An integrated approach to studying the relationship between anadromy and iteroparity in Atlantic salmon (*Salmo salar*)

By

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Abstract

Atlantic salmon is an anadromous species capable of spawning more than once during its lifecycle, being iteroparous. Increasing conservation concern has led to increased attention being paid to the potential short-term mitigating effects that iteroparous individuals may serve to populations suffering from low juvenile to adult recruitment. Despite the current research focus on repeat spawning Atlantic salmon, little is still known about how the iteroparous life cycle affects intrapopulation variation in marine movements and potential implications for population dynamics. The current thesis applied an integrated approach to studying the relationship between anadromy and iteroparity in Atlantic salmon from Newfoundland (Canada). Acoustic telemetry, scale pattern, stable isotope, fatty acid and mark-recapture analyses were included as analytical methods to investigate aspects of the relationship between iteroparity and anadromy and its importance in shaping the marine migratory patterns of Atlantic salmon. Using acoustic telemetry applied to 78 kelts and 90 smolts from two populations, I found that life stage influenced migratory movements and behaviour during the nearshore marine phase. Specifically, migratory movements were characterized by faster, more directed and less nocturnal movements by kelts as compared to smolts. These contrasts, however, varied by population, and the source of this variation was suggested to include responses to temporal and physical contrasts in the biotic and abiotic environment that shape the constraints imposed by trade-offs such as those between the need to reduce predation risk and increase growth and mass-gain. Using fatty acid (FA) and stable isotope analyses applied to 72 returning adults, I also found that life stage, as well as spawning history, influence migratory movements and dietary patterns during the at-sea phase. Specifically, significant differences in

FA composition and ratios of $\delta^{15}N$ in dorsal muscle tissue were identified, which supported the hypothesized divergent use of dietary sources among the different spawning history groups. Significant differences in FA composition, as well as lipid density, were also found among the different spawning histories in 69 post-spawned Atlantic salmon sampled as they emigrated from the river. Furthermore, patterns in lipid density were consistent with patterns in kelt return rates to consecutive repeat spawning. Consecutively spawned kelts and females had significantly higher lipid density than first-time spawned kelts and males, and consecutively spawned kelts and females experienced higher return rates compared to first-time spawned kelts and males. It was suggested that these spawning history related contrasts in energetic and nutritional state in post-spawned Atlantic salmon may be a carry-over effect of contrasts in the non-breeding area as affected by spawning-history dependent migration strategies, or alternatively, may represent an adaptive response to increased survival and recovery potential with age.

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Female Atlantic salmon in spawning colours (Photo by Kristin Bøe).

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Symbols

%C	Proportion of elemental carbon
%N	Proportion of elemental nitrogen
$\delta^{13}C$	Stable carbon stable isotope ratio
$\delta^{15}N$	Stable nitrogen stable isotope ratio
C:N	Percent elemental carbon to nitrogen ratio by mass
AIC	Akaike's information criterion
ALA	α-linolenic acid
AMPL	Acetone mobile polar lipids
AR	Alternate repeat spawner
CLD	Magnitude of seaward movements as represented by the change in linear distance from the river mouth per day
CR	Consecutive repeat spawner
CBR	Campbellton River
CR	Conne River
DHA	Docosahexaenoic acid
DOY	Day of year (Julian date)
DOM	Day of migration (Julian date)
EPA	Eicosapentaenoic acid
FA	Fatty acids
DPA	Clupanodonic acid
GLM	Generalized linear model
GLMM	Generalized linear mixed model
L_F	Fork length
M	Maiden spawner
MSW	Multiseawinter fish
NND	Nearest neighbour distance
PCA	Principal component analysis
PCO	Principal coordinate analysis
PERMANOVA	Permutational ANOVA
PL	Phospholipids
TAG	Triacylglycerol

List of Appendices

Co-authorship statement

I, Kristin Bøe, do hereby assert that my contributions, practical, intellectual, and philosophic, to the areas of; i) design and identification of the research proposal, ii) practical aspects of the research, iii) data analyses, and iv) manuscript preparation of this thesis are major. My status as the principal author of this thesis, as well as the published and unpublished works included therein are duly justified.

1 Chapter 1: Introduction and overview

1.1 General context of thesis

The work presented in this thesis aims to expand the current understanding of the role of iteroparity in shaping marine migratory patterns of anadromous Atlantic salmon (*Salmo salar*) using an interdisciplinary and integrated approach. Atlantic salmon has a considerable recreational, commercial and socio-cultural value and considerable effort, therefore, has been devoted to research and conservation of the species (Thorstad *et al.*, 2011; ICES, 2018). Due to logistical constraints, the majority of this effort has been devoted to the Atlantic salmon freshwater phase of the life history (Drenner *et al.*, 2012) which has resulted in improved conditions for survival and spawning in many populations (e.g. Forseth *et al.,* 2017) and added considerable knowledge of this part of the life cycle (Gibson, 1993; Bardonnet and Baglinière, 2000). Conversely, monitoring of Atlantic salmon populations have revealed that marine survival is in decline (Chaput, 2012a), with the causal mechanisms being poorly understood (Friedland *et al.*, 2014). Despite more recent advancement in methods studying migratory movements of aquatic organisms, the marine phase continues to be the least well understood part of the Atlantic salmon life cycle due to knowledge gaps surrounding spatiotemporal patterns of migration, behavior, and feeding ecology (Thorstad *et al.*, 2011; Chaput, 2012a; Renkawitz *et al.*, 2015).

