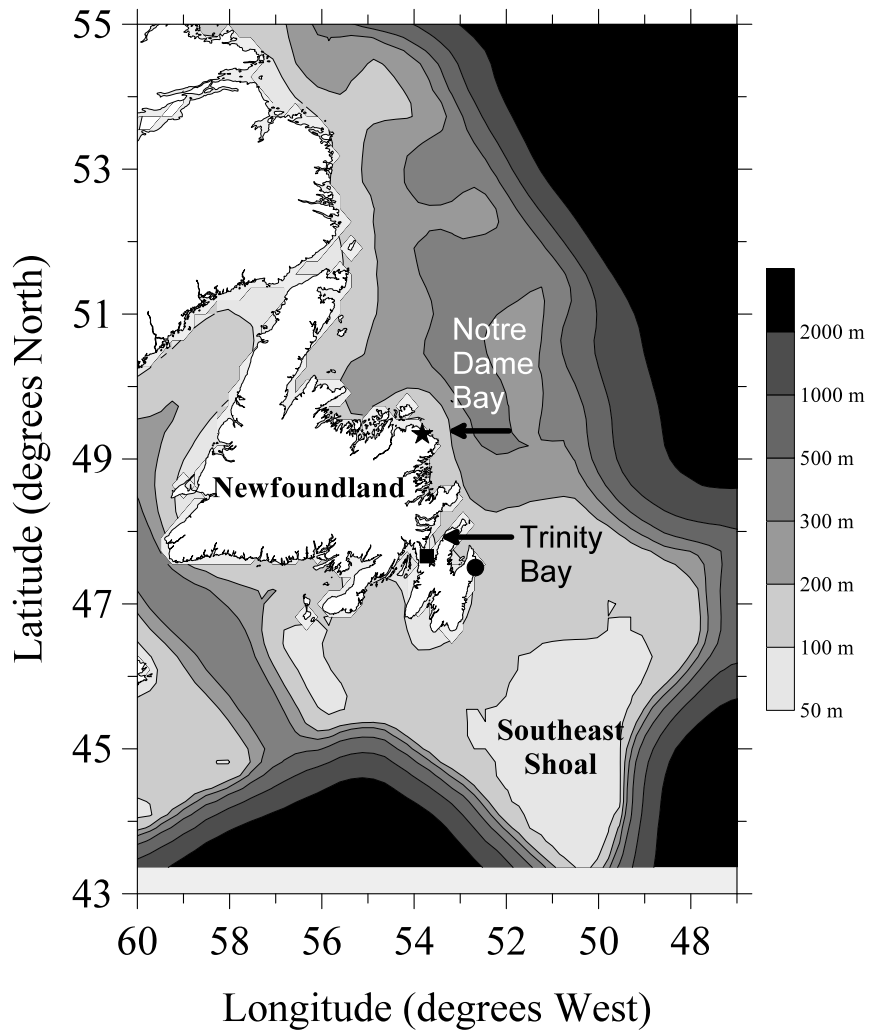


Figure 2.1: Map of Newfoundland with Bellevue Beach, Trinity Bay (*square*), Lumsden Beach, Notre Dame Bay (*star*) and the Ocean Sciences Centre, Logy Bay (*circle*)

seabed, unresponsive nature of the shoal to the equipment (Penton 2007). Based on this, in addition to the presence of eggs at the site and partially spent males and females in the samples, we are confident that the capelin collected were spawning at the demersal site. Capelin in spawning condition were collected from Bellevue Beach on 1-Aug using a 1.8 m cast net with 9.5 mm mesh (Wildco, USA).

Lumsden, Notre Dame Bay is located on the exposed northeast coast of Newfoundland (Fig. 2.1) where demersal sites (<40 m) are less than 4 km from shore (Penton and Davoren 2012). Despite differing depth and distance from shore between the two embayments, spawning sediment and temperatures are similar at demersal sites in both regions (Nakashima and Wheeler 2002; Penton and Davoren 2012). Adults were collected from the beach on 25-July using a 4.8 mm mesh dipnet (Frabill, USA). Capelin did not spawn at the Lumsden demersal sites in 2009, resulting in the common garden comparison of early life history stages from two beach sites (Bellevue Beach-BB; Lumsden Beach-LB) and one demersal site (Bellevue Demersal-BD).

Artificial fertilizations

To ensure that a minimum of six replicates were obtained for the CGE, 12 fertilizations were conducted at each site, crossing gametes from three males with one female for each half-sib group (n = 36 males and 12 females per site). As capelin eggs are adhesive, randomly selected females were dry-stripped into a tray of sterilized sediment (approximately 10 – 30 mm; collected at a known spawning beach (Therriault et al. 1996) and cleaned prior to fertilizations) while milt from three males was dry-stripped into a 500 mL plastic beaker. To activate the sperm, 250 mL UV-sterilized ($36\,000\ \mu\text{Ws}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$)

¹), filtered (0.35 μm) and treated (30 $\text{mg}\cdot\text{L}^{-1}$ Penicillin G and 50 $\text{mg}\cdot\text{L}^{-1}$ Streptomycin sulphate) sea water was added to the beaker. The water was poured over the eggs and sediment, let stand for three minutes and then decanted off. Fertilized eggs from the cross that adhered to the sediment were transferred to 500 mL glass containers with 300 mL filtered, UV-sterilized sea water. The containers were transported to the Ocean Sciences Centre (OSC; Fig. 2.1) by car (total transport time 2-4 h) in a 23 L insulated container with an ambient seawater (collected at the site) bath. Battery operated aerators were placed in each of the glass containers containing fertilized eggs. Males and females used to conduct the fertilizations were individually bagged and frozen for subsequent analysis.

Common Garden Experiments

At the OSC, controlled environmental chambers were maintained at three temperatures: cold (4.2 ± 0.2 (SD) $^{\circ}\text{C}$; representative of incubation temperatures experienced by eggs spawned at demersal sites in Notre Dame Bay; Penton et al. 2012), medium (7.4 ± 0.2 $^{\circ}\text{C}$) and warm (11.7 ± 3.5 $^{\circ}\text{C}$; representative of incubation temperatures experienced by eggs spawned at beach sites). In each chamber, 12 replicate trays held 24 glass jars (118 mL) containing 70 mL filtered, treated, UV-sterilized seawater that were submerged in a water bath to minimize fluctuations in temperature. HOBO temperature loggers (Onset Computer Corp., USA) were placed in a water bath in each chamber to monitor temperatures on an hourly basis throughout the experiment.

Upon returning to the laboratory from each site, eggs from the 12 half-sib groups were placed in the medium temperature chamber and after 48 hours, fertilizations from

each site were examined in random order. Using a stereomicroscope to examine the eggs, fertilization was confirmed by the presence of the formation of the blastodisk (Fridgeirsson 1976; Frank and Leggett 1981). The first six half-sib groups from each site where fertilization was successful were used for the experiment. One piece of sediment (containing 2-20 eggs) from each of the first six successfully fertilized half-sib groups was placed into one of the 118 mL glass jars in each of the 12 replicate trays in each chamber. Twelve replicates from each half-sib group were represented at all three temperatures (Fig. 2.2).

To minimize fungal growth and ensure sufficient oxygen to the incubating eggs, water in the jars was changed every second day and dead eggs were removed from sediment weekly after hatching commenced. Jars were checked daily for hatching and larvae were removed, placed on a microscope slide with a droplet of water that was subsequently siphoned off. Larvae were photographed using a digital camera (Olympus C-7070) mounted to a stereomicroscope (Olympus SZX7). All photographs included a 1 mm scale and ImageJ (v. 1.44; Abramoff et al. 2004) was used to measure the length of the larvae and the diameter of the oil globule. All measurements were taken by the same investigator (P.M.P.). Larvae found dead were not measured due to the 10-20% shrinkage in length associated with death (Litvak and Leggett 1992). The experiment was terminated on 23-Sept, approximately one month after predicted hatching time at the coldest temperature (Frank and Leggett 1981). Upon termination of the experiment, two, one and 25 live eggs remained at the warm, medium and cold temperatures, respectively.

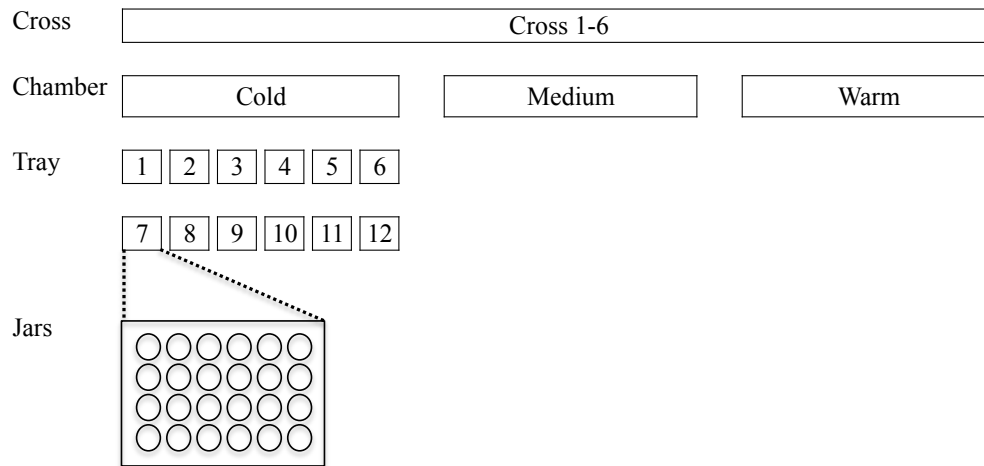


Figure 2.2: Common garden experiment study design schematic. Egg-bearing sediment from six successful crosses (1 female, 3 males) per site were separated into three environmental chambers (cold: 4.2 ± 0.2 °C; medium: 7.4 ± 0.2 °C; and warm: 11.7 ± 3.5 °C). In each chamber, one piece of sediment from each cross ($n = 2-20$ eggs) was placed into one 118 mL jar in each tray ($n = 12$ trays/chamber)

Parental Traits

Adult fish used in the fertilizations were thawed and total length ($L \pm 1\text{mm}$) and somatic weight (Body Mass (BM)-Gonad Mass (GM) $\pm 0.1\text{g}$) were recorded and Fulton's K ($((\text{BM}-\text{GM} \times L^{-3}) \times 10^3)$) was calculated for males and females. To determine egg size, ovaries were placed in glass jars containing modified Gilson's solution (% volume: 1.6 nitric acid [80], 1.9 glacial acetic acid, 10.8 Ethyl Alcohol [60], 85.7 water and 20 g mercuric chloride; Snyder 1983) for 61 - 62 and 211 - 214 days. Gilson's solution is known to cause shrinkage of eggs over time (Lowerre-Barbieri and Barbieri 1993) but there was no difference in egg size between the short (~60 days) and long (~210 days) preservation times (unpaired t-test, $t = 0.71$, $p = 0.49$). Each sample was shaken on a VWR Orbital Shaker for 16 – 112 hours at 150 rpm until eggs separated from ovarian tissue (Nakashima 1987). After eggs were loosened, each sample was poured over a 0.270 mm sieve to remove ovarian tissue. The sample was placed back into the jar and topped up with 70% ethanol for one week to harden the eggs. A random sample of 300-500 eggs were photographed and the diameter measured using ImageJ (v. 1.44; Abramoff et al. 2004). All photographs included a 1 mm scale and were measured by a single investigator (E.M.). If the sample appeared to be damaged (i.e. broken egg cases, discoloured or misshapen, etc.), the sample was not included in the analysis.

Statistical Analysis

With the exception of hatching success, all statistical analyses were based on half-sib group means ($n=6/\text{site}$) at each temperature (Hendry et al. 1998), using the average

trait values of larvae from each jar (n=12/half-sib group/temperature) as the replicate (range: 1 – 11 larvae/jar). Due to the unknown number of eggs in each jar at the start of the experiment, hatching success for each half-sib group was expressed as the proportion of replicate jars in which at least one larvae hatched at each temperature. These proportion data were arcsin transformed prior to statistical analysis.

A nested mixed-model analysis of variance (ANOVA) was used to determine the effects of temperature and site on time to hatch, hatching success, and length and oil globule volume at hatch using the following model:

$$Y = \mu + \text{temperature} + \text{site} + \text{half-sib group (site)} + \text{site} \times \text{temperature} + \varepsilon$$

Where Y is the trait of interest, μ is the group mean, temperature (cold, medium and warm) and site (BB, BD and LD) were set as fixed effects and half-sib group (i.e. cross), nested within site, was set as a random effect and ε is the error term. A significant interaction term (site x temperature) indicates different reaction norms and, thus, variation among sites due to genetic variability rather than phenotypic plasticity (Conover and Schultz 1997; Darwish and Hutchings 2009; Hutchings 2011). A single factor ANOVA was used to examine if parental traits varied among sites.

Prior to analyses, all traits were tested for normality and homogeneity of variance to meet the assumptions of linear models. Post-hoc comparisons were made using the Scheffe's test at a significance level of 0.05. All data analyses were generated using SAS software, Version 9.2 of the SAS System for Windows. Copyright © 2000 SAS Institute Inc., Cary, NC, USA.

RESULTS

Due to a lack of spawning at demersal sites in Notre Dame Bay in 2009, common garden experiments compared three sites: Bellevue Beach (BB), Bellevue Demersal (BD) and Lumsden Beach (LB). Across all temperatures, 541, 154 and 165 eggs hatched from Bellevue Beach (BB), Bellevue Demersal (BD) and Lumsden Beach (LB), respectively. Shrinkage upon death excluded 77 (BB), 33 (BD) and 28 (LB) larvae from length and oil globule volume measurements. Eggs did not hatch from one BB half-sib group at the warm treatment and from one BD half-sib group across all temperatures.

Parents used for the crosses did not differ significantly among sites in length, somatic weight or condition (Table 2.1) and average egg size of the females (0.70 ± 0.01 mm) did not differ among the three sites (one way ANOVA; $F_{[2, 12]} = 1.01$, $p = 0.3997$). There was no significant temperature by site interaction for any of the early life history traits investigated (Table 2.2), indicating that temperature reaction norms did not differ across sites due to genetic variability (Fig. 2.3). Therefore, we summarize below significant differences among sites and temperature treatments using within and among temperature Scheffe's post-hoc pairwise comparisons at a significance level of $p = 0.05$.

Patterns in hatching success and time to hatch were influenced by temperature and site (Table 2.2). Hatching success, expressed as presence-absence of larvae in each of the 12 replicates for each half-sib group at each temperature, was lowest at warm temperatures (Table 2.2, Fig. 2.3a). Elevation of hatching success reaction norms was significantly different among sites, with BB half-sib groups having higher hatching success at all temperatures (Fig. 2.3a). A decrease in time to hatch with increasing temperature occurred at BB only. At the warm temperature, LB and BD eggs took longer

Table 2.1: Mean (\pm SE) total length, somatic weight, condition (Fulton's K) of female and male capelin used in crosses as each site (Bellevue Beach-BB; Bellevue Demersal-BD; and Lumsden Beach-LB) for common garden experiments.

| Sex | Site | Variable | | |
|---------|----------------------|-------------------|--------------------|-----------------|
| | | Total length (cm) | Somatic weight (g) | Fulton's K |
| Females | BB (n=5) | 14.1 \pm 0.5 | 9.34 \pm 1.00 | 3.23 \pm 0.18 |
| | BD (n=6) | 14.1 \pm 0.6 | 9.77 \pm 1.29 | 3.49 \pm 0.46 |
| | LB (n=6) | 15.3 \pm 0.3 | 12.17 \pm 0.59 | 3.44 \pm 0.11 |
| | F _(2, 16) | 2.41 (NS) | 2.31 (NS) | 0.19 (NS) |
| Males | BB (n=18) | 15.7 \pm 0.2 | 20.73 \pm 0.84 | 5.20 \pm 0.19 |
| | BD (n=17) | 15.5 \pm 0.2 | 19.75 \pm 1.00 | 5.18 \pm 0.28 |
| | LB (n=18) | 16.0 \pm 0.2 | 21.93 \pm 0.90 | 5.65 \pm 0.27 |
| | F _(2, 52) | 1.01 (NS) | 1.41 (NS) | 1.19 (NS) |

Table 2.2: Summary statistics (F-values) for nested mixed-model ANOVA of influence of site and temperature on time to hatch, length at hatch, oil globule volume at hatch and hatching success. Site and temperature were set as fixed effects and half-sib group was set as a random effect.

| Effect | df | Trait | | | |
|-----------------------|----|---------------|-----------------|--------------------|------------------|
| | | Time to hatch | Length at hatch | Oil globule volume | Hatching Success |
| Temperature | 2 | 39.82* | 5.5* | 7.15* | 10.78* |
| Site | 2 | 8.99* | 1.07 | 6.11* | 23.20* |
| Half-sib group (Site) | 14 | 2.70* | 1.61 | 1.37 | 6.29* |
| Site x Temperature | 4 | 2.76 | 1.42 | 0.64 | 0.80 |

* $P < 0.05$

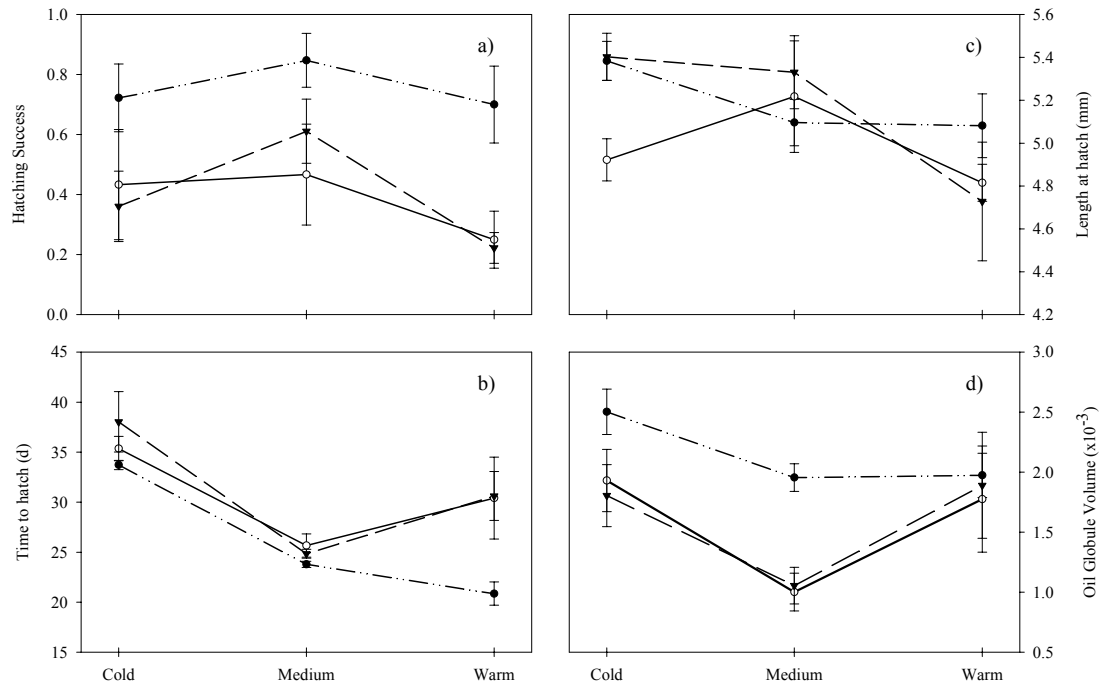


Figure 2.3: Reaction norms for a) hatching success, b) time to hatch, c) length at hatch, and d) oil globule volume at hatch for capelin (*Mallotus villosus*) crosses from Bellevue Beach (BB-closed circle, dashed and dotted line), Bellevue Demersal (BD-open circle, solid line) and Lumsden Beach (LB-triangle, dashed line) reared at three temperatures (cold: 4.2 ± 0.2 °C; medium: 7.4 ± 0.2 °C; and warm: 11.7 ± 3.5 °C). Data presented are site means \pm SE for each treatment.

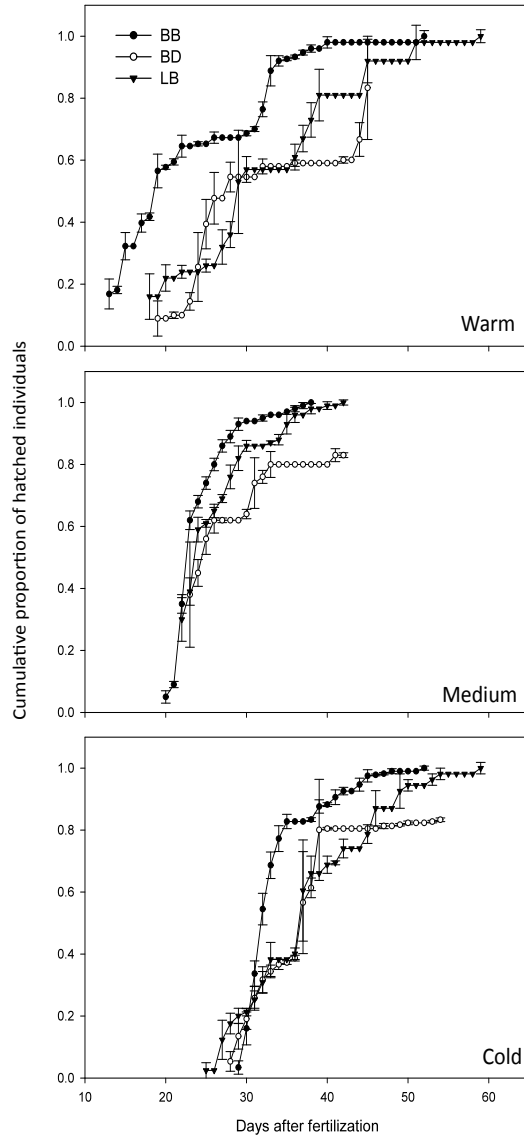


Figure 2.4: Cumulative proportion of hatched capelin eggs for Bellevue Beach (BB-*closed circle*), Bellevue Demersal (BD-*open circle*) and Lumsden Beach (LB-*closed triangle*) reared at three temperatures; cold (4.2 ± 0.2 °C), medium: (7.4 ± 0.2 °C) and warm (11.7 ± 3.5 °C). Hatching proportions are less than 1.0 in some cases due to half-sib groups that did not hatch

to hatch than at the medium temperature (Fig. 2.3b). Across all sites, hatching was synchronized at the medium temperature, with the majority of eggs (>60%) hatching within four days of the first hatched egg (Fig. 2.4). In contrast, hatching at the cold (25 – 59 d) and warm (13 – 59 d) temperatures was more protracted, consisting of two or more hatching peaks (Fig. 2.4).

Across all sites, larvae were smallest at the warm temperature (Fig. 2.3c) and oil globule volume was smallest at the medium temperature (Table 2.2, Fig. 2.3d). Within each temperature, length of larvae did not differ significantly among sites (Table 2.2, Fig. 2.3c). Oil globule volume was larger in BB larvae with the exception of the warm temperature where there were no differences among the sites (Table 2.2, Fig. 2.3d).

DISCUSSION

We provide experimental evidence that capelin early life history stages are not locally adapted to the thermal environment at beach and demersal sites. The lack of genetically-based differences support the facultative spawning strategy (Templeman 1948; Stergiou 1989; Dodson et al. 1991; Nakashima and Wheeler 2002), whereby fish spawn under thermal conditions suitable to meet the physiological requirements of both the spawning adults and critical early life history stages. We suggest that by spawning at both beach and demersal sites if temperature conditions are suitable, recruitment may be improved through the production of offspring that have variable traits at multiple locations and times (Lambert and Ware 1984). Favourable incubation temperatures are critical because they influence the characteristics that promote/suppress larval mortality rates, which can exceed 60% per day in capelin (Taggart and Leggett 1987).