In contrast to Pacific salmon, Atlantic salmon is capable of iteroparity, and individuals may spawn multiple times during their lifecycle (Ducharme, 1969). The potential short-term mitigating effects that iteroparous individuals may serve to populations suffering from low juvenile to adult recruitment is increasingly being recognized as important for their conservation (Niemela *et al.*, 2006; Reid and Chaput, 2012; Bordeleau *et al.*, 2019a). Despite the

current research focus (e.g. Reid and Chaput, 2012; Bordeleau *et al.*, 2019) on repeat spawning Atlantic salmon, little is still known how the iteroparous life cycle affects intra population variation in marine movements and potential implications for population dynamics (Bordeleau *et al.*, 2019a).

This thesis used an integrated approach to study the influence of iteroparity in shaping migratory dynamics in a North-American population using a population from Newfoundland (Canada) as a model system. Specifically, migratory behaviour, feeding ecology, and nutritional and energetic correlates to repeat spawning rates were investigated using four different methods. These analytical methods included: acoustic telemetry, scale pattern, stable isotopes, fatty acid and mark-recapture analyses, and provided an integrated approach to investigate aspects of the relationship between iteroparity and anadromy and its importance in shaping the marine migratory patterns of Atlantic salmon.

The thesis consists of a general introduction, followed by three data chapters and one data appendix. At the time of submission, Chapter 2 was published in the Canadian Journal of Fisheries and Aquatic Sciences (76: 2356-2376), chapter 3 accepted in ICES Journal of Marine Science (doi: 10.1093/icesjms/fsz168), chapter 4 submitted to the Journal of Animal ecology, pending review, and appendix 6.1 accepted in Conservation Physiology (10.1093/conphys/coz099) .

1.2 Animal migration

Understanding migration is important for understanding many ecological and evolutionary processes, as the phenomenon shapes the distribution of animals across time and space

(Chapman 2005), modulates population dynamics (Newton, 2006; Briedis and Bauer, 2018), and connect ecosystems (Bauer and Hoye, 2014). At the individual level, migration is an adaptive strategy where movements are employed to exploit multiple and/or heterogeneous environments to modulate growth, survival and/or reproduction (Dingle, 1996; Dingle and Drake, 2007; Jørgensen *et al.*, 2008). As such, migratory adaptations function by employing movement to exploit a changing and spatially extensive environment (Drake and Gatehouse, 1995; Dingle and Drake, 2007), and is a widespread phenomenon that is found in all major branches of the animal kingdom. As such, migration is a diverse phenomenon that takes place in a variety of taxa, medias, environments, and forms (Dingle and Drake, 2007). Despite this diversity, however, many of the challenges faced by migrating individuals are similar. To successfully reach a migratory destination and return, an organism needs appropriate mechanisms to mobilize physiology and morphology for the journey (e.g. energy stores), to ensure departure and arrival at the right time, and to ensure appropriate navigation and orientation towards destinations (Dingle, 2006). Thus, several different aspects of an animal's physiology and behavior need to function in a coordinated way (van Noordwijk *et al.*, 2006) and migration, therefore, is considered a complex trait that integrates specialized behaviours and a program of hormonal, metabolic, developmental, and neural control. This program preconditions individuals to migrate away from – or remain local within – its current habitat (Dingle, 1996), and can be explained by the genetic inheritance of correlated traits (Dingle, 2006; Pulido, 2007). By means of circadian and circannual rhythms, this migration syndrome (combination of specialized behaviours and adaptations) is in turn synchronized with the external environment and thereby the environmental factors influencing the long-term fitness

of migrations (Dingle, 1996). As such, migration does not depend solely on one adaptation, but relies on the integration of multiple adjustments in several traits.

Due to the similarity of many challenges faced by migrants, diverse animals may utilize somewhat similar tactics to meet their ends. For example, the majority of migratory species use fat to fuel their migrations (Dingle, 1996). Fat is more than twice as energy rich as carbohydrate or protein, and cost of transportation is thereby reduced by minimizing body mass (Weber, 2009). As such, diverse animals such as aphids (Aphidoidea spp.), Pacific salmon (Oncorhynchus spp.) and large mammals such as the grey whale (*Eschrichtius robustus*) accumulate large amounts of fats in preparation for migrations, and this accumulation usually occurs by means of diet shifts and the modification of metabolic pathways (Dingle, 1997). To facilitate transport of lipid constituents to working muscles at a rate required for long distance travels, birds, fish and insects also upregulate the expression of genes responsible for the production of fatty acid transport proteins during migration (Dingle 1997; Weber, 2009). As such, migration syndromes show many similarities among taxa, despite variation in phylogeny, migration medium, and forms.

In addition to involving a series of correlated physiological, behavioural, and neurological trait adaptations, migrations also tend to correlate with life history characteristics such as longevity, reproductive lifespan, fecundity and body size (Roff, 1991; Winemiller and Rose, 1992; Bohning-Gaese *et al.,* 2000). For example, egg production of long-distance migrating salmonids tends to be higher than for salmonids traveling shorter distances or not migrating at all (Kinnison *et al.,* 2001; Dingle, 2006). Similarly, in milkweed bugs, fecundity is higher in migratory compared to non-migratory forms (Dingle, 2006). This may in turn be explained by trade-offs such as that

between resources required to migrate and resources required to reproduce (e.g. Kinnison *et al.*, 2001). A migratory lifecycle needs to integrate these costs and benefits of migrating into optimal allocation strategies to maximize lifetime reproductive success. As such, Kinnison *et al.,* (2001) suggested that increased egg number in long distance migrants may be explained by an evolutionary compensation for the increased cost of migration compared to short distance migrants.

In many long-lived species, the migratory cycle is also repeated throughout the lifecycle. Iteroparous (repeat breeding) migrants is a term applied to species in which the migratory period of the breeding and nonbreeding life history stages is reiterated regularly, usually on an annual basis. Ramenofsky and Wingfield (2007) distinguished this migration pattern from that of ontogenetic migrations, which only occur once, and are not repeated. The ontogenetic form is most prominent in semelparous species (species that breed once and die), with the most common example being Pacific salmon that migrate to sea as juveniles where they grow to sexual maturity before returning to the natal river to spawn and die (Kinnison and Hendry, 2004). In iteroparous species, however, both ontogenetic and iteroparous migrations may occur in a lifecycle, as is the case for most repeat breeding anadromous salmonids. In these species, individuals first migrate to sea as juveniles where they sexually mature for the first time. As such, this migration is of an ontogenetic nature as the juvenile migration is only repeated once within the life span of an individual. Surviving spawners may, however, perform repeated migrations as adults, associated with the cyclic progression of non-breeding and breeding life-history stages (Klemetsen *et al.,* 2003).

As such, migration as a phenomenon functions within and across a series of biological organizational levels including behavior, physiology and life history (Dingle, 2006). An improved understanding of the proximate and ultimate mechanisms underlying migrations, therefore, requires integrating knowledge from all these levels.

1.3 The anadromous life cycle of Atlantic salmon

Central to the Atlantic salmon life cycle is anadromy where juveniles hatch in freshwater, migrate to sea and later return to spawn in freshwater. Depending on local temperatures and growth rates in the river juveniles spend from 1-5 years in freshwater before undergoing a physiological transformation for seawater tolerance at which point they are called smolts (Klemetsen *et al.*, 2003). During the transition from rivers through estuaries, predation appears to be a main natural contributor to smolt mortality, which can be high during this phase (Thorstad *et al.*, 2012). A nocturnal activity pattern is often observed during this migration phase, which is considered adaptive for reducing mortality by visual predators (Thorpe *et al.*, 1988; McCormick *et al.*, 1998). Survival and growth during the early marine phase are mediated by climate, as inferred by correlations between spring thermal habitat and annual spawning recruitment patterns (Friedland, 1998; Friedland *et al.*, 1999). During the marine phase, North American post-smolts (juvenile Atlantic salmon after ocean entry to the first winter at sea) are found in the Labrador Sea and off the west coast of Greenland in the Northwest Atlantic Ocean (Ritter, 1989), whereas European origin Atlantic salmon are found in the Norwegian (Holm *et al.*, 2003) and Barents Sea, although some feed as far west as Greenland (Jonsson and Jonsson, 2011). Atlantic salmon sexually mature after spending one (1SW) or multiple (MSW) winters at sea, and marine distribution patterns differ somewhat between the different maturation

strategies (Ritter, 1989; Soto *et al.*, 2018). Upon the initiation of sexual maturation, Atlantic salmon start the homing migration to natal rivers where they spawn. The accurate ability of anadromous salmonids to find their 'way home' is explained by imprinting to distinct odours of the natal stream as smolts, which is used as a cue by adults for homing after reaching coastal waters (Hasler and Wisby, 1951; Hasler *et al.*, 1978; Hansen and Jonsson, 1994). The return or spawning migration can, however, be divided into two distinct phases. First, fish must navigate the open ocean. Responses of salmonids to magnetic fields suggests that an inherited magnetic map facilitates navigation during the oceanic phase (Putman *et al.,* 2014). Olfaction is then used upon reaching the coast and freshwater to locate upstream spawning grounds. Natal homing is adaptive by ensuring the use of favorable spawning habitat over generations, while providing a mechanism to maintain population integrity and the evolution and transmission of local adaptations including migration syndromes (Secor, 2016a).