Larvae raised at temperatures between 4 and 7°C will likely have a higher probability of survival due to their large size and higher nutritional reserves. Larger larvae are thought to confer a fitness advantage due to their lower susceptibility to predation, one of the main contributors to mortality of early life history stages (Hutchings 1997). Although visual vertebrate predators (*Gasterosteus aculeatus*) select larger capelin larvae due to increased perception, encounter rates and caloric value (Litvak and Leggett 1992; Pepin et al. 1992; Bertram 1996), overall mortality rates of cohorts of larger capelin are lower. This is due to decreased probability of capture with increasing larval size (Pepin et al. 1992) and the lower number of larvae required to satiate vertebrate predators (Elliott and Leggett 1996). Further, the synchronous hatching at 7 °C may swamp predators, possibly conferring an additional survival advantage. Larger nutritional reserves upon hatching is also important because it reduces mortality due to predation (Frank and Leggett 1982) and is positively correlated with post-hatching lifespan (Chambers et al. 1989). By spawning between 4 and 7°C , whether that be at the beach or demersal habitat, capelin can produce large numbers of larvae with qualities that are known to offer higher probabilities of survival.

Capelin is considered a sea “canary” for climate change (Rose 2005) due to the impact of variations in temperature on many aspects of their biology (Stergiou 1991; Gjørseter 1998; Mowbray 2002; Carscadden and Frank 2002; Orlova et al. 2010). To cope with climate change and minimize the risk of extinction, species are expected to shift their distribution or adapt to new conditions (Hill et al. 2011). Capelin in the northwest Atlantic are predicted to experience a northward shift in range of 400 – 1800 km due to climate change (Rose 2005). We have demonstrated that the early life history

stages, suspected to be the most sensitive to climate change (Van Guelpen et al. 2007), do not exhibit adaptation. The ability to behaviourally seek refuge from warm conditions when necessary by spawning at demersal sites may prevent the need for extensive range shifts due to climate change.

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**CHAPTER 3: BODY SHAPE AND CONDITION RELATED TO SPAWNING HABITAT
USE BY CAPELIN (*MALLOTUS VILLOSUS*) IN TRINITY BAY, NEWFOUNDLAND**

ABSTRACT

Capelin (*Mallotus villosus*) in coastal Newfoundland are known to spawn on beaches but have been discovered spawning, often simultaneously, at demersal sites in the nearby (< 4 km) sub-tidal zone. Due to the lack of both a physical barrier and evidence of local adaptation to spawning habitats, we investigated the intrinsic factors that may influence habitat use. We found both morphometric (male) and condition-based (females) differences in spawning site use. Sex-based differences in traits reflect differing spawning behaviour – males that spawn multiple times throughout the season require a different set of traits to maximize lifetime reproductive success than females that spawn all eggs in one event. The occupation of beach habitat by large males with deep forked tails and females in good condition suggests that the beach must be a high quality habitat in some way. This may be due to lower predation risk on spawning adults despite higher physical demands of spawning relative to demersal sites. We speculate that capelin may select spawning habitat based on a trade-off between predation risk and energy available to maximize lifetime reproductive success.

INTRODUCTION

The choice of breeding habitat influences an individual's fitness through its direct impact on the survival of breeding adults and their developing progeny (Wootton 1998; Hendry et al. 2001; Krebs 2001). The occupation of particular breeding habitats may be influenced by fitness-related extrinsic stimuli, such as environmental cues (e.g. temperature and oxygen; McRae et al. 2012), conspecific density (Fretwell and Lucas 1970) and/or predator density (Suhonen et al. 1994; Claramunt et al. 2005). Intrinsic factors can also influence the choice of breeding habitat, with differences in size, body condition or morphology contributing to the use of a particular habitat (Facey and Grossman 1992; Blair et al. 1993). Sockeye salmon (*Oncorhynchus nerka*) that undergo long, difficult migrations up into rivers have higher energy densities, lower fecundity and small, fusiform bodies to reduce energetic costs relative to those salmon that have shorter migrations at lower elevations (Crossin et al. 2004). In some watersheds, sockeye salmon spawn in streams, as well as at nearby beaches. In contrast to males that spawn at beaches, males spawning in the streams have fusiform (streamlined) body shapes to allow more efficient swimming in faster flowing water, the ability to access more areas, and the prevention of stranding and decreased susceptibility to terrestrial predators (Quinn and Foote 1994; Hendry and Quinn 1997; Hendry et al. 2000).

Capelin (*Mallotus villosus*) are an important forage fish in the north Atlantic ecosystem (Carscadden and Vilhjalmsson 2002). In the northeast Atlantic, capelin are primarily deep-water spawners (Bakke and Bjørke 1973; Sætre and Gjørseter 1975; Davenport 1989) whereas capelin typically spawn on beaches on the eastern coast of Newfoundland. At the beach, capelin actively swim onto shore with incoming waves, at

which time they are left exposed in the terrestrial environment as they extrude eggs and sperm. If they are unable to swim out with the next wave, capelin can become stranded on the beach at high tide. In contrast to high wave action at the beach, current speeds at deep-water sites are low ($< 30\text{cm/s}$; Penton and Davoren 2012) but adults are more vulnerable to predation than at the beach, due to continuous exposure to high concentrations of predators such as humpback whales and many species of seabirds (Davoren 2007). Despite the physical demands of driving themselves onto and off of a terrestrial environment with variable wave action, the beach provides a refuge from predation for both the eggs and adults, often thought to be one of the primary advantages for beach spawning fish (DeMartini 1999).

The general spawning act is the same at both spawning habitats throughout their distribution. Males form dense aggregations near the spawning beds after extensive inshore migrations. When ripe (sexually mature and ready to reproduce), females join the males at the spawning beds and engage in the spawning act that lasts only seconds (Vilhjálmsón 1994). Spent (completed spawning) females leave the spawning areas while males rejoin the spawning school to complete several matings during the season (Fridgeirsson 1976). Capelin were typically thought to be semelparous (spawn during one season and die) but recent evidence for iteroparity (surviving to spawn in another year) in both male and female capelin (up to 13 and 50%, respectively; Shackell et al. 1994) is accumulating (Flynn et al. 2001; Christiansen et al. 2008).

Both extrinsic (i.e. temperature and wave height; Therriault et al. 1996; Carscadden et al. 1997; Davoren et al. 2012) and intrinsic (i.e. size; Carscadden et al. 1997; Vandeperre and Methven 2007) factors have been investigated to explain timing of

spawning in capelin. To date, spawning habitat use has only been examined in relation to extrinsic factors, with beach orientation (Nakashima and Taggart 2002), sediment size (Vilhjálmsson 1994; Nakashima and Taggart 2002; Penton and Davoren 2012) and temperature (Carscadden et al. 1989; Penton and Davoren 2012) identified as important factors.

In coastal Newfoundland, capelin spawn primarily on beaches but deep-water sites (less than 4 km from spawning beaches) have been reported in two coastal embayments (Nakashima and Wheeler 2002; Davoren et al. 2008). Both the beach and deep-water spawning sites are used (Nakashima and Wheeler 2002) but there is no evidence of genetic differentiation in adults (Appendix I) or local adaptation in early life history stages (Chapter 2 (Penton and Davoren 2013a)). Since individuals can presumably spawn in either habitat, this provides a unique opportunity to investigate intrinsic mechanisms of spawning habitat use.

The goal of this study is to examine intrinsic factors influencing spawning habitat use by investigating whether capelin spawning at nearby beach and demersal habitats in coastal Newfoundland possess a particular suite of traits that characterize habitat use. To better navigate the wave action and terrestrial exposure that is typical of beach spawning sites, we expect that capelin found spawning at the beach will (1) be in better condition and, (2) have body shape characteristics related to higher swimming ability than those that spawn at deep-water sites. Furthermore, we expect that any differences would be more pronounced in males because they spawn multiple times throughout the season whereas females extrude all eggs in one spawning event.

MATERIALS AND METHODS

Sampling

Trinity Bay is one of the two coastal regions where beach and demersal spawning are known to co-occur in Newfoundland (Fig. 3.1). Female capelin in spawning condition were sampled from one beach and one demersal site over two years and males were sampled at both habitats in 2009 only (Table 3.1). Spawning male and female capelin were collected at Bellevue Beach using a 6' cast net with 9.5 mm mesh (Wildco). At the beach in 2008, spawning started on 13-July and peaked the following day. Our collections of spawning females were made one day following peak spawning (Table 3.1). In 2009, spawning began at the beach on 20-Jul and peaked on 3-Aug. One hundred females were collected over two days, separated by more than a week (Table 3.1) because females on the first sampling date moved off the beach and were not accessible using the cast net. One hundred males were also collected in 2009 at the beach on 23-Jul. At the Bellevue demersal site, spawning capelin were collected using a modified crab pot (a 3' crab pot wrapped with 16 mm mesh) deployed from the Fisheries and Oceans research vessel, the *Narry Face*. At the deep-water site in 2008, 100 females were collected on 19-Jul and in 2009, 25 females and 100 males were collected on 24-Jul (Table 3.1). Fish were placed in individual bags and immediately frozen until processed in the laboratory, within nine months of collection. Spent fish (e.g. slack stomach or eggs coming out of the genital papilla) and samples that exhibited body torsion due to the freezing process were excluded from the analysis.

As the size of spawning capelin may change throughout the spawning season (e.g. Vandepierre and Methven 2007), we collected samples within both habitats at a similar

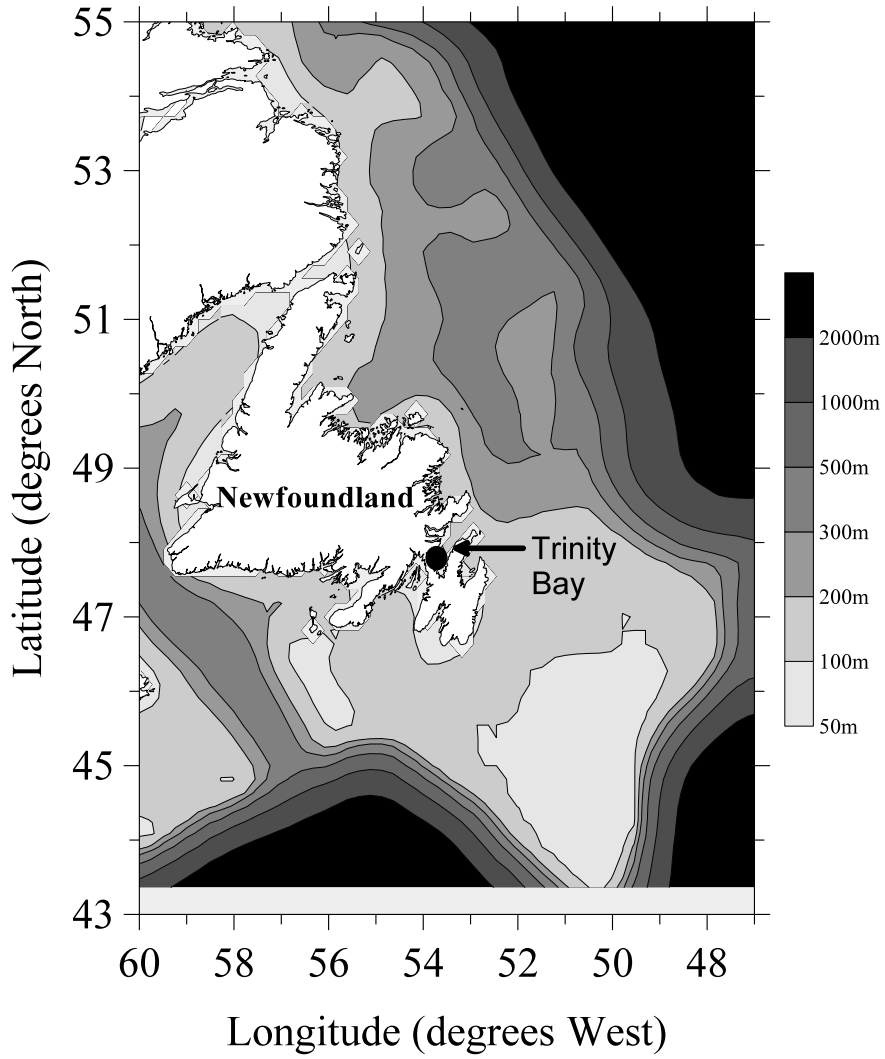


Figure 3.1: Map of Newfoundland with Trinity Bay and Bellevue area (*black circle*) indicated

Table 3.1: Summary of male and female capelin (*Mallotus villosus*) collected at beach and deep-water spawning habitats in Trinity Bay, Newfoundland in 2008 and 2009.

| Year | Habitat | Collection Date | Females | Males |
|------|------------|-----------------|---------|-------|
| 2008 | Beach | 15-Jul | 100 | N/A |
| | Deep-water | 19-Jul | 100 | N/A |
| 2009 | Beach | 23-Jul | 22 | 100 |
| | | 01-Aug | 78 | 0 |
| | Deep-water | 24-Jul | 25 | 100 |

N/A = males were not collected in 2008

time. The modified crab pot used at the demersal sites likely samples spawning fish only because spawning shoals at demersal sites are dense, tight to the seabed and unresponsive to equipment relative to more pelagic and responsive migrating shoals (Penton 2007). Combined with the presence of capelin eggs and spent capelin in the samples, samples from the crab pot primarily consisted of capelin spawning at the demersal site rather than those travelling to spawn at the beach.

Morphometrics

Thawed fish were placed on parchment paper on top of a Styrofoam board and pins were inserted at 16 landmarks on both male and female capelin (Roby et al. 1991, Fig. 3.2). All photographs were taken of the left side of the fish and included a reference ruler and label. TpsDig Software (v. 2.12 © Rohlf 2008) was used to obtain X and Y coordinates for the 16 landmarks at the insertion point of the pin in each photograph (Fig. 3.2), processed in a random order by the same investigator (P.M.P.). A truss network of 24 distances (Fig. 3.2) was calculated by transforming inter-landmark distances to linear distances in Excel using Pythagorean Theorem (Turan 1999).

Principal Component Analysis (PCA) - a multivariate analysis with no *a priori* grouping that reduces variables into a smaller number of components - was used to examine the truss network for potential body shape differences among spawning habitats. Principal components (PC) were extracted from the correlation matrix and those with an Eigenvalue greater than one were retained for future analysis (Quinn and Keough 2002; O'Rourke et al. 2005). The retained components were subjected to an orthogonal rotation to improve interpretability (Quinn and Keough 2002; O'Rourke et al. 2005). With

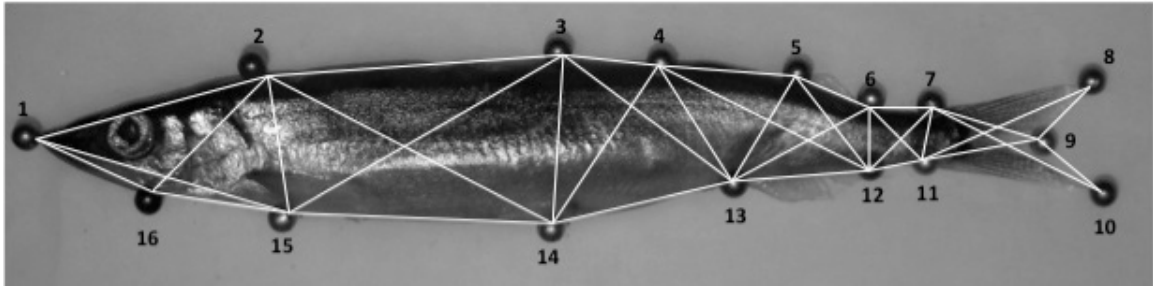


Figure 3.2: Location of 16 landmarks for a truss network (numbers) and morphometric distances (lines), indicated on a sexually mature female capelin. Landmarks include: (1) anterior tip of snout at lower jaw, (2) most posterior aspect of neurocranium (beginning of scales), (3) origin of dorsal fin, (4) insertion of dorsal fin, (5) origin of adipose fin, (6) insertion of adipose fin, (7) anterior attachment of dorsal membrane from caudal fin, (8) most posterior-dorsal point of naturally extended caudal fin, (9) base of middle caudal rays, (10) most posterior-ventral point of naturally extended caudal fin, (11) anterior attachment of ventral membrane from caudal fin, (12) insertion of anal fin, (13) origin of anal fin, (14) insertion of pelvic fin, (15) insertion of pectoral fin, and (16) most posterior point of maxillary.

rotation, any medium variable scores are adjusted so that they load either high or low on a component (Mazlum et al. 1999; Quinn and Keough 2002). A variable was said to load on a component if the loading was ≥ 0.3 (Elliott et al. 1995; Kocovsky et al. 2009).

Significance of differences were tested using a one-way (habitat) Analysis of Variance (ANOVA) on scores of each of the retained principal components (Janhunen et al. 2009).

Linear discriminant function, a multivariate analysis with *a priori* grouping, was used to test for site membership and to determine the percent of correctly classified individuals according to the significant truss variables by cross-validation classification.

Life history traits

After photographing the fish for morphometrics, total length ($L \pm 1$ mm), body mass ($BM \pm 0.1$ g) and ovary weight ($OWt \pm 0.1$ g) were measured for each fish. Somatic weight ($Wt - OWt \pm 0.1$ g), and Fulton's K ($(Wt - OW \cdot L^{-3}) \cdot 10^3$) were calculated for males and females, and Gonadosomatic Index ($GSI: OWt \cdot Wt^{-1} \cdot 100$) was calculated for females (McIntyre and Hutchings 2003). Prior to analyses, all traits were tested for normality and homogeneity of variance. Life history traits and condition indices were log transformed to meet the assumptions of linear models. All data analyses were generated using SAS software, Version 9.2 of the SAS System for Windows.

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RESULTS

Morphometrics

Sexes and years were analyzed separately due to sexual dimorphism of mature capelin and significant differences between years (one way ANOVA (year); $p < 0.0001$ for all measurements). Redundant measurements in the truss network are indicated by a high correlation (≥ 0.90) between the variables (Elliott et al. 1995). For females in 2008, eight variables (1-2, 2-14, 3-13, 3-14, 4-13, 5-13, 7-9 and 7-10) were considered redundant and removed from the analysis. Two variables (1-2 and 5-13) were removed from the analysis of females in 2009 and four variables (1-2, 3-13, 3-14 and 5-13) were removed from the analysis of males in 2009 due to redundancy.

In principal components analysis (PCA) of morphometric data, a shape-independent size component often exists, consisting of variable loadings that are similar in magnitude and direction (Mosimann 1970; Kocovsky et al. 2009). Variation was reduced to a single principal component (PC1) with loadings of similar magnitude and direction in 2008 females (range: 0.19-0.27), 2009 females (range: 0.15-0.24) and 2009 males (range: 0.17-0.25). The size components explained 76.01, 59.05 and 67.44% of the total variance in each analysis, respectively. Once size was accounted for in PC1, the remaining variation is due to shape (Jolicoeur and Mosimann 1960; Reyment et al. 1984).

For females in 2008, only one shape component had an eigenvalue greater than one (1.05), accounting for 6.6% of the total variation. Truss variables 8-9, 9-10 and 9-11 (Fig. 2) loaded on PC2, representing caudal fin shape. These differences could not be attributed to spawning habitat according to ANOVA results ($F_{1,154} = 0.66$, $p = 0.420$) and high misclassification rates (23.7-55.7%). Similarly, an ANOVA of PC scores in 2009

could not distinguish among females spawning in different habitats based on PC2 ($F_{1,93}=3.12$, $p=0.081$) and PC3 ($F_{1,93}=2.58$, $p=0.112$), which explained 7.0 and 5.7% of the total variance, respectively. PC2 was associated primarily with body depth at the position of both the pectoral (2-15) and pelvic (3-14) fins, as well as with the length of the dorsal caudal fin from the fork in the tail (8-9; Fig. 2). PC3 was also associated with one truss variable (9-11) describing caudal fin shape. Misclassification rates based on discriminant function analysis ranged from 1% for beach ($n=1$) to 100% for demersal ($n=19$).

For males, an ANOVA of PC2 scores was significant ($F_{1,155}=6.59$, $p=0.011$), distinguishing between beach and demersal body shape. PC2 explained 5.4% of the variation in body shape and was associated with the shape of the caudal fin, including measurements from the fork to the posterior point of the dorsal (8-9) and ventral (9-10) caudal rays and from the post-ventral point of the caudal fin to the anterior attachment of the dorsal caudal fin membrane. Beach spawning males had deeper forked tails than those spawning at demersal sites. PC2 was also associated with the distance running transversely from the posterior neurocranium to the insertion of the pelvic fin (2-14; Fig. 3.2). These traits resulted in classification rates of 63.9% ($n=53$) and 60.3% ($n=44$) for beach and demersal males, respectively.

Life history and condition

In females, life history traits and condition indices differed between spawning habitat but these differences were not consistent between years. In 2008, females spawning at the beach were longer ($F_{1,182}=26.00$, $p<0.0001$), heavier ($F_{1,182}=44.38$, $p<0.0001$), and in better condition, as indicated by Fulton's K ($F_{1,282}=29.90$, $p<0.0001$)

relative to those spawning demersally (Table 3.2). In 2009, there were no significant differences in life history traits or condition between early (23-Jul) and late (1-Aug) collections of beach spawning females so all females from the beach were combined for the habitat comparison. Life history traits did not differ between habitats, although beach females had higher Fulton's K ($F_{1, 114} = 4.89, p=0.03$). GSI did not differ between beach and demersal spawning females in either year. Males also differed between habitats in 2009, with beach males being longer ($F_{1, 195} = 5.93, p = 0.02$), and heavier ($F_{1, 195} = 4.19, p = 0.04$) than deep-water spawning males.

DISCUSSION

We investigated intrinsic factors related to spawning habitat use for capelin that spawn within two habitats that, while in close proximity to each other, differ greatly in the physical demands on spawning adults. Both male and female capelin possessed traits that are specific to beach or demersal spawning habitat, likely related to reducing energy expenditure during the spawning season. Habitat-related traits differed between males and females, reflecting sex-based priorities for maximum lifetime reproductive success. The preference for the beach habitat by both fast swimming males and females in good condition suggests that it is a high quality habitat or that it requires higher fitness capelin.

Males found spawning at the beach were longer and had deeper forked tails than those males that occupied demersal sites. Morphological traits that improve efficiency in a particular environment are well documented in fish. Fusiform bodies are often associated with energetically expensive environments (e.g. fast flowing streams; Hendry et al. 2000) and activities (e.g. long, difficult migrations; Crossin et al. 2004) because

Table 3.2: Summary of life history traits (length and weight) and condition indices (Fulton's K and Gonadosomatic Index (GSI) for female (2008, 2009) and male (2009) capelin in two spawning habitats (beach and demersal) in Trinity Bay, Newfoundland. Data presented are mean, range and sample size.

| Year/Sex | Habitat | Length (cm) | Weight (g) | Fulton's K | GSI (%) |
|-------------|----------|----------------|---------------|---------------|---------------|
| Females | | | | | |
| 2008 | Beach | 15.1 | 19.91 | 3.70 | 34.55 |
| | | (12.5-17.3) | (11.06-29.20) | (3.05-4.47) | (28.27-40.65) |
| | | n=88 | n=88 | n=88 | n=88 |
| | Demersal | 14.4 | 15.90 | 3.46 | 31.15 |
| (11.6-17.0) | | (8.00-27.20) | (2.88-4.22) | (14.13-43.09) | |
| | | n=95 | n=95 | n=95 | n=95 |
| 2009 | Beach | 13.9 | 14.01 | 3.51 | 31.92 |
| | | (11.7-16.1) | (7.60-23.60) | (2.83-5.08) | (18.24-40.23) |
| | | n=93 | n=92 | n=91 | n=87 |
| | Demersal | 13.8 | 13.34 | 3.33 | 31.50 |
| (11.2-15.9) | | (5.70-23.50) | (2.59-4.00) | (19.63-40.56) | |
| | | n=24 | n=24 | n=24 | n=24 |
| Males | | | | | |
| 2009 | Beach | 16.0 | 22.10 | 5.34 | |
| | | (13.8-17.6) | (12.10-30.20) | (4.78-6.18) | |
| | | n=98 | n=98 | n=98 | |
| | Demersal | 15.7 | 21.05 | 5.39 | |
| (13.2-17.7) | | (12.20-30.00) | (4.57-6.79) | | |
| | | n=98 | n=98 | n=98 | |

they reduce drag. Deep caudal peduncles, allowing increased burst speed, are also associated with fast flowing water (Janhunen et al. 2009). These traits are beneficial in strenuous environments because they improve swim efficiency, thereby conserving energy (Crossins et al. 2004). The deeper forked tails found in beach spawning male capelin suggests that swim speed is an important trait for spawning within this habitat. Although the overall purpose of the caudal fin is to thrust fish forward, its shape influences swim speed as well. For example, bluefin tuna (*Thunnus orientalis*) with deeper forked caudal fins have higher swim speeds than those with less forked tails (Tamura and Takagi 2009). A morphological trait associated with the ability to swim faster likely reduces the overall energy expenditure required for each push on and off the beach to spawn, as well as when holding off the beach between spawning events. Therefore, males with faster swimming capabilities may be more likely to perform multiple matings at beaches throughout the spawning season.

Energy expenditure also seemed important to females using the physically divergent spawning habitats, although this was not reflected in their body shape. Despite no habitat-related body shape differences in females, those females spawning at the beach had a higher body condition (i.e. Fulton's K) in both years. This index assumes that at a given length, fish that are heavier are in better condition. As such, Fulton's K can be considered a simple indicator of energy stores (Dibble and Meyerson 2012). It is possible that capelin at demersal sites have lower energy stores than those spawning at beach sites because they have used it for increased reproductive output. This is not the case; there is no habitat- or condition-related difference in GSI (this study) or fecundity (Chapter 4 (Penton and Davoren 2013b)). The use of the beach habitat by heavier, more

robust females with higher energy stores suggests that more energy is required to navigate the rigorous beach habitat during spawning. Therefore, females with higher energy stores may be more likely to survive beach spawning to reproduce in future years.

Although both traits are related to energy use during spawning, the habitat-related traits differ between sexes. These differences can be attributed to sex-based differences in life history (Huse 1998). In contrast to males that spawn with multiple females in a season, female capelin are total spawners, extruding all eggs in one event (Templeman 1948; Fridgeirsson 1976). As such, female capelin can increase their lifetime reproductive success by maintaining enough energy to commence feeding after spawning, whereas male fitness is increased if they can mate with two or more females in the same season (Huse 1998). Although capelin were historically thought to be semelparous (Murua and Saborido-Rey 2003), recent studies provide evidence of iteroparity (Huse 1998; Flynn et al. 2001; Christiansen et al. 2008), with up to 50% of female capelin in Newfoundland surviving to spawn in another year (Shackell et al. 1994). These sex-based differences in life history result in alternate priorities with respect to energy use during maturation and spawning.

As semelparous batch-spawners, male capelin should use their energy in such a way so that they can maximize the number of times they mate before death. With lower energy expenditure for each spawning event, males with deeper forked tails may have higher reproductive success at the beach relative to those individuals with less forked tails because they would be able to perform more matings at the rigorous beach habitat throughout the spawning period. As iteroparous total spawners, tail shape is unlikely to influence female reproductive success because they only spawn once at any given habitat

per year. Instead, actual energy stores would be more relevant to females whose priority is to survive to reproduce again. Females in poorer condition, with lower energy stores may be able to survive to reproduce again if they spawn at the less energetically demanding demersal spawning sites.

When raised in the laboratory at lower temperatures characteristic of demersal sites, hatching success is higher and larvae are larger (Penton and Davoren 2012). In the wild, demersal sites are characterized by lower mortality due to lower, less variable temperatures and consistent oxygen replenishment relative to the beach (Penton et al. 2012). Furthermore, environmental cues at the beach that have been demonstrated to influence recruitment (Frank and Leggett 1981, 1982, 1983) also operate at demersal sites (Penton and Davoren 2008). This evidence from both laboratory and natural settings indicates an environment that is conducive to the survival of early life history stages at deep-water spawning sites. Although the deep-water spawning habitat provides a more stable environment for both spawning adults and their early life history stages, fast swimming males and large, robust females spawn at the rigorous beach habitats. This suggests that there is some advantage to either the adults or their developing progeny that was not quantified in this study (e.g. fewer predators on adult capelin).

Capelin are a critical component in the diets of top vertebrate predators in the north Atlantic (Lavigne 1996; Carscadden and Vilhjalmsson 2002). When capelin arrive in coastal Newfoundland to spawn during the summer months, they are the primary prey of humpback (*Megaptera novaeangliae*) and minke (*Balaenoptera acutorostrata*) whales (Whitehead and Carscadden 1985), seabirds, including a capelin specialist, the common murre (*Uria aalge*) (Davoren and Montevecchi 2003) and Atlantic cod (*Gadus morhua*)

(Vandeperre and Methven 2007) and humans. These predators commonly aggregate at capelin demersal spawning sites on the northeast coast of Newfoundland (Davoren 2007). Although observations using underwater cameras reveal that diving seabirds primarily target solitary individuals that have broken off from the school, either to spawn or because they are in weakened condition post-spawning (pers. obs.), the risk of mortality due to predation at the demersal sites is likely higher than at the beach. The beach provides refuge from the large concentrations of predators found at demersal sites because, with the exception of groundfish predators (e.g. cod, sculpin, flounder), they are not able to follow capelin up onto the beach. The choice to spawn at either habitat, then, may be a trade-off between predator avoidance and maintaining enough energy to increase lifetime reproductive success. Those individual capelin, whether it be slow swimming males whose priority is to spawn multiple times in a single season, or females with lower energy stores that attempt to maintain enough energy stores to spawn in another year, that spawn within a less energetically demanding environment may be able to increase their lifetime reproductive success, despite the increased predation risk.

Demersal spawning in coastal Newfoundland is known to occur when beach temperatures are not suitable (Nakashima and Wheeler 2002; Davoren et al. 2012; Davoren in press) but we show that the use of this habitat may also represent a trade-off between energy available to increase reproductive success and risk of predation. The demersal sites then represent a good alternative that may ensure completion of the spawning act and enhance post-spawning survival for females that do not possess favourable traits for spawning within the rigorous beach habitat. Furthermore, because condition is not related to fecundity, egg size (Chapter 4 (Penton and Davoren 2013b)) or

stock productivity (Carscadden and Frank 2002) and early life history traits (e.g. egg survival, length at hatch) optimal when produced at 4 to 7°C (Penton et al. 2012; Chapter 2 (Penton and Davoren 2013a)), interannual variation in spawning habitat use may have little consequence for the population dynamics of this key forage fish.

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CHAPTER FOUR: MANUSCRIPT SUMMARY

Penton, P.M., and Davoren, G.K. 2013. Capelin (*Mallotus villosus*) fecundity in post-1990s coastal Newfoundland. *Marine Biology* **160**: 1625-1632
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For this manuscript, I formulated the question, designed the experiment and sampling protocol. I collected and with the exception of egg size measurement, I processed all samples. Further, I analyzed the data and wrote the manuscript. Dr. Gail Davoren provided financial and logistical support and provided comments on all versions of the manuscript prior to submission.

CAPELIN (*MALLOTUS VILLOSUS*) FECUNDITY IN POST-1990S COASTAL NEWFOUNDLAND

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ABSTRACT

In the 1990s, a cold water event was associated with drastic changes in the biology of Newfoundland capelin (*Mallotus villosus*), the key forage fish in the north Atlantic. In contrast to studies conducted prior to the 1990s, we report a lower maximum potential fecundity (7 616-42 880) and a weak relationship between fecundity and body size based on fecundity of 218 female capelin (12.3-16.9 cm) collected within two coastal regions of Newfoundland in 2008 and 2009. Further, using forward stepwise multiple regression and hierarchical partitioning, we conclude that life history traits (mass, somatic mass and egg size) and condition indices (Fulton's K, HSI and GSI) are not appropriate proxies for fecundity of capelin in our study area. Hierarchical partitioning revealed that egg size and condition indices suppress the variance in fecundity explained by other factors. Based on the insight it provides into the influence of traits on fecundity, we suggest that hierarchical partitioning is a powerful analysis technique that could be used in further investigations.

Keywords: capelin, *Mallotus villosus*, fecundity, condition, life history, hierarchical partitioning, multiple regression

INTRODUCTION

Capelin (*Mallotus villosus*) are a small and short-lived migratory schooling fish native to the northern hemisphere. Capelin are important as they are both a commercially fished species and a forage fish in the north Atlantic ecosystem (Carscadden and Vilhjálmsson 2002). As a major component in the diets of top vertebrate predators (e.g. Atlantic cod, marine mammals and seabirds), capelin transfer large amounts of energy from lower to upper trophic levels (Jangaard 1974; Vilhjálmsson 1994). Eggs are spawned in a single event and adhere to sediment at both beaches and in deeper water spawning sites throughout their circumpolar distribution. Capelin from the northeast Atlantic Ocean are typically demersal (oceanic sites located far from shore) spawners (Vilhjálmsson 1994), with the exception of local fjord populations that spawn on beaches (Davenport and Stene 1986). In the northwest Atlantic, capelin are thought to spawn primarily on coastal beaches (Templeman 1948). Environmental variability in the early life history stages of capelin are thought to influence recruitment (Frank and Carscadden 1989; Carscadden et al. 2000). Although a large body of work has examined the influence of the environment on survival of these vulnerable capelin life history stages in coastal Newfoundland (e.g. Frank and Leggett 1982a, 1982b; Leggett et al. 1984), comparatively less has focused on variability in egg production (Nakashima 1987), also a major source of variation in recruitment of marine fishes (Cushing 1995).

Potential fecundity - the total number of advance yolked oocytes matured per year (Hunter et al. 1992) - is an essential component of reproductive success because it is the maximum possible number of offspring that can be produced by an individual. Despite its importance, fecundity is not frequently monitored (Lambert 2008). Instead, due to a

strong relationship between body size and fecundity in many fish species (Kamler 2005), length or body mass is often used as an indicator of the reproductive potential of a population (Lambert 2008). Body size, however, is only one of many possible factors influencing fecundity and body size-fecundity relationships are variable both within and between species (e.g. McIntyre and Hutchings 2003; Kamler 2005; Nissling and Dahlman 2010), demonstrating the importance of assessing population-specific patterns. Multiple regression analysis is commonly used to determine if a particular set of factors (e.g. length, mass, condition, environmental conditions) can be used as proxies for fecundity as a quick and efficient means of assessing population reproductive potential (Lambert et al. 2003). Multiple regression analyses, however, may be confounded by highly collinear explanatory variables (multicollinearity), resulting in decreased statistical power and exclusion of important variables from the model (Graham 2003).

The majority of studies on capelin fecundity have been designed to examine spatial and/or temporal variations in fecundity rather than directly determining potential proxies. In addition to reports of a strong relationship with length in all studies (Table 4.1), fecundity in capelin has also been shown to be dependent on age (Winters 1971), population density (Galkin and Kovalev 1975) and stock. Capelin in the northeast Atlantic (i.e. Barents Sea and Iceland) are less fecund than capelin in Newfoundland (Table 4.1). It has been hypothesized that this life history difference is due to the contrasting spawning habitats between the northeast Atlantic (oceanic) and the northwest Atlantic (beach) (Jóhannsdóttir and Vilhjálmsson 1999). Lower fecundity of oceanic spawning capelin was recently supported in a fine-scale comparison of beach (Balsfjord, Norway) and oceanic (Barents Sea) spawners (Christiansen et al. 2008).

Table 4.1: Interstock differences in potential fecundity ranges and length-fecundity relationships (r^2 values) in capelin (*Mallotus villosus*) in the north Atlantic. N refers to the number of fish used in each analysis.

| Region | Stock | Total Length (cm) | Potential Fecundity | r^2 | N | Source |
|---------------------------|----------------------------|-------------------|---------------------|-------------------|-----|---------------------------------------|
| <i>Northeast Atlantic</i> | Barents Sea | 11.0-18.0 | 3 500-21 300 | 0.84 | 132 | Tereshchenko (2002) |
| | Barents Sea | 11.0-16.0 | 5 000-18 000 | 0.62 | 70 | Huse and Gjøsæter (1997) |
| | Barents Sea | 14.1-18.6 | 5 250-19 090 | 0.79 | 82 | Gjøsæter and Monstad (1973) |
| | Barents Sea | 13.0-16.0 | 5 256-48 336 | NR | 816 | Galkin and Kovalev (1975) |
| | Iceland | 13.0-18.0 | 6 432-23 135 | 0.78 | 198 | Jóhannsdóttir and Vilhjálmsson (1999) |
| <i>Northwest Atlantic</i> | Greenland | 11.1 ^a | 3 745-11 317 | 0.69 ^b | 18 | Hedeholm et al. (2011) |
| | Newfoundland (East coast) | 11.7-19.8 | 7 400-58 500 | 0.75-0.88 | 616 | Nakashima (1987) |
| | Newfoundland (Grand Banks) | 16.0-19.4 | 16 600-61 500 | 0.65 | 103 | Winters (1971) |
| | Newfoundland (East coast) | 13.2-20.7 | 16 000-52 000 | NR | 48 | Templeman (1948) |
| | Gulf of St. Lawrence | 12.5-17.4 | 7 448-28 794 | 0.18-0.28 | 129 | Grégoire (2004) |

^aMean value. Length range was not reported

^b r^2 value relates fecundity to gutted mass

NR-data was not reported

Capelin fecundity studies have been conducted across their distribution in the north Atlantic (Table 4.1), with the last Newfoundland study conducted in the 1980s (Nakashima 1987). In the 1990s, an anomalous cold water event in the northwest Atlantic was associated with dramatic changes in the biology of capelin in Newfoundland (Nakashima 1996). Younger and smaller fish dominated the spawning population and spawning was delayed and protracted (Nakashima 1996; Carscadden and Nakashima 1997). Delayed spawning, due to cold water during gonadal maturation (Nakashima 1996), is thought to have led to an increased occupation of demersal spawning sites in the nearshore region (sub-tidal) (Nakashima and Wheeler 2002). The purpose of this study is two-fold. First, we present the first report on the fecundity of this key forage fish since the 1990s. Second, we compare the utility of multiple regression with another statistical approach, hierarchical partitioning, to examine the potential use of life history traits and condition indices as a proxy for capelin fecundity and, thus, the reproductive potential of the spawning population.

MATERIALS AND METHODS

Sampling

Based on both observed differences in early life history traits (Penton and Davoren 2008; Penton et al. 2012) between beach and demersal (sub-tidal) sites in coastal Newfoundland and differences in fecundity between beach and oceanic spawners in the Barents Sea (Christiansen et al. 2008), we collected females from both beach and sub-tidal spawning sites. In 2008 and 2009, up to 100 gravid capelin in spawning condition were collected from one beach and one demersal site within two embayments (Bellevue,

Trinity Bay; Lumsden, Notre Dame Bay) where beach and demersal spawning occur in coastal Newfoundland (Table 4.2). Spawning capelin were collected at beach sites (Bellevue Beach-BB; Lumsden Beach-LB; Fig. 4.1) using a 6' cast net with 9.5 mm mesh (Wildco). At the Bellevue Demersal (BD) site, spawning capelin were collected using a modified crab pot (a 3' crab pot wrapped with 16 mm mesh) deployed from the Fisheries and Oceans research vessel, the *Narry Face*. At the Lumsden Demersal (LD) site, a tuck seine was deployed from a commercial fishing vessel, the *Lady Easton II*, to collect fish. Fish were placed in individual bags and immediately frozen until processed in the laboratory (within nine months of collection). Spent fish were excluded from collection. In Trinity Bay, capelin spawned in both habitats in both years of the study. In Notre Dame Bay, capelin spawned only at demersal sites in 2008 and only at beach sites in 2009.

Processing

Approximately 50% of the fish sampled from each site were randomly selected for fecundity analysis. Female capelin showing evidence of potential egg loss (e.g. body torsion, slack stomach, eggs coming out of the genital papilla) were excluded from processing. Females were thawed and total length ($L \pm 1$ mm), body mass ($Wt \pm 0.1$ g), ovary mass ($OWt \pm 0.1$ g) and liver mass ($LWt \pm 0.001$ g) were measured. Somatic mass ($Wt - OWt \pm 0.1$ g), Fulton's K ($(Wt - OWt \cdot L^{-3}) \cdot 10^3$), Gonadosomatic Index (GSI: $OWt \cdot Wt^{-1} \cdot 100$) and Hepatosomatic Index (HSI: $LWt \cdot Wt^{-1} \cdot 100$) were calculated. To determine fecundity, ovaries were placed in glass jars containing modified Gilson's solution (% volume: 1.6 nitric acid [80], 1.9 glacial acetic acid, 10.8 Ethyl Alcohol [60],

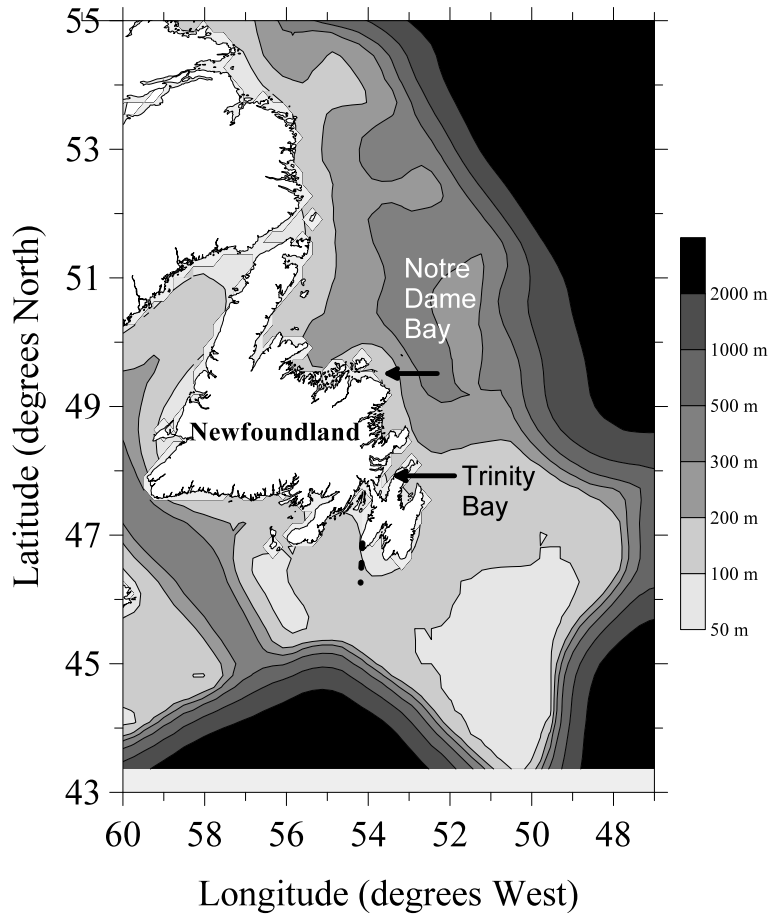


Figure 4.1: Map of Newfoundland, indicating the location of Trinity Bay (Bellevue spawning sites), Notre Dame Bay (Lumsden spawning sites) with depth indicated by *grey shading*.

85.7 water and 20 g mercuric chloride; Snyder 1983) for 62–214 days. Each sample was shaken on a VWR Orbital Shaker for 16–112 hours at 150 rpm until eggs separated from ovarian tissue (Nakashima 1987). After eggs were loosened, each sample was poured over a 0.270 mm sieve to remove ovarian tissue. The sample was placed back into the jar and topped up with 70% ethanol for one week to harden the eggs. Eggs were sub-sampled using a 1 L capacity Motoda splitter (Motoda 1959) and the final split (~200-300 eggs) was placed onto a Petri dish and eggs were counted using a dissecting microscope (Olympus SZX7). Eggs were photographed with a digital camera (Olympus C-7070) mounted to the microscope and the diameter was measured using ImageJ (v. 1.44; Abramoff et al. 2004). All photographs included a 1 mm scale and were measured by a single investigator (E.M.). Samples with discoloured or non-spherical eggs were considered damaged and removed from the analysis. Fecundity values greater than 2 standard deviations from the mean were considered outliers and were removed from further analyses (Hill and Lewicki 2006).

Statistical Analysis

Although length is frequently reported in relation to fecundity, body mass may be the more relevant body size metric (Koops et al. 2004). Statistical significance of linear regressions were used to examine the body size metric (length or body mass) most relevant to explaining the variance in fecundity. Differences in size-corrected fecundity among sites and years were subsequently examined using an analysis of covariance (ANCOVA) with the appropriate body size metric as a covariate.

Simple regressions were used to examine the univariate relationships of all condition and life history traits with fecundity. Forward stepwise multiple regression was used to examine the influence of life history traits and condition indices on patterns in fecundity. Variables both entered and stayed in the model at a significance level of 0.05. Variables were further examined using hierarchical partitioning (Quinn and Keough 2002). This method calculates independent and joint effects by determining the increased fit of a model when a variable is added relative to the same model without the variable (Chevan and Sutherland 1991). All possible models are considered and the influence of variables is averaged over hierarchies, removing multicollinearity problems that often occur when using a single model (MacNally 1996). High positive joint effects indicate collinear variables and negative joint effects indicate suppressor variables (Chevan and Sutherland 1991). Suppressor variables are those that mask the action of other variables such that their influence in a univariate correlation will appear to be zero (MacNally 1996).

All data analyses were generated using SAS software, Version 9.2 of the SAS System for Windows. Copyright © 2000 SAS Institute Inc., Cary, NC, USA. Prior to analyses, all traits were examined for normality (probability plots) and homogeneity of variance (Levene's test).

RESULTS

A total of 300 females were collected in 2008 (100 from each site) and 225 were collected in 2009 (BB: n=100; BD: n=25; LB: n=100). Four and 54 fish (samples were distributed across all sites and years) were removed from the analysis due to outliers and

damaged egg samples, respectively. Across sites and years, potential fecundities of 7 616–42 880 were observed in 218 female capelin. Females were 12.3–16.9 cm and ranged from 7.50 to 25.55 g (Table 4.2). Exploratory analyses determined that transformations were not necessary to meet the assumptions of linear models. The relationship between body length and total mass was strongly linear in both 2008 ($y = 3.5098x - 33.94$, $r^2 = 0.79$) and 2009 ($y = 3.3489x - 32.52$, $r^2 = 0.86$), regions and sites combined.