The anadromous migration strategy is believed to have evolved in response to the productivity differences between freshwater and marine, habitats in temperate climates, with increased availability of food resources at sea accelerating growth considerably and size at age in anadromous individuals (Gross *et al.*, 1988). Due to the positive correlation between female size and fecundity in fish (Heinimaa and Heinimaa, 2004), anadromy increases the reproductive output compared to residency while spawning in freshwater also ensures reduced mortality of eggs, embryos and juveniles (Gross *et al.*, 1988). For anadromy to be an evolutionary stable strategy, the proximate and ultimate cost of migration must be exceeded by the gains of using a second habitat, i.e. the lifetime product of reproductive success and survivorship must be higher for individuals undertaking ocean migrations compared to freshwater residency

(McDowall 1997). As such, the benefit of anadromy must offset the costs, which includes the elevated mortality due to increased predation during downstream migrations and at sea (Hvidsten and Lund, 1988; Dieperink, 2002; Jensen *et al.*, 2019), physiological costs of transitions across haloclines (McCormick and Saunders, 1987), and the energetic expense of migrations (Roff, 1988; Gilhousen, 1990; Jonsson and Jonsson, 2006). To minimize the proximate costs of migrations, specialized behavioural, morphological and physiological trait adaptations have developed to facilitate movements between habitats that differ in their biotic and abiotic properties (e.g. Thorpe and Morgan, 1978; Taylor and McPhail, 1985; McCormick *et al.*, 1998). Variation in anadromy/non-anadromy found within the salmonid genera, ranging from the limited salinity tolerance of lake trout (*Salvelinus namaycush*) that restricts them largely to freshwater and at most estuarine environments (Swanson *et al.*, 2010; Kissinger *et al.*, 2019), to the obligate anadromy of pink salmon (*Onchorhynchus gorbuscha*) were every individual migrates (Quinn, 2005), can thus be viewed as the result of variation in the benefits and costs of migrating as affected by phylogeny, environment, frequency, density and condition dependence (Hendry *et al.*, 2004).

Kinnison and Hendry (2004) characterized the Atlantic salmon migration strategy as 'extreme anadromy' to set them apart from facultative anadromous species, where migrations tend to be short in duration and distance, and where the occurrence of migration may vary greatly among populations, individuals and years (Rounsefell, 1958). Conversely, in Atlantic salmon populations having access to sea, most individuals perform migrations which may cover thousands of kilometers over the duration of years. Exceptions to anadromy occur in the form of precocial maturation characterised by juvenile males that forgoes anadromy to the

advantage of sexual maturation as parr in streams (Jonsson and Jonsson, 1993). This life history is linked to the Atlantic salmon mating system and differences in the relative costs and benefits of migration between the sexes. There is no strong relationship between size and fecundity in males, so that the advantage of a larger size at age confers an overall benefit to breeding females, whereas for males the benefit of large size derives from increased dominance and competitive advantages during intrasexual competition (Fleming, 1996). Depending on the density of similar breeding tactics on the spawning site, precocial parr can instead successfully reproduce by 'sneaking' access to females (Hutchings and Myers, 1994; Fleming, 1996).

The reliance on two different habitats for completing the life cycle increase the vulnerability to disturbances affecting migratory pathways through habitat fragmentation caused by anthropogenic influences such as hydropower dams and turbines (Lennox *et al.*, 2019). Historically, habitat fragmentation along with other anthropogenic stresses such as water acidification, agricultural pollution and land-use change resulted in reduced production in Atlantic salmon populations (Parrish *et al.*, 1998). Considerable research and conservation effort have now improved or stabilized conditions during the freshwater stage, which is no longer considered the most critical phase in the Atlantic salmon life history (Chaput, 2012b). Conversely, conditions during the marine phase appears to have worsened during the last decades. Despite closures of commercial at sea fisheries, the numbers of Atlantic salmon returning to spawn continues to decline or remain at historically low levels, a trend attributed to reduced survival at sea (Chaput, 2012b; ICES, 2018). The causal mechanisms, however, remains uncertain. Due to logistical difficulties of monitoring aquatic organisms and their environment over vast ocean distances, a comprehensive understanding of the marine phase of

the Atlantic salmon lifecycle is lacking, including details on stock specific migrations and feeding ecology at sea (Renkawitz *et al.*, 2015; Moriarty *et al.*, 2016; Strøm *et al.*, 2017). Because of the wide distributional ranges of Atlantic salmon at sea, populations can be exposed to multiple biotic and abiotic stressors acting at different areas and times so that the identification of critical factors influencing survival remains unclear. A growing body of literature, however, is demonstrating links between the growth and survival of Atlantic salmon with ocean climate and there is a consensus that altered ocean conditions affect Atlantic salmon performance at sea (Todd *et al.*, 2008). Increased sea surface temperatures associated with global warming has resulted in large-scale ecosystem and oceanographic changes in the North Atlantic which may affect Atlantic salmon directly or indirectly. Direct effects may include mechanisms acting on physiology, metabolism and phenology (Durant *et al.*, 2007), whereas indirect effects likely include modifications of the relationship between Atlantic salmon and their prey. As such, altered availability and/or quality of Atlantic salmon food resources may alter growth and survival at sea (Todd *et al.*, 2008; Renkawitz *et al.*, 2015).