Fecundity-body size relationships

Although often statistically significant, the linear relationship between fecundity and body size was not strong for either total length or total mass within each site in each year as well as with sites and years combined, as indicated by the low r^2 values of the regression relationships (Table 4.3). Body mass was used as the body metric covariate for further investigations because its relationship with fecundity was significant in more instances (Table 4.3). Despite spatial and interannual differences in female capelin body size (unpubl. data), there were no differences in the fecundity-body mass relationship among sites or between years (Fig. 4.2). An ANCOVA with body mass as the covariate revealed no differences in slope ($F_{1,206} = 0.88$, $p=0.35$; $F_{3,206} = 2.01$, $p = 0.11$) or y-intercepts ($F_{1,206} = 1.80$, $p = 0.18$; $F_{3,206} = 1.16$, $p = 0.33$) between years and among sites, respectively. The data were therefore pooled to examine the factors that influence fecundity of capelin in coastal Newfoundland.

Table 4.2: Summary of life history traits and condition indices for female capelin in two embayments of coastal Newfoundland in 2008 and 2009. Data presented are mean, range (in parentheses) and sample size (n = number of fish).

| Region | Habitat | Year | Collection Date | Total Length (cm) | Wet mass (g) | Fulton's K | HSI (%) | GSI (%) | Egg Size (mm) | Fecundity |
|-------------|-------------------|------|-----------------|-------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|-------------------------------|------------------------------------|
| Trinity Bay | Bellevue Beach | 2008 | 15-Jul | 14.8 (13.6-16.9) n = 36 | 18.99 (13.57-25.55) n = 35 | 3.684 (3.050-4.202) n = 37 | 0.797 (0.398-1.624) n = 37 | 33.16 (29.47-40.65) n = 37 | 0.67 (0.50-0.81) n = 35 | 24419 (11 200-39 552) n = 33 |
| | | 2009 | 23-Jul/01-Aug | 14.1 (12.4-16.1) n = 42 | 14.41 (8.50-23.60) n = 44 | 3.463 (2.952-4.129) n = 42 | 0.892 (0.352-1.587) n = 42 | 33.00 (23.64-40.09) n = 40 | 0.67 (0.60-0.77) n = 36 | 21 401 (9 280-38 144) n = 44 |
| | Bellevue Demersal | 2008 | 19-Jul | 14.6 (12.6-16.4) n = 35 | 16.53 (11.21-23.45) n = 35 | 3.470 (2.904-4.224) n = 35 | 0.767 (0.315-2.432) n = 33 | 34.02 (22.39-43.09) n = 35 | 0.71 (0.64-0.81) n = 31 | 21 620 (7 616-39 680) n = 35 |
| | | 2009 | 24-Jul | 13.9 (12.3-15.9) n = 15 | 13.37 (8.80-23.50) n = 16 | 3.354 (2.593-3.998) n = 16 | 0.946 (0.249-2.679) n = 12 | 32.83 (23.52-40.56) n = 15 | 0.63 (0.51-0.70) n = 13 | 28 500 (9 696-42 880) n = 16 |

Table 4.2 continued...

| Region | Habitat | Year | Collection Date | Total Length (cm) | Wet mass (g) | Fulton's K | HSI (%) | GSI (%) | Egg Size (mm) | Fecundity |
|----------------|------------------|------|-----------------|---------------------|------------------------|------------------------|------------------------|------------------------|---------------------|---------------------------|
| Notre Dame Bay | Lumsden Demersal | 2008 | 25-Jul | 14.9 (13.0-16.9) | 18.47 (11.64-25.49) | 3.715 (3.139-4.213) | 1.455 (0.704-2.441) | 32.09 (23.84-38.25) | 0.69 (0.62-0.80) | 24 483 (8 704-42 624) |
| | | | | n = 40 | n = 39 | n = 34 | n = 41 | n = 34 | n = 41 | |
| | Lumsden Beach | 2009 | 25-Jul | 14.3 (12.6-16.7) | 15.54 (9.90-24.50) | 3.507 (3.054-4.067) | 0.943 (0.289-2.297) | 33.01 (25.09-42.91) | 0.64 (0.50-0.73) | 25 781 (12 224-41 984) |
| | | | | n = 43 | n = 45 | n = 42 | n = 42 | n = 43 | n = 35 | n = 45 |

Table 4.3: Regression model parameters for the spatial and temporal relationships between capelin fecundity and body size (length and body mass) for each site and year in coastal Newfoundland and all combined.

| Year | Region | Spawning Habitat | Collection Date | Size Variable | n | r ² | p-value |
|--------------------------|----------|------------------|-----------------|---------------|-----|----------------|---------|
| 2008 | Bellevue | Beach | 15-Jul | Length | 35 | 0.085 | 0.085 |
| | | | | Body mass | 34 | 0.128 | 0.0351 |
| | | Demersal | 19-Jul | Length | 34 | 0.071 | 0.1229 |
| | | | | Body mass | 34 | 0.097 | 0.0681 |
| | Lumsden | Demersal | 25-Jul | Length | 38 | 0.162 | 0.011 |
| | | | | Body mass | 39 | 0.286 | 0.0004 |
| 2009 | Bellevue | Beach | 23-Jul/01-Aug | Length | 41 | 0.369 | <0.0001 |
| | | | | Body mass | 43 | 0.472 | <0.0001 |
| | | Demersal | 24-Jul | Length | 14 | 0.010 | 0.7197 |
| | | | | Body mass | 15 | 0.036 | 0.4804 |
| | Lumsden | Beach | 25-Jul | Length | 42 | 0.283 | 0.0002 |
| | | | | Body mass | 44 | 0.207 | 0.0017 |
| Sites and years combined | | | | Length | 204 | 0.127 | <0.0001 |
| | | | | Body mass | 209 | 0.147 | <0.0001 |

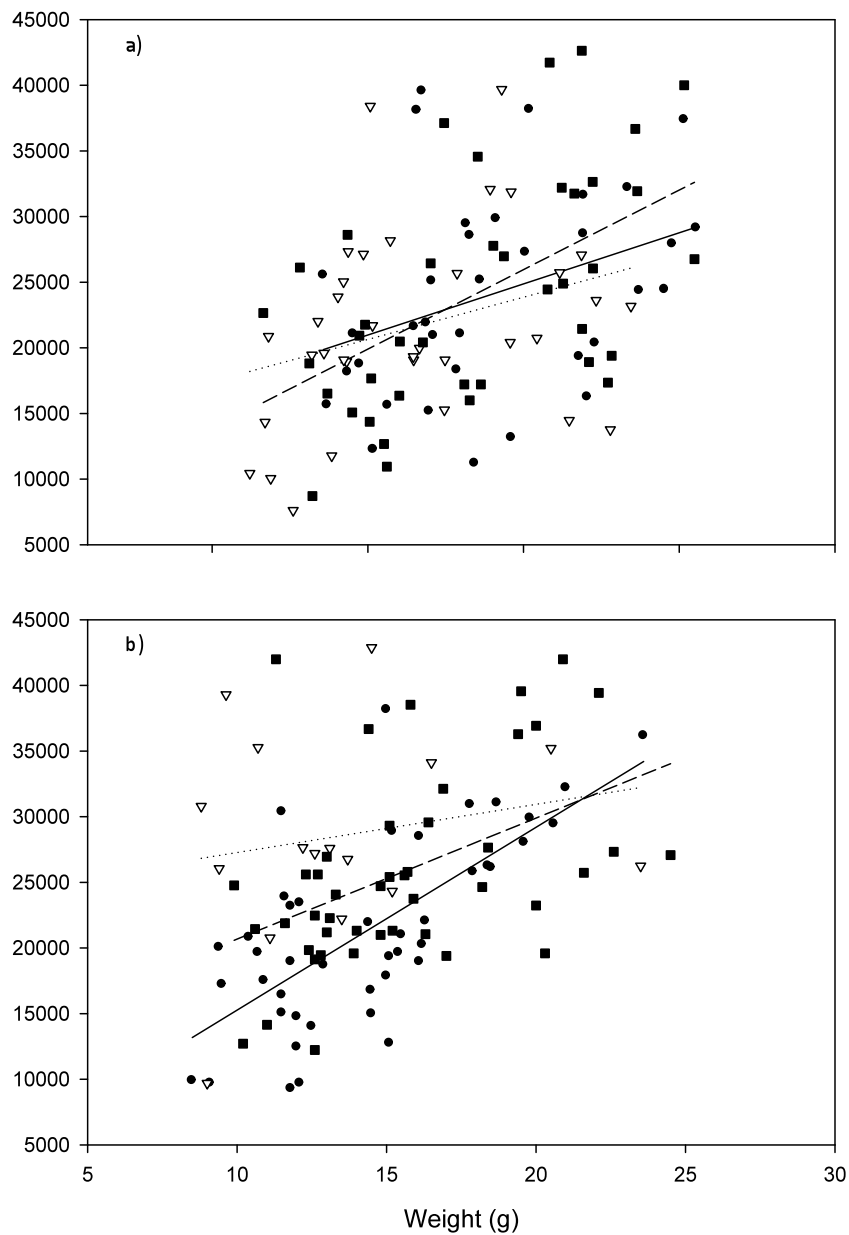


Figure 4.2: Relationships between potential fecundity and body mass for capelin

(*Mallotus villosus*) in a) 2008 and b) 2009. Each sampling location is indicated: Bellevue Beach (BB; *circle*), Bellevue Demersal (BD; *inverted triangle*), Lumsden (*box*) Demersal (2008) and Beach (2009)

Table 4.4: r^2 and p-values of univariate regressions of life history traits and condition indices against fecundity with sites and years pooled.

| Trait | r^2 | p |
|--------------|--------|-----------|
| Body mass | 0.1465 | <0.0001 * |
| Somatic mass | 0.1010 | <0.0001 |
| Fulton's K | 0.0026 | 0.4598 |
| GSI | 0.0371 | 0.0050 * |
| HSI | 0.0097 | 0.1659 |
| Egg size | 0.0147 | 0.1026 * |
| N | 156 | |
| Full Model | 0.3592 | |

* indicates significant variables in Stepwise Multiple Regression

Factors influencing fecundity

Despite significant relationships, the strength of the univariate relationship with fecundity was low for all life history traits ($r^2 < 0.14$, Table 4.4). Similarly, the proportion of variation in fecundity explained by condition indices (Fulton's K, HSI) was low and not significant (Table 4.4). The stepwise multiple regression retained body mass, GSI and egg size at a significance of 0.05 for a final model $r^2 = 0.36$ (Table 4.4). The univariate relationship between body mass and GSI was not significant ($r^2 = 0.01$, $p = 0.1421$). Increasing egg size with larger body size was significant but the relationship was weak ($r^2 = 0.10$, $p < 0.0001$).

Hierarchical partitioning demonstrated that the independent effects of the variables were low but fairly evenly distributed (Fig. 4.3). The high positive joint effects of length, body mass and somatic mass relative to their independent effects (Fig. 4.3) indicated that these variables are collinear. The joint effects of both condition indices were equal in magnitude to independent effects but were negative, indicating the role of Fulton's K and HSI as suppressor variables. Egg size, one of the variables retained in the final stepwise multiple regression model (Table 4.4), had the highest independent effect on fecundity but the negative joint effect that was equal in magnitude identified it as a suppressor variable (Fig. 4.3). GSI was the only variable whose influence on fecundity was completely independent (Fig. 4.3).

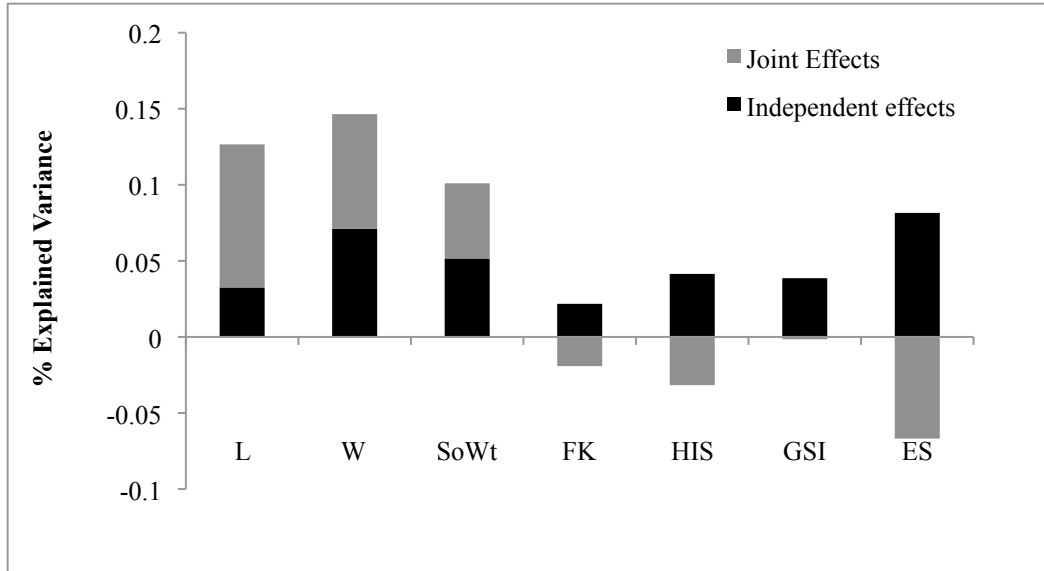


Figure 4.3: Percentage distribution of the independent and joint contributions of the variables used to explain fecundity in capelin, years and sites combined. Variables include length (*L*), body mass (*W*), somatic weight (*SoWt*), Fulton's K (*FK*), Hepatosomatic Index (*HSI*), Gonadosomatic Index (*GSI*) and egg size (*ES*)

DISCUSSION

Potential fecundity of female capelin collected for this study (7 616–42 880) is within the range reported for capelin in coastal Newfoundland prior to the 1990s (Table 4.1). The maximum potential fecundity in this study is lower than previously reported for capelin (Templeman 1948; Nakashima 1987), but is consistent with smaller-sized capelin since the 1990s (Carscadden and Nakashima 1997). Despite length, body mass and condition differences in capelin among sites and years, there was no difference in size-adjusted fecundity between females in the two years of this study and similar to a previous study (Nakashima 1987), there were no regional differences in fecundity. The major contrast of this study with that of Winters (1971) and Nakashima (1987), as well as with studies conducted in the northeast Atlantic (Table 4.1), is the weak relationship between fecundity and length. Despite statistically significant relationships with body size, fecundity and egg size were highly variable. We observed both upper and lower ends of the range in fecundity and egg size in both small and larger females.

Age and an iteroparous reproductive strategy (surviving to spawn in another year; Wootton 1990) are two factors that may potentially contribute to the weaker relationships we observed between fecundity and body size in this study. First, Winters (1971) reported increasing fecundity with older female capelin. Therefore, it is possible that for a given length range, the high and low fecundities result from a mix of older and younger capelin, respectively. Second, repeat spawning fish are thought to have higher fecundity because they are presumably older and larger (Daniel et al. 1993; Green 2008; Wright and Trippel 2009; Thorstad et al. 2010), but determinants of fecundity can differ between first time and repeat spawners. In Atlantic cod (*Gadus morhua*), environmental factors

have been demonstrated to influence fecundity differentially; fecundity in first time spawners is determined by body size at the start of the spawning season and in repeat spawners, fecundity is related to both body size and food conditions during vitellogenesis (Kjesbu et al. 1991; Kjesbu and Holm 1994). Although capelin have been typically considered semelparous, dying after the spawning event (Jangaard 1974; Friis-Rødel and Kannevorf 2002; Murua and Saborido-Rey 2003), support for an iteroparous strategy in capelin is accumulating (reviewed in Flynn et al. 2001). Changes in fishing regulations (Joikokko and Jutila 2005) and the age distribution of spawning populations (Morgan et al. 2007) have been shown to increase the proportion of repeat spawning fish. It is possible that in addition to smaller and younger capelin in the spawning population (Carscadden and Nakashima 1997), changes in capelin biology in the 1990s may have included an increased proportion of repeat spawners. Indeed, the number of recovering spawning females increased from 20% in 1995 to >50% in 1996 and 1997 (Flynn et al. 2001). If changes in capelin biology in the 1990s included an increased proportion of repeat spawners whose fecundity similarly differs depending upon spawning experience, this may explain the weak relationship of length with fecundity in capelin in coastal Newfoundland and should be examined further.

The similarity in size-based fecundity among habitats, regions and years permitted pooling all data to examine the influence of life history traits and condition on capelin fecundity in coastal Newfoundland using different statistical techniques. A low proportion of variance in fecundity was explained by life history traits (< 15%) and condition indices (<5%) using univariate regressions. Lambert et al. (2003) established a minimum 65% explained variance for models to successfully act as proxies for fecundity

based on the substantial increase in predictive power for models with $r^2 > 0.65$. Based on this criteria, the model that retained body mass, GSI and egg size using Stepwise Multiple Regression cannot be used as a proxy for fecundity in capelin in our study area.

The selection of models based on regressions can be complicated by multiple interactions among traits or factors of interest (Quinn and Keough 2002). We used the life history traits and condition indices, albeit with low explanatory power in this study, to investigate the value of using hierarchical partitioning to examine patterns in fecundity. Using univariate regressions, length, body mass and somatic mass were the only traits that explained more than 10% of the variation in fecundity. The independent influence of these traits (3-7%), revealed using hierarchical partitioning, however, were similar to the other life history traits (4-8%) and condition indices (2-4%). The collinear nature of these variables, therefore, confounds results in univariate regressions. In this study, the highest amount of independent influence on fecundity was due to body mass. Although this corresponded to the highest size-based trait using univariate regression, this may not always be the case. Hierarchical partitioning also demonstrated that egg size, one of three traits retained in the multiple regression, has negative joint effects equal in magnitude to its independent effects on fecundity variation. This identifies egg size as a suppressor variable and its potential role in masking the contribution of another trait to variation in fecundity (Chevan and Sutherland 1991). Although condition indices were not retained in the multiple regression model, Fulton's K and HSI were also identified as suppressor variables. The low explanatory power of these traits in the current study does not require further attention. If r^2 values were higher, however, the potential of

hierarchical partitioning to reveal important information and insight, such as demonstrated here, would be critical to understanding patterns in fecundity.

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CHAPTER 5: INVESTIGATING CAPELIN AND OTHER COMMERCIAL FISH SPECIES FOR VARIABILITY AS A STRATEGY FOR AN UNPREDICTABLE ENVIRONMENT

ABSTRACT

In coastal Newfoundland, the spawning of capelin (*Mallotus villosus*) at alternate habitats in the 1990's is associated with unfavourable oceanic conditions during that time. The facultative occupation of these two habitats remains persistent despite the divergent environmental conditions that both adults and incubating embryos must endure. This chapter tests for evidence of a bet-hedging strategy in capelin through the examination of the variation in offspring size (egg and larvae) and time to hatch across multiple scales of analysis. For all three traits, *within-female* variability was greater than population-level variation (i.e. *among-female*), and higher than the individual variability in early life history traits reported for capelin in the 1980's. For capelin, the production of a cohort of offspring that is variable in trait values would ensure some survival if spawning occurs in either of the alternate habitats. This individual-level variability has implications for the survival and recruitment of capelin. Trait means are frequently used for fisheries management but individual variability should be considered because it could provide insight into the biology of this ecologically important marine fish species.

INTRODUCTION

Across a variety of scales, variability can shape evolution, population biology and ecosystem processes. Variation in individual traits is important in evolutionary ecology because it is a fundamental requirement for natural selection (Darwin 1956).

Intraspecific trait variation in discrete spawning populations of the Bristol Bay sockeye salmon complex results in stability of meta-population sizes (Portfolio effect: Hilborn et al. 2003; Schindler et al. 2010).

Specialist species are common in stable and predictable environments, possessing a suite of traits that allow them to maximize performance, and thus, maximize fitness in that environment (Peers et al. 2012). As environmental uncertainty increases; however, females producing offspring that are specialized for specific conditions run the risk of reproductive failure (Kassen 2002; Marshall et al. 2008). Instead, generalist and bet-hedging strategies are proposed to be successful for species that face environmental uncertainty (Donaldson-Matasci et al. 2008). Generalists can occupy a variety of environments but do not attain the same fitness as the specialist occupying the same environment. Generalists are the ‘jack of all trades, master of none’ (Donaldson-Matasci et al. 2008). Alternatively, diversified bet-hedging produces phenotypically diverse offspring (not genetic in nature) within each brood (Einum and Fleming 2004). When environmental conditions become unpredictable or if females cannot determine the conditions their offspring will encounter (Imperfect Information Hypothesis; Koops et al. 2003), producing offspring with variable traits (i.e. within-female variability is higher than among-female variability) ensures that at least some offspring are successful. This minimizes reproductive failure, thereby increasing maternal fitness compared to those

females that produce invariable offspring in conditions that are not suitable for them (Kassen 2002; Donaldson-Matasci et al. 2008; Marshall et al. 2008). Variable within-brood hatching times increases maternal fitness in a variety of taxa, including birds (Amundsen and Slagsvold 1998), fish (Trippel et al. 1997; Green 2008), amphibians (Thumm and Mahony 2002) and insects (Smiseth et al. 2006). Similarly, within a specific set of environmental conditions, within-female offspring size (i.e. egg size and size at hatch) variability increases maternal fitness in insects (Bradford and Roff 1993), amphibians (Crump 1981) and fish (Koops et al. 2003; Morrongiello et al. 2012).

Capelin, the main forage fish in the north Atlantic, spawns adhesive demersal eggs at beach and sub-tidal habitats within two embayments (Notre Dame Bay and Trinity Bay) in coastal Newfoundland (Penton and Davoren 2012). Evidence suggests that capelin are not locally adapted to divergent environmental conditions at these beach and sub-tidal spawning habitats (Chapter 2 (Penton and Davoren 2013a); Appendix I). Instead, spawning habitat use in these embayments appears to be facultative (Chapter 3), with some individuals sampling environmental conditions at both habitats prior to spawning (Davoren 2013). Temperature is the main feature that differs between the two habitats and, thus, likely is used as a cue for spawning habitat selection (Davoren 2013) as this will determine thermal suitability of habitats for incubating embryos. In support, although incubating embryos can survive temperatures down to -6°C (Davenport 1989), adult capelin at spawning grounds have suspended spawning if temperatures decrease below 2°C (Carscadden et al. 1989; Nakashima and Wheeler 2002; Penton and Davoren 2012) or above 12°C (Nakashima and Wheeler 2002), suggesting these are the upper and lower thermal cues for spawning adults. High temperatures (14°C) are similarly not

conducive to normal egg development and survival (Frank and Leggett 1981; Penton and Davoren 2012; Chapter 2 (Penton and Davoren 2013a)). At the beach, temperatures are not only warmer, but also more variable than sub-tidal spawning sites. For example, there was a 26°C difference between maximum and minimum hourly temperatures during incubation at a capelin spawning beach in coastal Newfoundland (Penton et al. 2012). This was in contrast to differences of 6.7, 10.3 and 10.4°C at three deep-water spawning sites in the same embayment in the same year (Penton et al. 2012).

Temperature during gonadal development ultimately determines the timing of inshore migrations to spawning grounds (Carscadden et al. 1997), arrival at the spawning grounds (Regular et al. 2008) and timing of spawning (Davoren et al. 2012). The availability of beach and/or sub-tidal spawning habitats is therefore dependent on a combination of these interannual variations in arrival at inshore spawning grounds and the temperatures at beach and sub-tidal spawning habitats when they arrive. Depending on these conditions, there are three potential options for availability of spawning habitats, for instance in Notre Dame Bay (2003-2010, Davoren et al. 2012), when capelin arrive inshore to spawn: beach only (e.g. only beach habitat was used in 2009), sub-tidal spawning sites only (e.g. beach habitat was not used in 2006 and 2008), or both beach and sub-tidal sites (e.g. both habitats were used in 2003-2005). During gonadal development (February to June) in offshore waters, it is unlikely that female capelin are able to predict what habitats will be available, and therefore in what conditions their offspring will develop.

Given (1) the lack of specialization to beach or sub-tidal spawning habitats, (2) the thermal variability both within and between these habitats, and (3) the inability of

females to predict spawning habitat availability, the objective of this paper is to investigate whether capelin use a bet-hedging strategy to deal with uncertain and unpredictable environmental conditions within spawning habitats. To do this, traits were quantified, including variation in capelin egg size, time to hatch and size at hatch. In accordance with a bet-hedging strategy, I predict that variability within individual female capelin will be higher than among females (Marshall et al. 2008). Further, I conducted a literature search to examine this variability in relation to other commercially important fish species by quantifying variation in offspring size (egg size and size at hatch). I predict that bet-hedging strategies will be more prevalent in capelin and other short-lived forage fish species due to their decreased number of opportunities to reproduce relative to long-lived species. As egg development in fish is largely dependent on temperature (Blaxter 1988; Pepin 1991; Rombough 1997) and is well-documented for capelin (Frank and Leggett 1981; Penton et al. 2012; Præbel et al. 2013), I also examine individual variability in the time to hatch across all temperatures in the context of average time to hatch in published studies.

MATERIALS AND METHODS

Egg size and early life history traits

Female and male capelin in spawning condition were collected from three sites in coastal Newfoundland (2 embayments – Lumsden Beach-LB, Notre Dame Bay and Bellevue Beach-BB and Bellevue Demersal-BD, Trinity Bay). For detailed information on the sampling sites, collection methods and laboratory conditions, refer to Chapter 2 (Penton and Davoren 2013a). In brief, artificially fertilized eggs were raised in the lab

and females (6/site) were frozen for subsequent analysis. The eggs of each female were fertilized with sperm from three males, creating 18 half-sib families. Eggs were incubated at 4, 7 and 12°C in the laboratory and monitored daily until all eggs had hatched. Newly hatched larvae, as well as eggs remaining in the ovaries of females used for fertilizations (for detailed information on ovary processing, see Chapter 4 (Penton and Davoren (2013b))), were photographed and measured using ImageJ (v. 1.44; Abramoff et al. 2004).

A total of 18 half-sib families were reared in the three temperature treatments, producing 720 larvae. As the purpose was to examine variation in early life history traits, half-sib families that produced less than five larvae were excluded from the analysis. More than 60% of the half-sib families at the warm temperature did not meet these criteria, excluding the entire warm temperature treatment from the analysis. In the cold treatment, 7 half-sib families produced less than five larvae and these families were also excluded from further analysis.

For each female within each temperature, mean, standard deviation (SD) and coefficient of variation [$CV=100*(SD/mean)$] was calculated for: egg size, time to hatch and length at hatch. For each trait, *within-female* variability was calculated by first determining the CV for each individual female, and then averaging these CVs across all females. *Among-female* variability was calculated by determining the CV of all females combined (Morrongiello et al. 2012).

Literature review

Offspring size variability was examined for commercially important marine fish (Appendix II: Table A2.1 and A2.2). The literature search was focused on forage fish (herring, sandlance, capelin, anchovy, menhaden and sardine; Pikitch et al. 2012) and other commercial fish outlined in 2012 Quota Reports (Fisheries and Oceans Canada: plaice, hake, halibut, mackerel, monkfish, swordfish, flounder, tuna and haddock). Studies that presented data allowing calculation of CV (coefficient of variation) were included (i.e. CV, mean \pm SD, sample size and mean \pm SE, or raw data that were extracted from graphs or tables). Egg diameter and length at hatch were the most commonly reported measures of offspring size in the literature. Studies that used weight or volume as a metric of offspring size were excluded from the analysis because the CV of these measurements are not equivalent to body length (Chambers and Leggett 1996; Marshall and Keough 2007). Following criteria for my study on capelin, I only included studies that calculated means using a minimum of five eggs or larvae.

The majority of data were compiled from studies that examined regional- or temperature-driven differences among offspring traits. Each CV presented in tables and figures is therefore associated with a different collection site and/or temperature treatment. To exclude variability caused by fluctuating temperatures, larval size at hatch was only included if data were obtained from rearing experiments at controlled temperatures. To examine among-female (or within-population) variability in offspring size, only data collected from two or more females was included. For eggs collected in surface plankton tows, the number of females used to produce those eggs is assumed to

be high (Chambers and Leggett 1996). Studies examining within-female (individual) variability were included according to the same criteria but are presented separately.

In the interest of examining patterns in offspring size variability between small forage fish and larger, long-lived fish, CV's are presented as a function of body length. The size of the females used to produce the early life history stages was not reported in all studies (e.g. eggs collected in plankton tows). The maximum body length reported for each species is used as it is a good indicator of many life history parameters (Froese and Pauly 2013). The climatic region and maximum length (L_{max}) for each fish species (Appendix II: Table A2.1 and A2.2) was obtained from FishBase (Froese and Pauly 2013). In accordance with the studies that report separate means for offspring size from different geographical areas, CV's for eggs and larvae from Trinity Bay and Notre Dame Bay were calculated separately. Data collected from these sites were combined with data extracted from the literature for a broader comparison.

Data on time to hatch for capelin was also extracted from previously reported studies. In all of these studies, a single parameter is reported for development time (average time to hatch or time to first hatch) at a given temperature. Similar to the larger analysis on offspring size of marine fish, datapoints for capelin time to hatching represent population means (among-female means), experimental conditions (e.g. salinity, temperature) or field conditions (e.g. tidal zone, spawning habitat). Data from these studies are incorporated with the following data from a lab experiment (Chapter 2 (Penton and Davoren 2013a)): average time to hatch (one population mean within each temperature) and time to hatch for each individual larva during the experiment from all

three temperature treatments. Data from the 12°C treatment was included because for this portion of the chapter, within-female variation was not the measurement of interest.

RESULTS

Egg size

The diameter of 2 563 eggs from nine females used for the crosses ranged from 0.50 to 0.94 mm, with an overall mean of 0.67 mm. Data for females BB4 and BB1 were excluded from the analysis because egg samples were damaged. Egg size distributions were similar across all nine females and CV's were similar within each half-sib group, ranging from 7.6 to 9.7% (Fig. 5.1). Within-female variability in egg size (CV = 8.6%) was higher than among-female variability (CV = 4.0%).

Twenty-three studies reported appropriate data on among-female variation in egg size for 11 commercially important marine fish species (Appendix II, Table A2.1). The maximum body length (L_{\max}) reported for these species ranged from 20 to 300 cm (Fig. 5.2; Table A2.1). There was no clear relationship between L_{\max} and among-female egg size variation. With the exception of Pacific bluefin tuna and Pacific halibut with CV > 25% (one datapoint per species), CV was less than 10% for all species (Fig. 5.2). Among-female egg size variation for capelin in this study (Trinity Bay: 9.3%; Notre Dame Bay: 8.6%) was the highest of those fish with a CV less than 10%. The 3 forage fish species (capelin, anchoveta and gulf menhaden) did not have a wide range in CV's, in contrast to cod, mackerel, flounder and haddock (Fig. 5.2).

Seven studies reported data on within-female variation in egg size for six species of commercially important marine fish (Table 5.1). These data were available from more

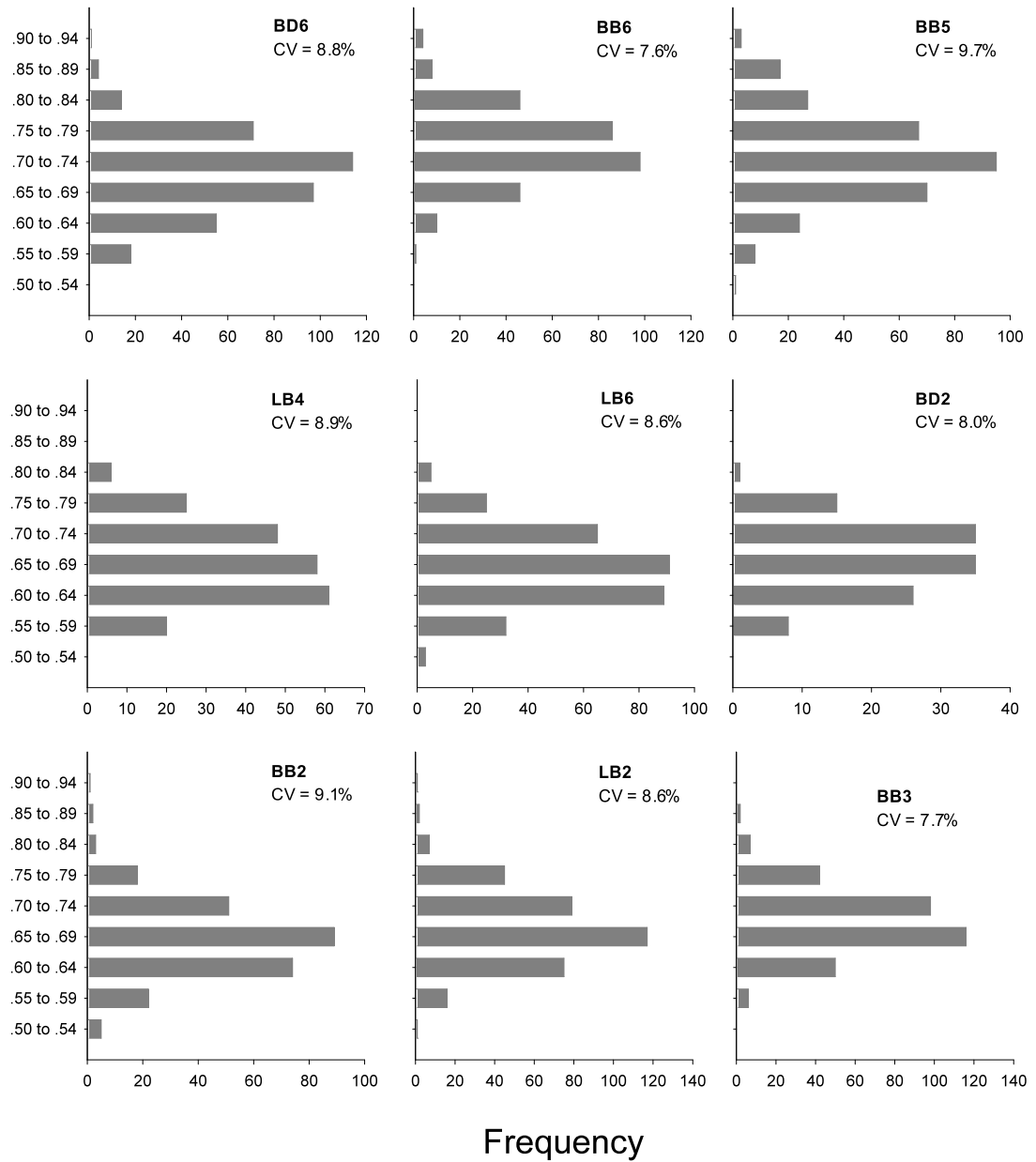


Figure 5.1: Variation in egg size for nine female capelin from Newfoundland. Female ID and CV (coefficient of variation) are indicated on each chart.

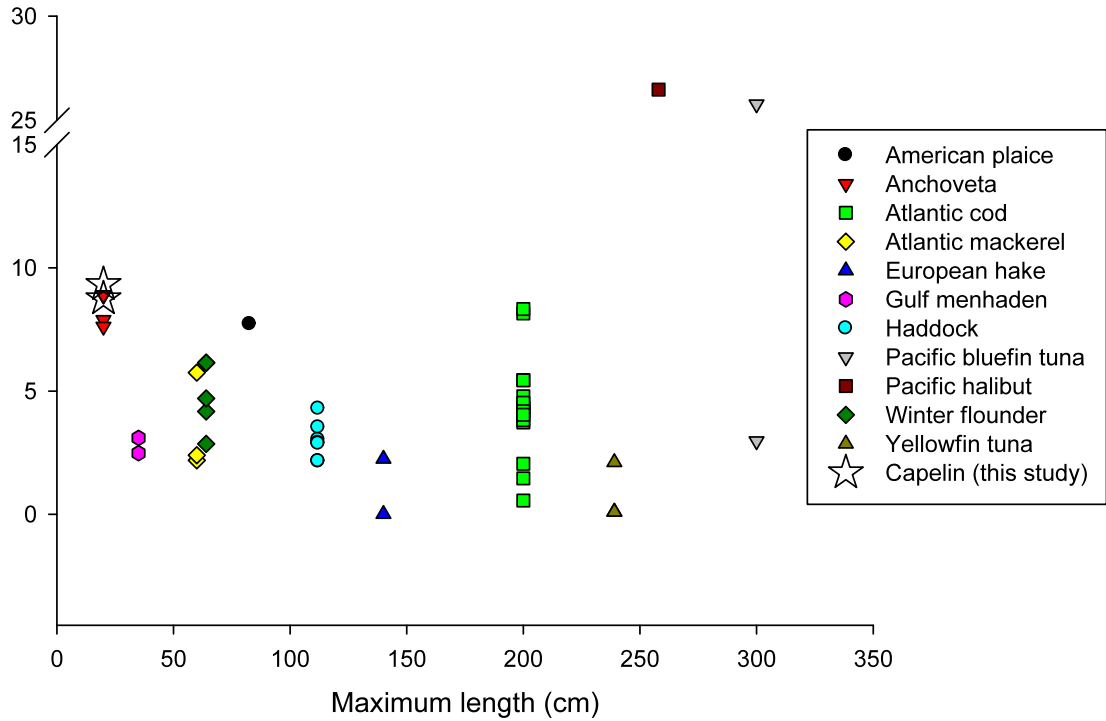


Figure 5.2: Egg size variation in marine commercial fish, and capelin (this study). See Table A2.1 for scientific names and data associated with each point. Capelin data from this study are presented as mean among-female CV for each region (Notre Dame Bay, Trinity Bay).

Table 5.1: Within-female variation (coefficient of variation, CV %) in offspring size of commercially important marine fish.

| Trait | Species | CV (%) range | Source |
|--------------------|--------------------------------------|---------------|-----------------------------|
| Egg Diameter | Atlantic cod | 0.9 – 2.1 | Chambers and Leggett (1996) |
| | <i>Gadus morhua</i> | 2.8 – 7.4 | Chambers and Waiwood (1996) |
| | | 1.35 – 11.63 | Browman et al. (2003) |
| | Atlantic halibut | 0.97 | Blaxter et al. (1983) |
| | <i>Hippoglossus hippoglossus</i> | | |
| | Capelin | 2.5 – 5.2 | Chambers and Leggett (1996) |
| | <i>Mallotus villosus</i> | 7.6 – 9.1 | This study |
| Pacific sand lance | | 8 | Pinto (1984) |
| | <i>Ammodytes personatus</i> | | |
| | Winter flounder | 2.4 – 4.3 | Chambers and Leggett (1996) |
| | <i>Pseudopleuronectes americanus</i> | 10.25 – 11.05 | Butts and Litvak (2007) |
| | Yellowtail flounder | 2.32 – 4.26 | Benoit and Pepin (1999) |
| | <i>Thunnus albacares</i> | | |
| Length at hatch | Atlantic halibut | 3.10 – 3.55 | Pittman et al. (1990) |
| | <i>Hippoglossus hippoglossus</i> | 3.75 | Blaxter et al. (1983) |
| | Atlantic herring | 1.24 – 2.17 | Bang et al. (2006) |
| | <i>Clupea harengus</i> | | |
| | Pacific sand lance | 4.91 | Pinto (1984) |
| | <i>Ammodytes personatus</i> | | |
| Winter flounder | | 3.98 – 4.43 | Bertram et al. (1993) |
| | <i>Pseudopleuronectes americanus</i> | 2.23 – 6.30 | Fraboulet et al. (2009) |

than one study for Atlantic cod and Winter flounder. For Atlantic cod, the range in CV was variable within the study for all three studies, and between all three studies. The range was variable between studies on Winter flounder and within each study. The difference in the range of CV's for this study on capelin was similar to that reported by Chambers and Leggett (1996) (Table 5.1) but the values of the within-female CV's in this study were much higher.

Time to hatch

Time to hatch (TTH) was highly variable within each half-sib group at 4°C (CV = 8.5 to 21.0%; Fig. 5.3) and at 7°C (CV = 8.6 to 22.4%; Fig. 5.4) and patterns were not similar among females. The range in time between first and last hatched larvae for each female was 7-33 days at 7°C, and 11-25 days at 4°C. Mean TTH across all half-sib groups was 33 days at 4°C and 41 days at 7°C. Half-sib family means spanned over a seven-day period at both 4°C (29-36 days) and 7°C (22-29 days) and over a 46-day period at 12°C (13-59 days). Within-female variability in hatching time was higher than among-female variability at both 4°C (within-female CV = 14.3%, among-female CV = 6.1%) and 7°C (within-female CV = 15.5%, among-female CV = 6.0%). At the high temperature, among-female CV (42.3%) was much higher than within females (1.25%).

Egg development rates (average TTH and time to first hatch) in this study at all three temperatures (all sites combined) were comparable to values reported in other studies (Fig. 5.5). The range of development duration at each of the three temperatures in this study; however, was high, with some larvae hatching as late as 50 days post-fertilization (Fig. 5.5).

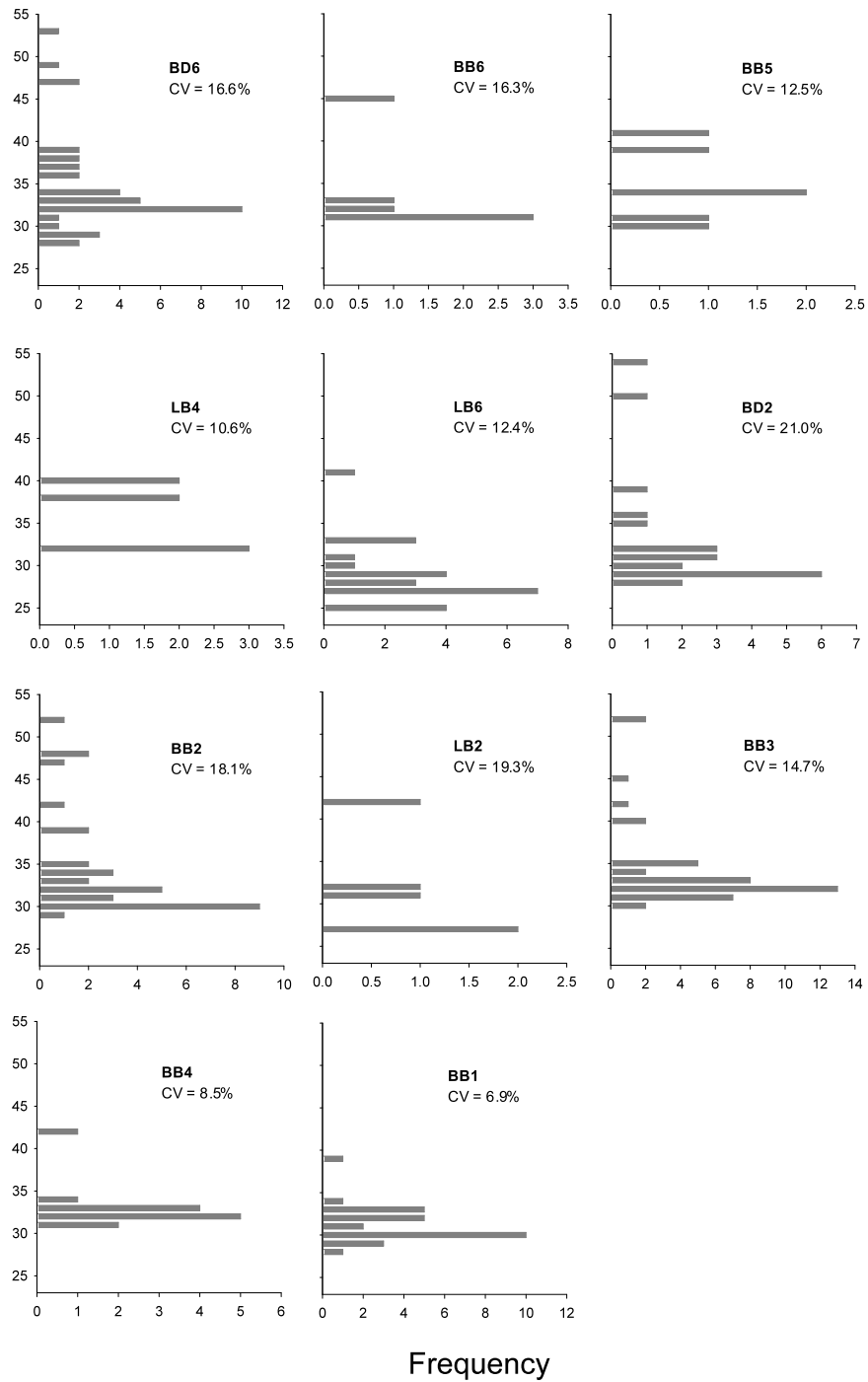


Figure 5.3: Variation in time to hatch for 11 half-sib groups raised at 4°C. Female ID and CV (coefficient of variation) are indicated on each chart.