1.4 The role of iteroparity in the Atlantic salmon lifecycle

The anadromous migration strategy is closely interlinked/correlated with other life history traits (Hutchings and Morris, 1985; Roff, 1991) and anadromy is, therefore, rarely viewed as a discrete trait. Rather it is a complex suite of life history characteristics which are bound by various trade-offs. The most prominent life history trade-off in animal lifecycles involves the cost of reproduction on survival, growth and maintenance, with current reproduction thought to negatively impact longevity and reproduction in the future (Williams, 1966). As survival and reproduction are the key components of fitness, the manner in which an organism allocates

limited resources to reproduction, the reproductive effort, is hence subject to strong selection (Stearns, 1992).

In nature one can identify two main reproductive types that reflect two very different allocation strategies. Semelparity implies the maximum possible reproductive effort in a single reproductive event with death as a by-product (Stearns 1992), often as a direct result of endocrine and other changes which accompany the physiological commitment to reproduce (Kirkwood and Rose, 1991). Models assuming an accelerating dependency between reproductive effort and effective fecundity (measured as the number of offspring that survives to reproduce) predicts semelparity to be beneficial when costs of reproduction are high even at low levels of reproductive effort (Schaffer and Schaffer, 1977; Schaffer, 2004). Conversely, iteroparity describes a life history capable of repeated breeding throughout the lifecycle. The benefits of repeat breeding (iteroparity) include selection for bet-hedging for juvenile survival in highly variable environments, and age-related increases in fecundity for organisms with indeterminate growth (Fleming & Reynolds, 2003).

By summarizing published data, Fleming (1998) identified a positive relationship between the degree of anadromy and reproductive effort within the Salmonidae. The species referred to as ‘extreme migrants’ by Kinnison and Hendry (2004) included the five species of Pacific salmon and Atlantic salmon and, showed the highest reproductive effort in terms of female gonadal investment and the percent of total energy expended during reproduction (Fleming, 1998). In contrast to the other ‘extreme migrants’ however, Atlantic salmon are iteroparous and have the capacity for repeat spawning. Thus, some Atlantic salmon survive spawning and repeat the marine feeding migration to restore depleted energy stores for new reproductive events

(Fleming, 1996). By comparing species and population pairs of resident-, and anadromous forms within the group of iteroparous salmonids, Fleming (1998) identified an inverse relationship between anadromy and the probability of repeat breeding. The relationship was interpreted as a negative impact of the increased energy expenditure associated with anadromous migration on post spawning survival. Furthermore, anadromous females tend to invest more heavily in the production of eggs compared to non-anadromous forms (Fleming, 1998). Higher gonadal investment of anadromous forms is consistent with life history theory which predicts that low probabilities of repeat breeding should favor an increasing investment into current reproduction at the expense of post-breeding survival (Schaffer, 2004). Thus, if parity is viewed as a continuum with semelparity at one end and iteroparity at the other (Crespi and Teo, 2002), this trait is perhaps the most strongly associated with the complex set of life history traits that constitutes anadromy.

In Atlantic salmon, there are two different repeat spawning strategies. Consecutive spawners breed annually, spending on average a few months at sea between breeding events (Klemetsen *et al.*, 2003). Alternate spawners is a term applied to individuals that skip breeding and consequently spend more than one full year at sea before returning to repeat spawn (Klemetsen *et al.*, 2003) (Figure 1.1). Atlantic salmon may display both repeat spawning strategies within a lifecycle. Post-spawned individuals may migrate to sea in fall immediately after spawning, or in spring after overwintering in freshwater habitat, a choice thought to be influenced by individual variation in post-spawner state, the availability of overwintering habitat in freshwater, and winter conditions at sea (Niemela *et al.*, 2000; Halttunen *et al.*, 2013). Due to the high reproductive investment of Atlantic salmon, reducing spawner energy

values by up to 70 % (Jonsson *et al.*, 1997), the occurrence of repeat spawning is generally low, with on average less than 10 % of individuals returning to spawn again (Fleming, 1998). Considerable between and within population variation in repeat breeding, however, exists (Jonsson *et al.*, 1991; Klemetsen *et al.*, 2003; Dempson *et al.*, 2004), which may at least in part be attributed to variation in the costs of reproduction. Males on average are less likely to repeat breed than females, likely as a result of intense sexual selection leading to injury, infection and ultimately lower survival (Fleming, 1996; Jonsson and Jonsson, 2011). Size-dependent energy costs during reproduction also appear to be linked to variation in survival and post-breeding recovery potential among populations (Jonsson *et al.*, 1991, 1997; Jonsson and Jonsson, 2003). Smaller fish (generally 1SW salmon) use a lower relative amount of energy to spawn and tend to spawn annually, whereas larger MSW fish spend relatively more energy and are more likely to spawn biennially, implying they require more time to restore depleted somatic reserves (Jonsson and Jonsson, 2011). As such, there is a relationship between iteroparity and the degree of anadromy within Atlantic salmon insofar as shorter migrations by 1SW fish are associated with higher repeat spawning rates.

As a result of growing conservation concerns, increased attention is now being paid to the potential short-term mitigation effects that iteroparous individuals may have on populations suffering from low juvenile to adult recruitment (e.g. Reid and Chaput, 2012; Bordeleau *et al.*, 2019b). As such, repeat spawners may have an important contribution to population productivity and genetic diversity in years of low smolt-to-adult recruitment. Despite this, understanding of the iteroparous life cycle of anadromous Atlantic salmon is still incomplete. For example, a cohort of seaward migrating fish may consist of a mixture of juvenile and adult

individuals which differs in size, experience, motivation, migration consequences, and potentially migration route. Few studies, however, have investigated the role of ontogeny in shaping intrapopulation variation in migratory movement patterns among Atlantic salmon monitored under the same environmental conditions. Furthermore, although repeat spawning may be associated with contrasts in spatiotemporal migration patterns (i.e. consecutive v. alternate repeat spawners) little is known about the degree of spatial connectivity among and within maiden and repeat spawning Atlantic salmon at sea, and how connectivity affects the environmental factors experienced by spawning history dependent migration strategies. Previous work on other organisms has shown that divergent migration patterns may result in differential mortality and carry-over effects that can have concomitant consequences for the dynamics and resilience of populations (Briedis and Bauer, 2018). Lastly, a mechanistic understanding of the physiological and energetic factors that affect post-breeding recovery in Atlantic salmon, including the importance of post-spawning energetic state in determining repeat spawning rates, is not well developed. As size and life history are intimately correlated in Atlantic salmon (i.e. age at maturity and number of breeding events), the potential effects of life history versus the energetic size effects on post-spawner state and repeat breeding rates have not been disentangled.

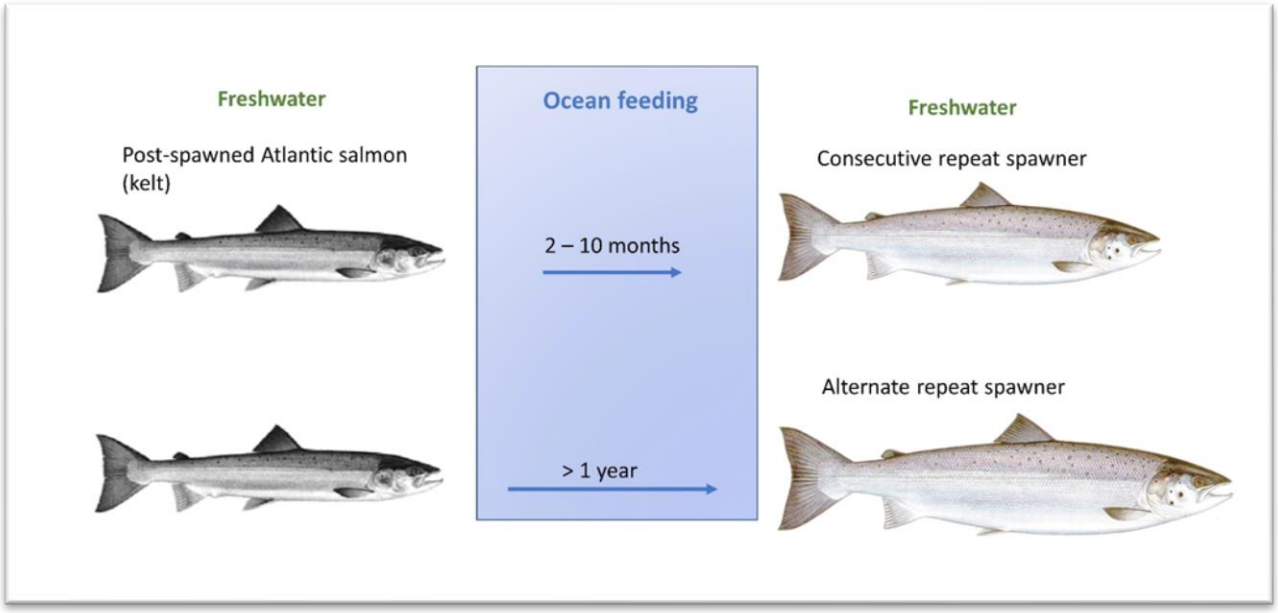

Figure 1.1: The consecutive and alternate repeat spawning strategies of iteroparous Atlantic salmon.