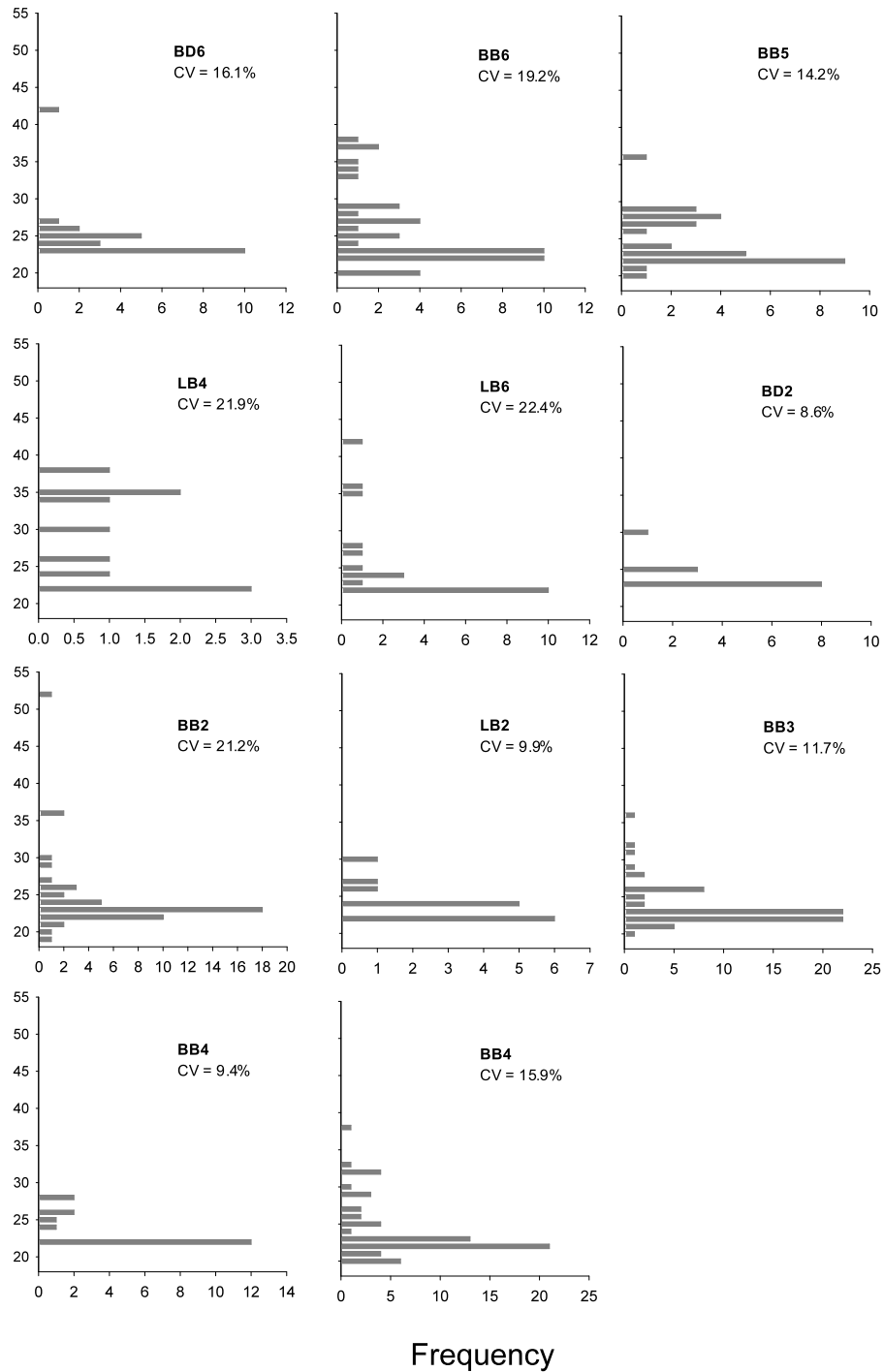


Figure 5.4: Variation in time to hatch for 11 half-sib groups raised at 7°C. Female ID and CV (coefficient of variation) are indicated on each chart.

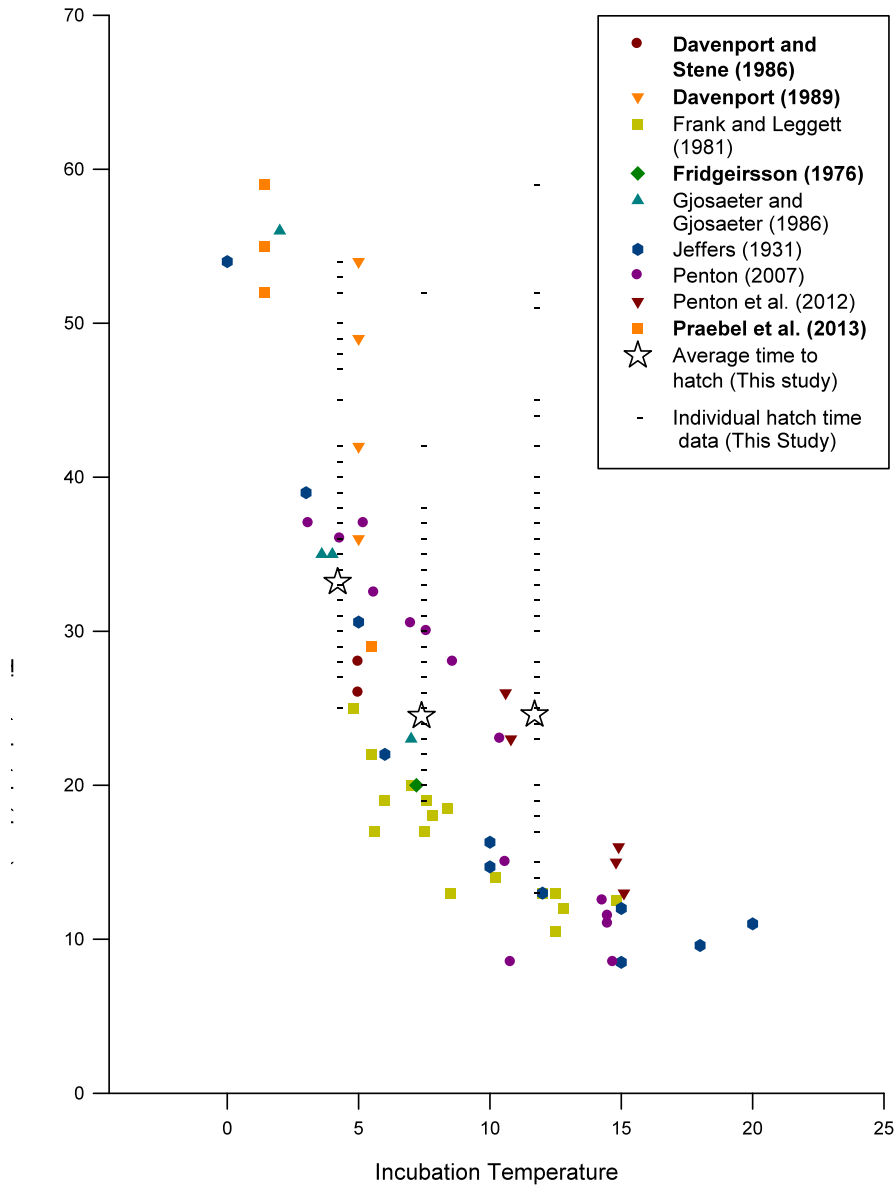


Figure 5.5: Relationship between time to hatch (days) and incubation temperature ($^{\circ}$ C) for capelin, including this study and previously published data. Data from this study includes average time to hatch and hatch time of all larvae produced at 4, 7 and 12 $^{\circ}$ C. Data available from previous publications are average time to hatch or time to first hatch (these studies are in bold in the legend).

Length at hatch

Mean length at hatch across all larvae was 5.038 mm at 7°C and 5.279 mm at 4°C. The range of half-sib group means was similar between the 7°C (4.550-5.725 mm) and 4°C (4.824-5.754 mm) treatments and the variability in length at hatch was similar at both 4°C (CV = 5.4 - 17.1%; Fig. 5.6) and 7°C (CV = 8.7 - 16.8%; Fig. 5.7), higher than egg size variation. Comparing the same half-sib group between the two temperatures did not reveal a clear trend of increased variability at a particular temperature. Within-female variation in length at hatch at both 4°C (within-female CV = 11.2%) and 7°C (within-female CV = 13.4%) was higher than among-female variation at both temperatures (CV = 5.3% at 4°C, 5.1% at 7°C).

Twenty-four studies on 16 commercially important marine fish species provided among-female variation in length at hatch data (Table A2.2). For forage fish ($L_{\max} < 50\text{cm}$), the variation for length at hatch was wide, ranging from of CV of 1 to 15% (Fig. 5.8) and was higher for temperate species (i.e. capelin: 12%; herring: 8%) relative to tropical species (range: 3-5%; Fig. 5.8; Table A2.2). With the exception of haddock, larger, long-lived fish ($L_{\max} > 50\text{cm}$) had CV's less than 6% (Fig. 5.8). Of the forage fish species, capelin in this study had the highest CV values and variability in length at hatch for capelin from both regions and the two temperature treatments were higher than reported in two previous capelin studies (Table A2.2).

Six studies on four commercially important fish reported within-female variation in size at hatch (Table 5.1). Across species, CV ranged from 1.24 to 6.3%, lower than the range observed for within-female variation in egg size.

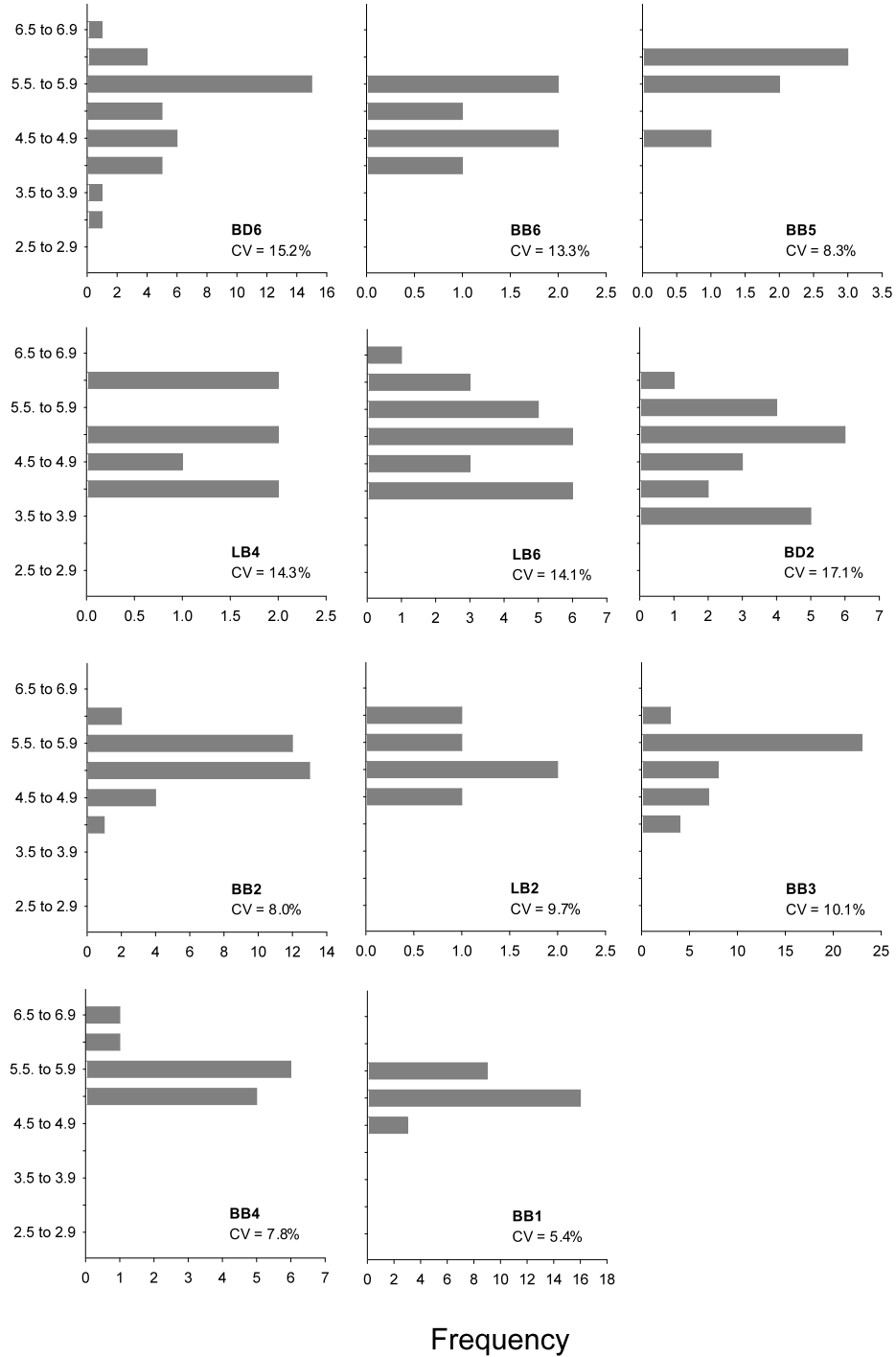


Figure 5.6: Variation in length at hatch for 11 half-sib groups raised at 4°C. Female ID and CV (coefficient of variation) are indicated on each chart.

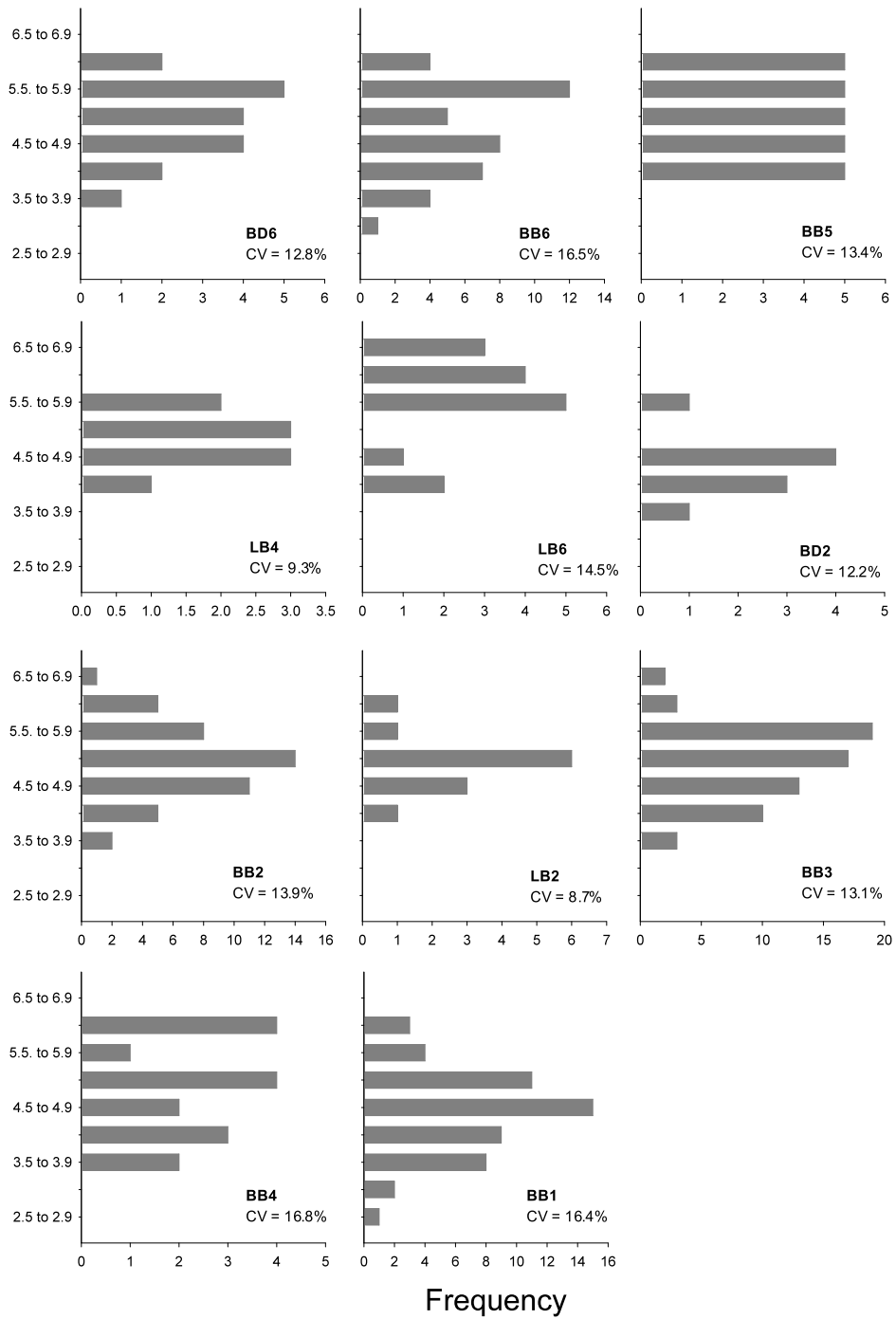


Figure 5.7: Variation in length at hatch for 11 half-sib groups raised at 7°C. Female ID and CV (coefficient of variation) are indicated on each chart.

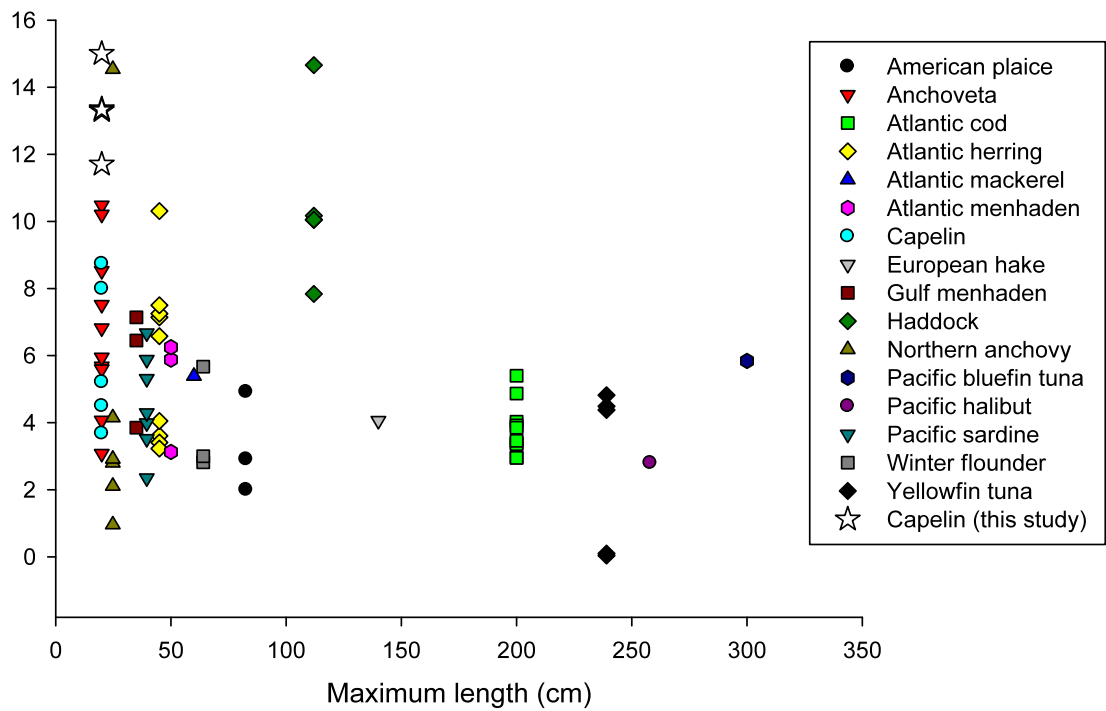


Figure 5.8: Variation in length at hatch for commercially important marine fish. See Table A2.2 for scientific names and data associated with each point. Capelin data from this study are presented as mean among-female CV for the 4 and 7 °C temperature treatments from each region (Notre Dame Bay, Trinity Bay).

DISCUSSION

By quantifying individual- and population-level variability in capelin early life history traits under controlled lab conditions (Chapter 2 (Penton and Davoren 2013a)), I found that within-female variability in traits was higher than the variability among-females. This is consistent with a diversified bet-hedging strategy that increases maternal fitness through the production of variable offspring in response to uncertain environmental conditions (Kassen 2002; Donaldson-Matasci et al. 2008, Marshall et al. 2008). This variability in capelin provides further insight into the mechanisms of the temporally persistent facultative spawning at two environmentally divergent habitats during a period of major changes in capelin biology. In support, within-female variation in offspring size was higher than among-female variation not only in this study, but was also higher than in the 1980's (Chambers and Leggett 1996), when beach spawning appeared to be the norm and timing of spawning was predictable.

Diversified bet-hedging should ensure that at least some individuals are suited for success in unpredictable environments (Koops et al. 2003; Crean and Marshall 2009). Bet-hedging studies typically look for patterns in variation across a range of environments that differ in predictability. For example, Marshall and others (2008) found evidence of diversified bet-hedging by comparing offspring size variation across 5 phyla of marine invertebrates, that were subject to varying degrees of environmental uncertainty. Individual females of a freshwater fish produced more variable egg sizes across streams of increasingly extreme conditions (Morrongiello et al. 2012). With the exception of anomalous data from two studies, capelin demonstrated high variability in offspring size when compared with other marine fish species. Comparing variability

across a range of climates and life history strategies did not reveal any clear trends with the exception that capelin and herring, the two temperate forage fish species, had a higher range in variability than the tropical forage fish species. As environmental conditions are expected to be more variable at higher latitudes (Koops et al. 2003), these findings also are consistent with the hypothesis that bet-hedging is a common phenomenon in uncertain environments.

Given the high levels of uncertainty surrounding present-day capelin biology, understanding processes operating at the egg and larval stage that could influence survival is critical because year class strength of capelin in Newfoundland is determined within the first two weeks of hatching (Carscadden et al. 2013). Hatching time is an important early life history trait and although variability in hatching time has the potential to influence recruitment, it is often overlooked (Green 2008). Synchronous hatching, stimulated by an environmental cue, has been shown to influence recruitment in a variety of taxa, including capelin (Frank and Leggett 1983). In coastal Newfoundland, wind events stimulate synchronous emergence of capelin larvae into an environment that is conducive to survival because of low predator and high prey concentrations (Coastal Water Mass Replacement; Frank and Leggett 1982). This is considered to be one of the few long-lasting environment-recruitment models in marine fisheries (Leggett and Frank 2008). However, this relationship has not been examined since the changes in capelin biology in the 1990's (Carscadden et al. 2013). An examination of larval emergence mechanisms at sub-tidal sites in the mid-2000's revealed that despite the presence of an environmental cue, larval emergence was passive (Penton and Davoren 2008). If environmental cues became unpredictable in the 1990's, the continued synchronous

hatching and emergence of individuals could result in complete reproductive failure. Instead, high variation in hatch time may increase cohort survival, thereby reducing variation in year class strength.

Offspring size is understood to be a major contributor to recruitment in fish (Chambers and Leggett 1996; Johnson et al. 2010) but inherent in that assumption is that the larger larvae have higher probabilities of survival relative to smaller larvae. Larger larvae are thought to contribute more to recruitment because they are able to find food and evade predators more easily than smaller larvae (Bigger-is-Better hypothesis: Cowan and Shaw 2002; Leggett and Frank 2008). This theory results from datasets aggregated at higher (e.g. species) levels of organization and like other paradigms in fisheries biology, does not always apply to individuals (Chambers 1993; Pepin and Miller 1993; Leggett and Frank 2008). While it is true that larger larvae will have bigger mouth gapes (e.g. increased food availability) and can escape more quickly from predators (e.g. increased burst speeds), larger larvae are also subject to increased predation rates due to their increased conspicuousness to predators and higher dietary value (Pepin et al. 1992, Elliott and Leggett 1997). In this study, it is demonstrated that females produce cohorts that are highly variable in size, relative to both other marine fish species and to levels of variability in capelin reported in the 1980's (Chambers and Leggett 1996). If predator-prey fields in the nearshore region have shifted (see above), it seems likely that variability in both offspring size and hatching time will buffer the impact of a weakened environment-recruitment relationship.

Understanding the recent changes in capelin biology (as the key forages fish in the north Atlantic) and monitoring future changes to both the mean and variation of trait

values will be critical to manage ecosystem structure and function. I suggest that variability of early life history traits observed both within and between families produced by individual females is critical for capelin to be successful across the range of temperatures they experience in nature. Many other aspects of capelin life history are also highly variable, including timing of spawning (varying up to 3 weeks over a period of 3 years; Davoren et al. 2013) and fecundity-at-length (Penton and Davoren 2013b (Chapter 4)). The positive relationship between fecundity and length that was typical in capelin prior to the 1990's (Nakashima 1987) has weakened, with females in each length-class producing highly variable numbers of eggs. Although population-level means demonstrate that fecundity is much lower now than prior to the 1990's, it obscures the variation that may have an impact on reproductive success. In populations that show high levels of variation in traits, individuals will respond differently than the population mean (Bradford and Roff 1993), demonstrating the need for incorporating this variability into modeling of year-class strength. Population models using this variation can provide very different outcomes, through the inclusion of frequency dependent effects (Bolnick et al. 2003), thereby providing a more complete understanding of the biological system under investigation.