1.5 Analysing patterns of anadromy and iteroparity

Traditionally, the study of marine fish migrations was enabled through commercial and scientific catch data, and conventional tagging (Harden Jones, 1968; Leggett, 1977) which provided information on the locations of spawning, feeding and wintering areas (Hjort, 1914) and gross population movements between freshwater and marine habitats (Harden Jones, 1968; Quinn, 2005; Secor, 2016a). For Atlantic salmon, scale pattern analysis, enabled by the correlation between fish size, somatic growth and the deposition and arrangement of scale circuli (Dahl, 1910), also provided researchers with a tool to estimate latitudinal origins, as well as age, life history and growth information from intercepted fish (e.g. Reddin, 1985; Friedland and Reddin, 2000). Together, these methods were instrumental in describing natal homing as well as general distributions and habitat use at sea of Atlantic salmon of different life stages

(Jacobsen *et al.*, 2012; Reddin *et al.*, 2012; Maoileidigh *et al.*, 2018). Coupled with stomach content analysis, fishery data also provided coarse information on feeding ecology at sea, and the main prey types ingested by Atlantic salmon (Templeman, 1967, 1968; Lear, 1973; Renkawitz *et al.*, 2015). Several methodological issues, however, constrain the quality and detail of the information obtained. For example, tag-recapture studies do not provide information about movements during the time an animal is at liberty (Bolle *et al.*, 2005), and are reliant on a significant chance of recapture which is generally not the case (Thorstad *et al.*, 2014). Furthermore, spatiotemporal bias in sampling and harvest effort complicates interpretation of the data obtained as locational information may be more affected by distribution of fishing vessels than the movement of fish (Bolle *et al.*, 2005; Thorstad *et al.*, 2014). The use of stomach content analysis in turn only provides a snapshot of recent dietary information, while being subject to the same spatiotemporal bias as noted above. The study of migratory ecology of anadromous fish has, therefore, been greatly facilitated by the development of two different methods, namely biotelemetry, and what has been referred to as 'life cycle tracers' (Secor, 2016b).

The advent of telemetry methods (reviewed in Thorstad *et al.*, 2014) enabled the monitoring of long and short term movement of fish and the simultaneous gathering of data on their immediate environment that provided detailed information on habitat use, survival, and behavior (Hockersmith and Beeman, 2012). Conversely, life cycle tracers is a collective term used for biological (e.g. parasites) or biochemical (e.g. otolith microchemistry) tracers that provide retrospective information on the habitat movements and/or assimilated diet during parts or entire phases of life cycles (Secor, 2016b). These tracers includes analyses of stable

isotopes and fatty acids (FA) (Campana, 1999; Dalsgaard *et al.*, 2003; Rubenstein and Hobson, 2004; Boecklen *et al.*, 2011), as well as genetic markers (e.g. Boustany *et al.*, 2008). Telemetry methods considerably expanded the knowledge of the smolt migration within rivers and the early marine phase, and identified the latter stage as critical for many populations during the marine phase (Drenner *et al.*, 2012; Thorstad *et al.*, 2012). Stable isotope analyses (SIA) improved knowledge on inter-, and intra-population variation in inferred marine movements (Sinnatamby *et al.*, 2009; Dempson *et al.*, 2010; Mackenzie *et al.*, 2011; Kelly *et al.*, 2019), as well as diet and trophic status (Johnson and Schindler, 2009; Dixon *et al.*, 2012). Fatty acid analysis is rapidly being developed as a biomarker tool to further elucidate spatiotemporal patterns of feeding, and information on dietary origins of major prey resources consumed. This is possible because of the strong phylogenetic and trophic patterns that exist in FA synthetic capacity among organisms (Bell and Tocher, 2009), where unique FAs and FA compositions found in primary, and some secondary, producers are bioaccumulated through the food chain (Iverson, 2009).

Despite these methodological advances, however, the study of fish migrations is still affected by logistical constraints. Although life cycle tracers have the advantage over telemetry insofar as they can cover time frames on the scale of seasons, years, or entire lifecycles, tracers typically entail less precise and detailed information on spatial histories (Hobson, 1999). Furthermore, the use of life cycle tracers as 'geolocation' tags often require existence of baseline information of the natural distribution of the biomarkers of the migration environment of fishes, which can be difficult to obtain (Trueman *et al.*, 2012; McMahon *et al.*, 2013). Location data inferred by acoustic telemetry are in turn dictated by the location and numbers