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CHAPTER SIX: GENERAL DISCUSSION

In the face of climate change, some species are motivated to move into new habitats to minimize extinction risk (Holt 1990; Aitken et al. 2008). Gaining an understanding of the causes and consequences of excursions into new habitats is imperative because global temperatures have already risen over the last decade and additional warming of more than 6.4 °C is expected over the next 100 years (NRC 2010). In the 1990's, a cooling event in the northwest Atlantic led to many changes in the biology of capelin, the forage fish responsible for transferring large amounts of energy from lower trophic levels to top predators (Carscadden and Vilhjalmsson 2002). During this time, capelin in Newfoundland were documented at alternate spawning habitats but the mechanisms underlying the use of an environmentally divergent habitat were unknown. Facultative spawning, that is the movement to spawn in cooler waters when conditions are warm, had been proposed for alternate habitat use (Templeman 1948; Nakashima and Wheeler 2002) but had not been tested. Alternatively, if the divergent environments experienced by capelin at the habitats cause phenotypic variation in heritable, fitness-related traits, adaptive divergence could occur (Conover and Schultz 1997). In other fish, divergent timing, location and thermal habitat of spawning resulted in locally adapted and genetically distinct populations on short time scales (Hendry et al. 2001).

In Notre Dame Bay on the exposed northeast coast of Newfoundland, two temporally distinct (8 – 12 days; Penton et al. 2012) spawning runs of capelin are associated with beach and sub-tidal spawning habitats (Davoren et al. 2007). These spawning habitats are dramatically different with the beach being a highly variable

environment due to unpredictable magnitudes of wave action, inundation with water, solar radiation and oxygen concentrations (Frank and Leggett 1981; Præbel et al. 2009). In contrast, adults at sub-tidal spawning sites are not exposed to the terrestrial environment, embryos experience colder but less variable temperature regimes (Penton et al. 2012) and currents are sufficient to replenish oxygen to the egg layer (Stergiou 1989; Penton 2007). Field-based investigations of early life history stages in Notre Dame Bay provided evidence for divergence in fitness-related egg development strategies (Penton et al. 2012) and larval emergence mechanisms (Penton and Davoren 2008).

The goal of this dissertation was to gain an understanding of the mechanisms underlying excursions of capelin into environmentally divergent habitats during localized warm conditions. Due to their role in the north Atlantic food web, changes in capelin biology in response to changing temperatures has had major implications for ecosystem processes, coining them as a “sea canary” for climate change (Rose 2005). An increased understanding of the recent changes to their spawning biology is imperative because despite an unknown spawning biomass since the 1990’s, the occurrence of sub-tidal spawning is not incorporated into capelin stock assessments (DFO 2011). A secondary goal of this research was to provide insight into the responses of capelin to warming sea temperatures, especially since capelin in Newfoundland are thought to be the first stock throughout their north Atlantic distribution to respond to these changes (Carscadden et al. 2013).

An experiment, combined with comparative analyses of morphometrics, condition indices, maternal traits and variation in early life history traits were used to address the goal of this dissertation. The benefit of this combination of techniques is that together,

they have the potential not only to provide strong evidence for or against local adaptation to environments with different selection pressures, but can also provide a comprehensive insight into mechanisms of alternate habitat use. Results of my dissertation research demonstrate that rather than adaptation to local conditions, capelin exhibit variation in many life history traits and spawning habitat decisions that allow them to utilize both of the divergent inshore spawning habitats.

In a lab experiment (Chapter 2), I raised capelin eggs from beach and sub-tidal sites at three different temperatures to mimic conditions experienced by capelin at the two sites in nature (Penton et al. 2012). These common garden experiments are frequently used to distinguish between genetic variability and phenotypic plasticity in early life history traits (Beacham and Murray 1986; Hendry et al. 1998; Haugen and Vøllestad 2000; Conover et al. 2006). The lack of difference in reaction norms between early life history stages from beach and sub-tidal spawning sites provided support for facultative spawning. The findings from this chapter are further supported by a lack of genetic differentiation between beach and sub-tidal spawning adults (Appendix I) and the results of a recent tagging study (Davoren 2012).

Raising the early life history stages at this range of temperatures also provided insight into the mechanisms surrounding the utilization of the two habitats. The larvae produced at both 4 and 7°C had higher hatching success and hatched at a larger size with more nutritional reserves than larvae at 12°C. This suggests that if capelin can utilize alternate habitats to produce eggs in temperatures more suitable for survival (i.e. shift to sub-tidal habitat when beaches become too warm), this may maximize recruitment and minimize population-level impacts of climate change.

A comparative analysis of body shape and condition between adults at the two spawning habitats provided further support for facultative spawning (Chapter 3). Morphometrics in fish are commonly used for two purposes: to determine how body shape is adapted to divergent environments (Arbour et al. 2001; Janhunnen et al. 2009; Sheehan et al. 2005), and to distinguish among commercially important fish stocks (Cadrin 2000; Kocovsky et al. 2009). Studies using morphometrics for stock differentiation rarely relate differences in traits to a particular environment. Supported by the lack of genetic divergence between beach and sub-tidal spawning adults (Appendix I), capelin body shape was not adapted to a particular habitat. To extend the analysis, I combined morphometric and condition indices to examine if these intrinsic factors influence the use of a particular habitat. Capelin spawning at the beach sites were better equipped for the physical demands of spawning at the exposed beach environment. Females were in good condition and males had deeply forked tails. With these traits, the cost of increased predation at sub-tidal spawning environments may exceed the cost of an energetically demanding spawning environment when sub-tidal sites are available. This suggests that capelin may select spawning habitat based on a trade-off between predation risk and energy available to maximize lifetime reproductive success.

Potential fecundity is a major contributor to the reproductive success of an individual but is largely underreported (Lambert 2008). Although spawning-habitat based differences in fecundity could have major implications because it would require different management, I did not find any habitat-based differences in fecundity (Chapter 4). Therefore, I combined all females to examine the current status of the reproductive potential of capelin because, despite changes in capelin biology in the 1990's, it has not

been examined in Newfoundland since the 1980's. Maximum potential fecundity was found to be lower than in the previous study and the relationship between fecundity and body size has weakened, negating its use as a proxy for fecundity in present-day coastal Newfoundland. Life history traits (mass, somatic mass and egg size) and condition indices (Fulton's K, HSI and GSI) also failed as possible proxies for fecundity of capelin in the study area.

My investigations up until this point in the dissertation supported the facultative use of spawning habitats (Chapters 2 & 3). Given that the habitats are environmentally divergent and both are not available in all years (Davoren et al. 2012), I investigated bet-hedging as a strategy that would allow them to produce viable offspring in either habitat (Chapter 5). Diversified bet-hedging is the within-female production of phenotypically diverse offspring (Einum and Fleming 2004). Higher within-female variation in offspring size and developmental duration compared to among-female variation supports diversified bet-hedging in capelin, a strategy that would allow them to spawn in thermally available habitats without experiencing reproductive failure.

Overall, the results of the studies conducted for this dissertation provide the first support for facultative spawning in coastal Newfoundland and high variability in all life history traits examined. Although the facultative spawning hypothesis that was first proposed in the 1940's has been used to explain sub-tidal spawning in recent years, this has not received direct support until now. Capelin that use these divergent spawning habitats, therefore, are not reproductively isolated sub-populations and do not differ in important biological traits, such as fecundity. These findings support the current management of beach and sub-tidally spawning capelin as a single unit. The high

variability in egg size, fecundity, duration of egg development and larval size at hatch however, demonstrates that management decisions based on population means will not be applicable to all individuals. Both trait means and their variation should be considered when making decisions about managing the fishery of this ecologically important fish, especially at a time when high levels of uncertainty dictate a precautionary management approach.

My results also provide insight into predictions surrounding the response of capelin to climate change. At present, the two major predictions about how capelin in Newfoundland will respond to climate change are that they will shift their distribution 400-1800 km northward (Rose 2005) and early life history stages will be the most sensitive to climate change due to adhesive eggs and a beach spawning strategy (VanGuelpen et al. 2007). My dissertation can provide further insight into predictions surrounding the responses of capelin to climate change because they suggest that capelin may be more tolerant to climate change than previously expected. Although capelin have already begun to expand their distribution into the arctic (Gaston et al. 2003; Provencher et al. 2012), capelin may also remain at their southernmost distribution in Newfoundland by seeking refuge from warming spawning conditions in deeper, colder waters. In addition, high variability in all life history traits rather than specialization to a specific habitat will buffer the impact of warming temperatures by preventing complete reproductive failure. Together, these characteristics suggest that this key forage fish will have a high tolerance to climate change whether they remain in their current range and or shift farther north.

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APPENDIX I: MANUSCRIPT SUMMARY

Penton, P.M., McFarlane, C.T., Docker, M.F., Davoren, G.K. (in prep). Lack of genetic divergence in capelin (*Mallotus villosus* - Osmeridae) spawning at beach versus sub-tidal habitats in coastal embayments of coastal Newfoundland.

For this manuscript, I formulated the question, designed the sampling protocol and collected fin clip samples in the field. I conducted preliminary screening of molecular markers and C.T. McFarlane processed and analyzed all samples to completion. C.T. McFarlane wrote the molecular methods and results and I wrote the introduction, collection methods and discussion. All authors provided comments and revisions on two versions of the manuscript. Dr. Gail Davoren and Dr. Margaret Docker provided financial and logistical support.

**LACK OF GENETIC DIVERGENCE IN CAPELIN (*MALLOTUS VILLOSUS* -
OSMERIDAE) SPAWNING AT BEACH VERSUS SUB-TIDAL HABITATS IN COASTAL
EMBAYMENTS OF NEWFOUNDLAND**

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Running Title: no divergence between capelin spawning sites

ABSTRACT

Capelin (*Mallotus villosus*) – a focal forage fish in the north Atlantic – spawn on both beach and demersal (deep-water) sites throughout their circumpolar distribution. Although these habitats rarely occur in close proximity, demersal spawning sites within 4 km of beach spawning sites (sub-tidal) have recently been discovered in two coastal embayments in Newfoundland, Canada. The physical environment differs considerably between beach and demersal spawning sites, creating the potential for local adaptation and divergence of capelin from the two habitats, but this has never been investigated on a fine spatial scale. We use eight microsatellite loci to test for genetic divergence between capelin spawning at beach and sub-tidal sites within these two coastal regions in Newfoundland. We found no genetic differentiation between fish spawning at beach and sub-tidal sites or between the two regions. The results from this fine-scale study are in agreement with the lack of habitat-based structure found in other studies examining beach and demersal sites separated by a larger geographic area. We suggest that instead of showing site fidelity and local adaptation, capelin employ a generalist strategy that may be more successful in an unpredictable environment.

Keywords: capelin, *Mallotus villosus*, reproduction, habitat, microsatellites, population structure

INTRODUCTION

Capelin (*Mallotus villosus*), small, short lived (3–6 year), migratory, schooling fishes native to the northern hemisphere, are an essential component in the diets of seabirds, marine mammals and large predatory fish such as Atlantic cod (*Gadus morhua*) (Lavigne 1996; Friis-Rødel and Kannevorff 2002; Davoren and Montevecchi 2003). Capelin spend most of their annual cycle feeding in offshore waters; at maturation they undergo extensive inshore migrations to spawning grounds at either beach or demersal (at depths of up to 90 m) sites (Carscadden and Nakashima 1997). Previous genetic studies concluded that capelin in the north Atlantic are subdivided into two distinct clades: the northwest Atlantic (Newfoundland, St. Lawrence Estuary and Hudson's Bay) and northeast Atlantic (Iceland and Barents Sea) (e.g., Dodson et al. 2007; Præbel et al. 2008; Colbeck et al. 2011). These geographically delineated clades show differences in vertebral number, fecundity, egg size and spawning mode (Stergiou 1989). Capelin from the northeast Atlantic Ocean are generally demersal (oceanic) spawners (Vilhjálmsson 1994), with the exception of local fjord populations that spawn on beaches (Davenport and Stene 1986). Those in the northwest Atlantic were thought to primarily spawn on coastal beaches, with the exception of an offshore demersal spawning site located on the Grand Banks (Southeast Shoal, Carscadden et al. 1989). Throughout their circumpolar distribution, beach and demersal sites do not typically occur in close proximity. Recently, however, persistent demersal spawning sites have been discovered adjacent to spawning beaches (sub-tidal) at two coastal Newfoundland locations (Nakashima and Wheeler 2002; Davoren et al. 2008).

Capelin eggs adhere to the sediment for the duration of the incubation period (Gjøsæter and Gjøsæter 1986), leaving early life history stages directly subject to the environmental features of the spawning habitat. These features differ dramatically between beach and demersal spawning sites, creating the potential for divergent selection pressures. The beach is a highly variable environment subject to unpredictable daily fluctuations in wave action and inundation with water and solar radiation, all of which influence the temperature, salinity and oxygen concentrations experienced by incubating eggs (Frank and Leggett 1981; Christiansen et al. 2008; Præbel et al. 2009). Beach spawning adults can become stranded at high tide and thus are subject to intense predation by vertebrates, including humans (Jangaard 1974). At demersal sites, adults are not exposed to the terrestrial environment, currents are sufficient to replenish oxygen through the egg layer (Stergiou 1989; Penton 2007) and incubation temperatures are less variable (Præbel et al. 2009; Penton et al. 2012). Analysis of life history traits suggest that capelin from the few beach spawning populations in the fjords of the northeast Atlantic are smaller, mature earlier, have smaller eggs and are iteroparous compared to their demersal counterparts (Christiansen et al. 2008). Differences in fitness-related traits have also been reported between the two habitats in coastal Newfoundland; at the beach, eggs experienced high mortality (4–85%) and developed faster relative to the demersal sites in the sub-tidal zone (Penton et al. 2012), and larval emergence mechanisms that can be used to distinguish capelin stocks (Fortier et al. 1987) differ between the habitats (Frank and Leggett 1982; Penton and Davoren 2008), suggesting potential for local adaptation and divergence.

It was generally thought that an absence of physical barriers in the marine environment precludes the evolution of local adaptation in marine organisms due to high gene flow among locations (Bohonak 1999; Conover et al. 2006; Hauser and Carvalho 2008). Empirical evidence for ecologically-based divergent natural selection in marine fish, however, continues to accumulate (e.g., rainbow smelt (*Osmerus mordax*; Coulson et al. 2006), Atlantic silverside (*Menidia menidia*; Clarke et al. 2010), Atlantic cod (*Gadus morhua*; Grabowski et al. 2011)). Ecological speciation can occur when organisms exposed to divergent selective regimes at some stage of their life evolve reproductive isolation as a by-product of adaptation (Schluter 2001). For example, behavioral, ecological, or other phenotypic differences between individuals from divergent habitats can lead to assortative mating, even in the absence of physical barriers, and survival, growth, or reproductive success of immigrants from one habitat to another can be reduced relative to the better adapted resident population (Hendry and Day 2005; Schluter and Conte 2009). Reproductive isolation between individuals from divergent habitats can be rapid; Hendry et al. (2000) presented evidence for the evolution of reproductive isolation in sockeye salmon (*Oncorhynchus nerka*) colonizing different spawning habitats – river and lake beach – after only 13 generations.

The determination of genetically distinct reproductive units is essential for management of commercial fisheries to ensure more vulnerable distinct spawning groups are not overfished (Reiss et al. 2009). Genetic studies on spatial scales relevant to fisheries management are available for only two commercial fish species in the northeast Atlantic (i.e. Atlantic cod and herring (*Clupea harengus*); Reiss et al. 2009). In contrast to cod and herring (for which there are at least 20-50 published genetic studies on a

variety of spatial scales; Reiss et al. 2009), fewer than ten population genetic studies have been conducted on capelin in the north Atlantic. The majority of these investigations have been on scales spanning their north Atlantic distribution (e.g., Præbel et al. 2008; Colbeck et al. 2011) or using mitochondrial DNA markers (e.g., Dodson et al. 1991, 2007), restricting their potential to examine finer scale reproductive units, such as between habitat types in close geographic proximity. Whereas mtDNA tends to show historic patterns of reproductive isolation and colonization, microsatellites have a relatively fast mutation rate which makes them effective for detecting recent, fine-scale divergence (Selkoe and Toonen 2006). As capelin is the focal forage fish species in the northwest Atlantic, on which most top predators rely for prey (Lavigne 1996), it is important to understand whether beach and sub-tidally spawning fish constitute a single or separate gene pools for sound fisheries management. Using eight microsatellite loci, we thus tested for genetic differentiation between capelin from two spawning habitats (i.e. beach and sub-tidal) in close proximity within two Newfoundland embayments.

MATERIALS AND METHODS

Sample collection

Capelin were collected from two beach and two sub-tidal spawning locations in two coastal regions of Newfoundland: Trinity Bay and Notre Dame Bay (Fig. A1.1). These are the only two regions in Newfoundland where beach and sub-tidal spawning are known to occur in close proximity. Within Trinity Bay, spawning occurs at Bellevue Beach and at relatively shallow (<20 m) sub-tidal sites that are located less than 1 km

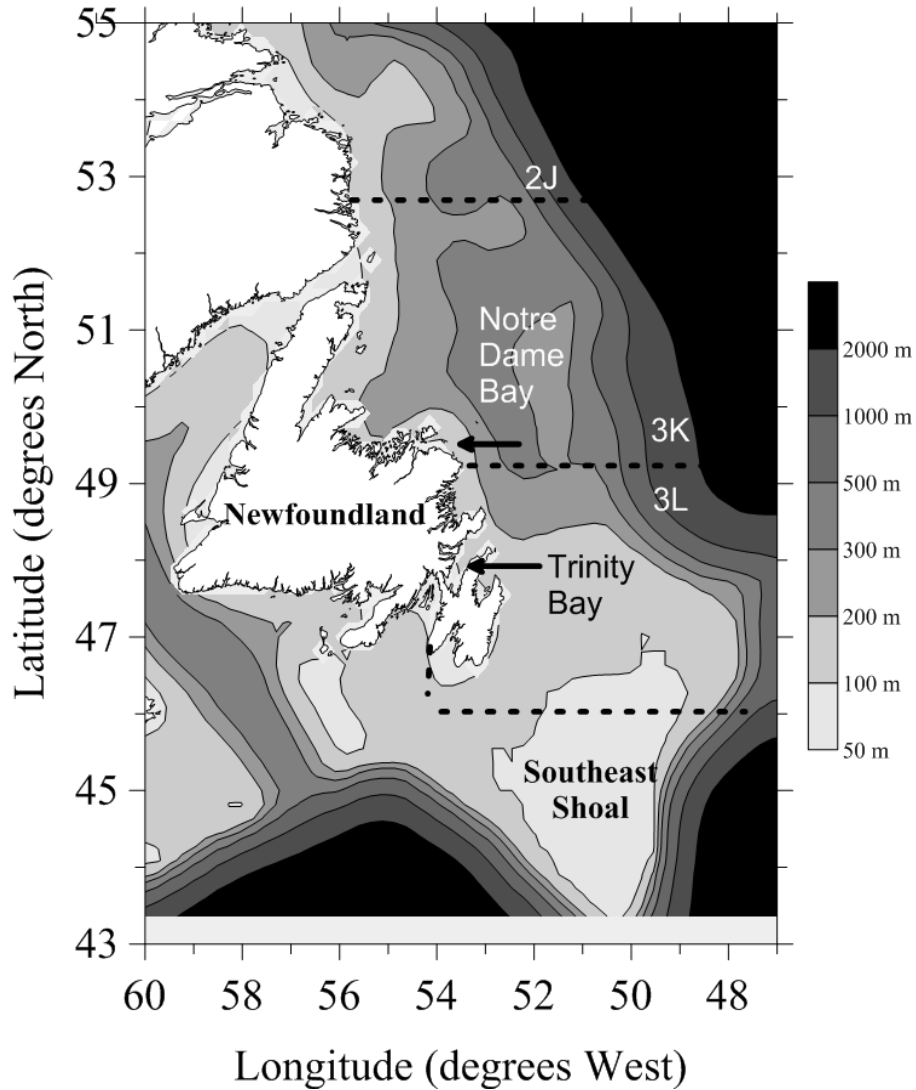


Figure A1.1: Map of Newfoundland, indicating the location of Trinity Bay (Bellevue Beach), Notre Dame Bay (Lumsden Beach) and the Southeast Shoal, subdivided into Northwest Atlantic Fishery Organization Convention Areas 2J, 3K and 3L with depth indicated by gray shading.

from shore (Nakashima and Wheeler 2002). In this region, temperatures and timing of spawning overlap between beach and sub-tidal sites (Nakashima and Wheeler 2002). In Notre Dame Bay, Lumsden Beach and the sub-tidal spawning sites are located along the exposed northeast coast (Fig. A1.1); compared to Trinity Bay, the sub-tidal spawning sites are up to 38 m deep and are farther from shore (up to 4 km; Penton 2007; Penton and Davoren 2008). In this region, two temporally distinct spawning runs of capelin occur, separated by 1–2 weeks and beach temperatures are high and variable relative to sub-tidal sites (Penton et al. 2012).

Spawning fish were sampled for molecular analyses at spawning sites, as these fish represent the ‘reproductive unit’. At beaches, capelin were collected using a 1.8 m cast net with 9.5 mm mesh (Wildco, USA) or a dip net with 4.8 mm mesh (Frabill, USA). At the Bellevue sub-tidal site, capelin were collected using a modified crab pot deployed from the *Narry Face*, a Fisheries and Oceans Canada research vessel; at the Lumsden sub-tidal site, a tuck seine deployed from the *Lady Easton II* was used to collect fish in spawning condition. In 2008, 50 gravid females were collected from sub-tidal sites in both regions but due to a lack of spawning at Lumsden Beach, 50 females were collected from Bellevue Beach only (Table A1.1). In 2009, 25 sexually mature capelin of each sex were collected from Lumsden and Bellevue beaches. Sub-tidal collections were made only at Bellevue due to the lack of spawning at the Lumsden sub-tidal site in 2009 (Table A1.1). Both sexes were collected in the second year of the study to ensure that local adaptation and genetic divergence was not sex dependent because males are thought to select spawning habitat (Vilhjálmsson 1994). Dorsal fin clips were taken and stored in 70% ethanol.

Table A1.1: Summary of samples collected for molecular analysis at beach and demersal (sub-tidal) spawning sites in both the Trinity Bay (Bellevue) and Notre Dame Bay (Lumsden) regions on the east coast of Newfoundland in 2008 and 2009. Spawning male capelin were not collected in 2008. NS indicates no spawning by capelin at a site.

| Year | Location | Habitat | Collection Date | Females | Males |
|------|----------|----------|-----------------|---------|-------|
| 2008 | Bellevue | Beach | 15-Jul | 50 | - |
| | | Demersal | 19-Jul | 50 | - |
| | Lumsden | Beach | NS | 0 | - |
| | | Demersal | 25-Jul | 50 | - |
| 2009 | Bellevue | Beach | 23-Jul /01-Aug | 26 | 0 |
| | | Demersal | 24-Jul | 25 | 25 |
| | Lumsden | Beach | 25-Jul | 25 | 26 |
| | | Demersal | NS | 0 | 0 |

Genetic analysis

All DNA was extracted using the Wizard[®] Genomic DNA purification kit (Promega), following manufacturer's instructions. The samples were then genotyped at eight microsatellite loci: Mav135, Mav9, Mav53, Mav38 and Mav17 (Røed et al. 2003), and *Mvi9*, *Mvi5*, and *Mvi16* (Gordos et al. 2005). Polymerase chain reaction (PCR) was used to amplify the microsatellite fragments. PCR were done in 10 μ L volumes with 1 \times PCR Buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 2.5 mM MgCl₂, 0.2 mM dNTP, 0.4 μ M of each primer including fluorescently labeled forward primers (Sigma and Applied Biosystems), and 0.2 units of *Taq* DNA polymerase (Invitrogen). The PCR reaction conditions were: initial denaturation at 94 °C for 2 min followed by 35 cycles of 30 s denaturation at 94 °C, annealing at temperatures ranging from 45–65 °C for 30 s (Table A1.2; Røed et al. 2003; Gordos et al. 2005), extension for 30 s at 72 °C and a final extension at 72 °C for 5 min. Analysis of microsatellite fragments was done on an Applied Biosystems 3730xl genetic analyzer and allele sizes were determined with GeneMapper 4.0 (Applied Biosystems). Arlequin (Excoffier et al. 2005) was used to implement the following: Hardy-Weinberg equilibrium (HWE) was tested at each locus using an approximation of Fisher's exact test; expected heterozygosity (H_E); an exact test of population differentiation and F_{ST} were calculated for pairwise population comparisons; and P -values were calculated with a permutation test. The samples were divided by spawning site, sampling location, year collected and sex for pairwise comparisons. Simulations were used to test the power of our dataset to detect very small values of F_{ST} with the program POWSIM (Ryman and Palm 2006). Four simulated populations representing the Lumsden beach and sub-tidal and Bellevue beach and sub-

tidal spawning sites were drawn from our data and allowed to drift for a number of generations. The statistical power testing a hypothetical estimate of the true level of differentiation was then estimated for a range of values (Ryman and Palm 2006).

Structure v2.3.2 (Pritchard et al. 2000) was used to determine whether Bayesian model based clustering could determine whether there were any groups of genetically similar capelin. The model was run assuming admixture, correlated allele frequencies between subpopulations, and not using any prior population information. The program was run at values of k ranging from 1 to 8 with a burn-in of 50,000 steps and 50,000 steps; each k was replicated 20 times. The most likely k was chosen by the highest value of $P(k | \text{our data})$ (Pritchard et al. 2000).

RESULTS

The number of individuals genotyped ranged from 230 to 268 per locus. A Fisher's exact test found that only *Mvi5* was in HWE; most loci are out of HWE due to homozygote excess (Table A1.2). Two of these loci (Mav9 and Mav53) are potentially suffering from large allele dropout because they do not cover the high end of the size range reported by Røed et al. (2003). Removing these loci from the analysis, however, did not change the results (see below), and the F_{ST} values for the loci that were not in HWE were consistent with those from *Mvi5*. Values of F_{ST} ranged from -0.001 to 0.005 with all 8 loci (-0.003 to 0.004 when Mav9 and Mav53 were omitted) but none were significantly different from zero and none of the exact tests showed significant difference in any pairwise comparison (Table A1.3). There is therefore no significant genetic

Table A1.2: Assessment of genetic diversity at eight microsatellite loci previously developed in capelin. For each locus annealing temperature (T_a), number of individuals genotyped (n), number of alleles (n_a), size range of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_E) were determined. Arlequin (Excoffier et al. 2005) was used to test for loci not in HWE, indicated by *.

| Locus | T_a | n | n_a | Size Range | H_o | H_E |
|---------------------------|-------|-----|-------|------------|--------|-------|
| Mav135 ¹ | 51 | 262 | 16 | 89-121 | 0.830* | 0.900 |
| <i>Mvi9</i> ² | 65 | 268 | 29 | 92-208 | 0.812* | 0.955 |
| Mav9 ¹ | 51 | 255 | 30 | 172-208 | 0.613* | 0.931 |
| Mav53 ¹ | 45 | 262 | 32 | 80-144 | 0.750* | 0.951 |
| <i>Mvi5</i> ² | 56 | 230 | 16 | 96-144 | 0.714* | 0.859 |
| Mav38 ¹ | 46 | 266 | 12 | 138-168 | 0.562* | 0.750 |
| <i>Mvi16</i> ² | 63 | 252 | 50 | 164-348 | 0.782* | 0.974 |
| Mav17 ¹ | 55 | 253 | 15 | 205-243 | 0.772 | 0.797 |

Source of primers: 1 – Røed et al. (2003), 2 – Gordos et al. (2005)

Table A1.3: Pairwise F_{ST} values (below the diagonal) were calculated with Arlequin (Excoffier et al. 2005) and P -values are indicated above the diagonal; an exact test also showed no significant pairwise differences. Populations (for 2008 and 2009) are: Bellevue Beach (BB); Bellevue Demersal (BD); Lumsden Demersal (LD); Lumsden Beach (LB).

| | BB 08 | BD 08 | LD 08 | BB 09 | BD 09 | LB 09 |
|-------|-------|--------|-------|-------|-------|-------|
| BB 08 | - | 0.586 | 0.937 | 0.432 | 0.396 | 0.775 |
| BD 08 | 0 | - | 0.928 | 0.144 | 0.099 | 0.937 |
| LD 08 | 0.002 | -0.002 | - | 0.441 | 0.108 | 0.802 |
| BB 09 | 0.002 | 0.005 | 0.003 | - | 0.441 | 0.577 |
| BD 09 | 0.002 | 0.004 | 0.004 | 0.001 | - | 0.586 |
| LB 09 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | - |

differentiation between regions (Bellevue and Lumsden), year, or spawning habitat (beach or sub-tidal). There are also no significant differences based on sex at any spawning location; the maximum pairwise F_{ST} was 0.03 between beach spawning males and sub-tidal spawning females at Bellevue Beach ($P = 0.008$) and the minimum was -0.002. Structure determined that there is most likely 1 cluster ($P(k | x) = 1$). POWSIM simulations of statistical power suggested that the sample sizes, number of loci and the specific loci chosen were sufficient to discover very low values of F_{ST} . The study design gives a >90% probability of detecting $F_{ST} > 0.002$.

DISCUSSION

Despite divergent environmental conditions experienced by capelin embryos and adults in beach versus demersal spawning sites, we found no evidence of genetic differentiation between capelin spawning in these different habitats within coastal embayments of Newfoundland. Previous studies examining genetic structure of capelin in the north Atlantic have not found differences between beach and demersal spawning habitats, but this question has never been directly addressed on a fine geographic scale. Using mitochondrial DNA data, Dodson et al. (1991) found no significant haplotype frequency differences between six beach spawning populations in Newfoundland and a demersal spawning population from the Southeast Shoal, located approximately 350 km from the coast (Carscadden et al. 1989). Similarly, Mork and Friis-Sørensen (1983) found no significant allozyme frequency differences between capelin from beach and demersal sites in the northeast Atlantic separated by more than 200 km, but mtDNA and allozymes generally do not provide sufficient resolution for examining recent divergence

(Sunnucks 2000). Microsatellites – with their relatively fast mutation rate – are the “marker of choice” for population genetic studies (Sunnucks 2000). Although significant genetic differentiation in capelin was not found using microsatellites from distant (> 200 km) beach and demersal sites in the northeast Atlantic (Präbel et al. 2008), it has been suggested that fine-scale genetic structure within regions, specifically reproductive modes, should be examined further. The current study examined genetic differences between replicate beach and sub-tidally spawning capelin in close proximity (<4 km apart) with microsatellite loci shown to have the power to detect very small values of F_{ST} . Although the majority of loci used in this study were out of HWE, F_{ST} is relatively robust to violations of HWE (Weir and Cockerham 1984) and the consistent values of F_{ST} produced across all loci suggest that there is no systematic bias. Although Structure is less robust to violations of HWE (Pritchard et al. 2000), the results were consistent with those found with F_{ST} .

Despite evidence of local adaptation in other marine species with high dispersal capabilities and an absence of physical barriers (Bohonak 1999; Conover et al. 2006; Hauser and Carvalho 2008), our results suggest that capelin in coastal Newfoundland are not locally adapted to conditions specific to beach and sub-tidal spawning habitats. Common in many examples of ecological speciation is a limited ability to disperse (e.g., rough periwinkle (*Littorina saxatilis*); Grahame et al. 2006) or high site fidelity (e.g., sockeye salmon (*Oncorhynchus nerka*; Hendry et al. 2000). High gene flow or low heritability in timing and location of spawning are two possible reasons for the lack of genetic structure observed in this study. Larvae from spawning sites across coastal Newfoundland are predicted to passively drift offshore where immature and maturing

capelin are thought to overwinter together in offshore feeding grounds (Templeman 1948; Dodson et al. 1991). There is also a high potential for mixing during inshore migrations to spawning grounds. Mixing of mature fish inshore prior to spawning is thought to be extensive over large areas (NAFO divisions 3L and 3K; Fig. 1), as capelin arrive first at the southeast coast of Newfoundland and move northward along the coast, entering large bays with suitable spawning beaches (Nakashima 1992). Heritability in timing and location of spawning is thought to promote local adaptation and divergence in salmon (Hendry and Day 2005). Although philopatry has been suggested in a beach spawning capelin population in the fjords of Norway (Christiansen et al. 2008), this population is not thought to migrate out of the fjord system. In capelin, high dispersal capabilities and mixing combined with a general lack of evidence of site fidelity emphasizes the likelihood of gene flow.

As predicted by Dodson et al. (1991), it appears that spawning site choice in coastal Newfoundland is facultative (e.g., that capelin spawn at sub-tidal sites when beach temperatures are too warm; Templeman 1948). That individual capelin show a high degree of variability in many of the traits needed to both reproduce (e.g., fecundity and egg size; Christiansen et al. 2008) and develop (e.g., eggs survive at temperatures from -4 to 22 °C; Davenport and Stene 1986) in conditions experienced at both beach and demersal spawning environments suggests that capelin are ecological generalists, capable of successfully raising offspring under a wide range of environmental conditions (Penton et al. 2012; Præbel et al. 2009). Such a generalist strategy (instead of showing site fidelity and local adaptation) is predicted to be highly advantageous for species living in an unpredictable environment (Kassen 2002).

The present study suggests that capelin spawning in different habitats in coastal Newfoundland can be managed as a single stock without risking impact on recruitment or loss of genetic diversity (Carvalho and Hauser 1994). Although lack of evidence of genetic differentiation, of course, is not evidence of genetic homogeneity, microsatellite loci shown to have the power to detect very small values of F_{ST} showed no genetic divergence between the habitats or regions examined. Although genetically distinct reproductive units should be the first requirement for stock identification (Reiss et al. 2009), a holistic approach is thought to increase the probability of correct stock identification (Begg and Waldman 1999). However, the lack of genetic structure in this study should be corroborated by additional stock identification techniques (e.g., morphometrics, meristics, tagging, life history traits, otolith microchemistry; Coyle 1998; Begg and Waldman 1999).

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**APPENDIX II: DATA FOR EXAMINING VARIATION IN OFFSPRING SIZE OF
COMMERCIALY IMPORTANT MARINE FISHES**

Table A2.1: Overview of within-population egg size variation in commercially important marine fish. CV (coefficient of variation) of egg size was calculated from data reported in the documents indicated throughout the table. Basic biological information (climate and L_{\max} (the maximum length reported for the species)) is taken from FishBase.org.

| Species | Climate | L_{\max} (cm) | Egg Size (mm) | CV (%) | Source of Broodstock | Source |
|--|-------------|-----------------|---------------|--------|----------------------|---------------------------------------|
| American plaice <i>Hippoglossoides platossoides</i> | Temperate | 82.6 | 1.731 | 7.72 | USA | Howell and Caldwell (1984) |
| Anchoveta <i>Engraulis ringens</i> | Subtropical | 20 | 0.266 | 7.89 | Chile | Llanos-Rivera and Castro (2006) |
| | | | 0.259 | 8.88 | | |
| | | | 0.302 | 7.62 | | |
| | | | 0.292 | 8.90 | | |
| Atlantic cod <i>Gadus morhua</i> | Temperate | 200 | 1.37 | 1.46 | Iceland | Libungan (2009) |
| | | | 1.46 | 2.05 | Iceland | Marteinsdottir and Steinarsson (1998) |
| | | | 1.34 | 3.73 | | |
| | | | 1.43 | 4.20 | | |
| | | | 1.46 | 4.79 | | |
| | | | 1.62 | 4.32 | Baltic Sea | Vallin and Nissling (2000) |

Table A2.1 continued...

| Species | Climate | L _{max} (cm) | Egg Size (mm) | CV (%) | Source of Broodstock | Source |
|---|-------------|-----------------------|---------------|--------|----------------------|--------------------------------|
| Atlantic cod con't <i>Gadus morhua</i> | Temperate | 200 | 1.67 | 4.31 | Scotian Shelf | Miller et al. (1995) |
| | | | 1.35 | 8.15 | | |
| | | | 1.45 | 5.45 | | |
| | | | 1.36 | 3.82 | | |
| | | | 1.39 | 4.53 | | |
| | | | 1.53 | 4.18 | | |
| | | | 1.51 | 4.04 | | |
| | | | 1.68 | 8.33 | | |
| | | | 1.60 | 5.44 | | |
| | | | 1.57 | 0.56 | | |
| Atlantic mackerel <i>Scomber scombrus</i> | Temperate | 60 | 1.252 | 5.75 | Canada | Lanctot (1980) |
| | | | 1.235 | 2.19 | Bay of Biscay | Mendiola et al. (2007) |
| | | | 1.209 | 2.40 | | |
| European hake <i>Merluccius merluccius</i> | Temperate | 140 | 1.067 | 2.25 | Spain | Sanchez et al. (2012) |
| | | | 1.07 | 0.01 | Norway | Bjelland and Skiftesvik (2006) |
| Gulf menhaden <i>Brevoortia patronus</i> | Subtropical | 35 | 1.21 | 2.48 | Gulf of Mexico | Powell (1993) |
| | | | 1.29 | 3.10 | USA | Hettler (1983) |

Table A2.1 continued...

| Species | Climate | L _{max} (cm) | Egg Size (mm) | CV (%) | Source of Broodstock | Source |
|---|-------------|-----------------------|---------------|--------|----------------------|-------------------------|
| Haddock <i>Melanogrammus aeglefinus</i> | Temperate | 112 | 1.395 | 2.15 | Scotland | Hislop (1988) |
| | | | 1.39 | 2.16 | | |
| | | | 1.32 | 3.03 | | |
| | | | 1.38 | 2.90 | | |
| | | | 1.395 | 2.87 | | |
| | | | 1.415 | 3.53 | | |
| 1.4 | 4.29 | | | | | |
| Pacific bluefin tuna <i>Thunnus orientalis</i> | Subtropical | 300 | 1.01 | 2.97 | Japan | Sawada et al. (2005) |
| | | | 0.97 | 25.77 | Japan | Miyashita et al (2000) |
| Pacific halibut <i>Hippoglossus stenolepis</i> | Temperate | 258 | 3.4 | 26.47 | Canada | McFarlane et al (1991) |
| Winter flounder <i>Pseudopleuronectes americanus</i> | Temperate | 64 | 0.70 | 2.86 | Gulf of St. Lawrence | Fraboulet et al. (2009) |
| | | | 0.72 | 4.17 | | |
| | | | 0.65 | 6.15 | Canada | Butts and Litvak (2007) |
| | | | 0.65 | 6.15 | | |
| | | | 0.66 | 4.7 | | |
| Yellowfin tuna <i>Thunnus albacares</i> | Tropical | 239 | 1 | 0.10 | Japan | Marguiles et al (2001) |
| | | | 0.97 | 0.10 | Parana | Marguiles et al (2007) |

Table A2.2: Overview of within-population size-at-hatch variation in commercially important marine fish. CV (coefficient of variation) of egg size was calculated from data reported in the documents indicated throughout the table. Basic biological information (climate and L_{\max} (the maximum length reported for the species)) is taken from FishBase.org.

| Species | Climate | L_{\max} (cm) | T (°C) | Length at hatch (mm) | CV (%) | Source of Broodstock | Source |
|--|-------------|-----------------|--------|----------------------|--------|----------------------|---------------------------------|
| American plaice <i>Hippoglossoides platossoides</i> | Temperate | 82.6 | 2.0 | 4.35 | 2.00 | USA | Howell and Caldwell (1984) |
| | | | 6.0 | 4.87 | 2.91 | | |
| | | | 10.0 | 4.23 | 4.92 | | |
| Anchoveta <i>Engraulis ringens</i> | Subtropical | 20 | 10.0 | 2.58 | 8.53 | Chile | Llanos-Rivera and Castro (2006) |
| | | | 12.0 | 2.64 | 5.68 | | |
| | | | 15.0 | 2.50 | 5.60 | | |
| | | | 18.0 | 2.29 | 10.48 | | |
| | | | 20.0 | 2.20 | 6.82 | | |
| | | | 10.0 | 2.69 | 5.95 | | |
| | | | 12.0 | 2.70 | 4.07 | | |
| | | | 15.0 | 2.60 | 3.08 | | |
| | | | 18.0 | 2.66 | 7.52 | | |
| | | | 20.0 | 2.35 | 10.21 | | |

Table A2.2 continued...

| Species | Climate | L _{max} (cm) | T (°C) | Length at hatch (mm) | CV (%) | Source of Broodstock | Source | |
|--|-----------|-----------------------|--------|----------------------|--------|----------------------|---------------------------|---------------------------------------|
| Atlantic cod <i>Gadus morhua</i> | Temperate | 200 | 1.0 | 3.82 | 3.40 | Sweden | Nissling (2004) | |
| | | | 2.9 | 3.72 | 4.03 | | | |
| | | | 7.3 | 3.72 | 2.96 | | | |
| | | | 9.0 | 3.73 | 2.95 | | | |
| | | | 10.8 | 3.57 | 3.36 | Iceland | | Libungan (2009) |
| | | | 8.0 | 4.93 | 4.87 | | | |
| | | | 8.0 | 4.63 | 5.40 | Iceland | | Marteinsdottir and Steinarsson (1998) |
| | | | 7.0 | 4.63 | 3.46 | | | |
| | | | 7.0 | 4.59 | 3.92 | | | |
| 7.0 | 4.42 | 3.85 | | | | | | |
| Atlantic herring <i>Clupea harengus</i> | Temperate | 45 | 8.0 | 6.40 | 10.31 | Kiel | Blaxter and Hempel (1963) | |
| | | | 8.0 | 7.00 | 7.14 | Buchan | | |
| | | | 8.0 | 6.90 | 7.25 | Dogger | | |
| | | | 8.0 | 8.00 | 7.50 | Norway | | |
| | | | 8.0 | 7.60 | 6.58 | Clyde | | |
| | | | 9.1 | 8.30 | 3.61 | Canada | | Gary Kingston (1983) |
| | | | 9.9 | 7.40 | 4.05 | | | |
| | | | 7.0 | 8.80 | 3.41 | Irish sea | | Geffen (2002) |
| | | | 7.0 | 9.30 | 3.23 | | | |

Table A2.2 continued...

| Species | Climate | L _{max} (cm) | T (°C) | Length at hatch (mm) | CV (%) | Source of Broodstock | Source |
|---|-------------|-----------------------|--------|----------------------|--------|----------------------|--------------------------------|
| Atlantic mackerel <i>Scomber scombrus</i> | Temperate | 60 | | 3.71 | 5.39 | Bay of Biscay | Mendiola et al. (2007) |
| Atlantic menhaden <i>Brevoortia tyrannus</i> | Subtropical | 50 | 16.0 | 3.40 | 5.88 | Atlantic | Powell (1993) |
| | | | 20.0 | 3.20 | 6.25 | Atlantic | |
| | | | 24.0 | 3.20 | 3.13 | Atlantic | |
| Capelin <i>Mallotus villosus</i> | Temperate | 20 | 3.6 | 7.09 | 8.74 | Barents Sea | Gjosaeter and Gjosaeter (1986) |
| | | | 3.6 | 7.68 | 5.21 | | |
| | | | 3.6 | 7.88 | 3.68 | | |
| | | | 3.6 | 7.56 | 4.50 | | |
| | | | 9.5 | - | 8.00 | Canada | Chambers et al. (1989) |
| European hake <i>Merluccius merluccius</i> | Temperate | 140 | 14.0 | 3.20 | 4.06 | Spain | Sanchez et al. (2012) |
| Gulf menhaden <i>Brevoortia patronus</i> | Subtropical | 35 | 16.0 | 3.10 | 6.45 | USA | Powell (1993) |
| | | | 20.0 | 2.60 | 3.85 | | |
| | | | 24.0 | 2.80 | 7.14 | | |

Table A2.2 continued...

| Species | Climate | L _{max} (cm) | T (°C) | Length at hatch (mm) | CV (%) | Source of Broodstock | Source |
|---|-------------|-----------------------|--------|----------------------|--------|----------------------|------------------------|
| Haddock <i>Melanogrammus aeglefinus</i> | Temperate | 112 | 2.0 | 4.21 | 7.84 | Canada | Martell et al. (2005) |
| | | | 4.0 | 4.18 | 10.05 | | |
| | | | 6.0 | 4.23 | 10.17 | | |
| | | | 8.0 | 4.16 | 14.66 | | |
| | | | 10.0 | 3.98 | 10.05 | | |
| Northern anchovy <i>Engraulis mordax</i> | Subtropical | 24.8 | 13.5 | 3.11 | 0.96 | California | Lo (1983) |
| | | | 14.0 | 2.27 | 14.54 | | |
| | | | 15.0 | 2.86 | 2.80 | | |
| | | | 16.2 | 2.84 | 2.11 | | |
| | | | 17.0 | 2.41 | 4.15 | | |
| | | | 20.0 | 2.40 | 2.92 | | |
| Pacific bluefin tuna <i>Thunnus orientalis</i> | Subtropical | 300 | 25.0 | 3.08 | 5.84 | Japan | Sawada et al. (2005) |
| Pacific halibut <i>Hippoglossus stenolepis</i> | Temperate | 258 | 6.0 | 7.50 | 2.80 | Vancouver | McFarlane et al (1991) |

Table A2.2 continued...

| Species | Climate | L _{max} (cm) | T (°C) | Length at hatch (mm) | CV (%) | Source of Broodstock | Source |
|---|-------------|-----------------------|--------|----------------------|--------|----------------------|-------------------------|
| Pacific sardine <i>Sardinops sagax</i> | Subtropical | 39.5 | 11.0 | 3.76 | 3.99 | USA | Lasker (1964) |
| | | | 12.0 | 3.77 | 5.31 | | |
| | | | 13.0 | 3.73 | 4.29 | | |
| | | | 14.0 | 3.98 | 3.52 | | |
| | | | 16.0 | 3.75 | 6.67 | | |
| | | | 16.8 | 3.74 | 5.88 | | |
| | | | 20.5 | 5.10 | 2.35 | | |
| Winter flounder <i>Pseudopleuronectes americanus</i> | Temperate | 64 | 9.0 | 2.82 | 5.67 | Canada | Fraboulet et al. (2009) |
| | | | 9.0 | 3.19 | 2.82 | Canada | Chambers et al. (1988) |
| | | | 5 | 3.83 | 3.0 | | |
| Yellowfin tuna <i>Thunnus albacares</i> | Tropical | 239 | 27.6 | 2.61 | 0.04 | Panama | Marguiles et al (2007) |
| | | | 26.0 | 2.51 | 4.38 | Japan | Wexler et al (2001) |
| | | | 26.0 | 2.28 | 4.82 | | |
| | | | 29.0 | 2.45 | 4.49 | | |
| | | | 26.0 | 2.51 | 0.10 | Japan | Marguiles et al. (2001) |

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