of receivers that can be placed in a study area. Battery life constraints restricts the size and life stages of which tags can be applied, as well as the duration of the study. Acoustic telemetry thus tends to describe fragments of migration trajectories and life cycles (Secor, 2016b) and the majority of research efforts on anadromous salmonids have historically focused on the freshwater and early (coastal) marine phase (Drenner *et al.*, 2012). Indirect position estimates based on telemetry, on the other hand, does not require external receivers as geographic position is estimated through external environmental data such as solar angles, temperature, and current patterns (Thorstad *et al.*, 2014). Instead, sensor data are logged by the tag which needs to be retrieved by physical recaptures or by mechanisms that release the apparatus from individual fish and relay the information via satellites. The former is limited by low recapture probability, whereas the latter is in large part restricted by size; only large bodied fish can carry the big load of a tag and release apparatus (Thorstad *et al.*, 2014). Each methodological problem creates issues with representability as the information obtained is often patchily distributed in time and space and is derived from a few individuals. As such, there is an increasing awareness that only through integrative and interdisciplinary research will it be possible to understand mechanistic and ultimate bases (Cooke *et al.*, 2008) and the interactions between biological levels of organization in the complex phenomenon of migrations (Bowlin *et al.*, 2010).

1.6 Thesis objectives and chapter structure

The general aim of this thesis was to develop a comprehensive picture of the role of iteroparity in shaping migratory patterns using an interdisciplinary approach applied to Atlantic salmon from the Northwest Atlantic, Newfoundland. By combining methods such as acoustic telemetry,

stable isotope and lipid analyses, I sought to meet the following objectives: i) characterize the role of life stage (juvenile vs. adults) in shaping migratory movement patterns during the early marine phase (Chapter 2), ii) characterize the role of life stage (maturing vs. re-maturing) and life history (repeat spawning strategy) in shaping marine diet and spatial resource use (chapter 2 & 3), and iii) explore energetic and nutritional drivers of variation in repeated migrations (i.e. post-spawner return rates) (chapter 4) using two Atlantic salmon populations from the Northwest Atlantic. It is hoped reported findings will make a significant contribution to the current understanding of the migratory dynamics of repeat spawning Atlantic salmon, which remains limited, as well as improve knowledge of the life history related sources of variation in migratory dynamics within Atlantic salmon populations. Lastly, it is hoped that this research will be useful for tailoring management and conservation efforts towards sustaining the contribution of repeat spawning salmon to overall population productivity.

In chapter 2, I contrasted Atlantic salmon marine migratory movements in the early marine phase between juvenile (smolts) and veteran (post-spawned adults) migrants using acoustic telemetry. The investigation was carried out on two separate populations from Newfoundland (Canada) where the main objective was to explore the effect of ontogeny on migratory patterns such as diel movements, residency times and movement responses to the thermal habitat.

In chapter 3, I continued to investigate the influence of life stage, including spawning history, on the marine movements of Atlantic salmon with a focus on the at-sea phase of the life history. As logistical constraints precluded the use of telemetry for long-term investigations of multiple life-stages over vast ocean distances, the chapter applied chemical tracers in the form of stable isotopes and fatty acid analyses to retrospectively infer likely feeding area use and

dietary contrasts among groups of Atlantic salmon, based on existing knowledge of fatty acid and stable isotope ratio variations within the foodwebs of the putative feeding areas for North American origin Atlantic salmon.

In chapter 4, I investigated factors associated with repeat spawning in Atlantic salmon with a focus on energetic and nutritional state. Here I hypothesized that differential reproductive investment between the sexes, as well as differences in spawning histories, would result in contrasts in post-reproductive state and differences in marine return-rates. Scale pattern analyses were combined with non-lethal biopsies to assess correlations between life history and nutritional state as inferred by lipid density and fatty acid composition. Mark-recapture analysis was then applied to obtain individual information on post-spawner reproductive fates.

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2 Chapter 2: The influence of temperature and life stage in shaping migratory patterns during the early marine phase of two Newfoundland (Canada) Atlantic salmon (*Salmo salar*) populations

Co-authorship statement

This research was developed and carried out by Kristin Bøe, together with the supervisory assistance of Ian Fleming and Michael Power, and the guidance of Martha Robertson, Brian Dempson and Corey Morris. All authors discussed the results and contributed to the final manuscripts.

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2.1 Abstract

Owing to the iteroparous nature of Atlantic salmon, a seaward migrating cohort may consist of juveniles and adults that differ in size, maturity, experience, and in the motivation and consequences of migratory movements. Few studies have investigated the role of ontogeny in shaping intra-population variability in movement patterns among Atlantic salmon monitored under the same environmental conditions. This study contrasted the movements of smolts and kelts in two Canadian (Newfoundland) populations from marine entry through coastal embayments and quantified the influence of local water temperatures on movement patterns. Significant differences in migration routes, migration speed, and diel movements between smolts and kelts were present. Kelt generally displayed faster, more directed and less nocturnal movements compared to smolts. Temperature influenced seaward movement positively, as well as the degree of diurnal movement. Prolonged nearshore residency by smolts and kelts in the two embayments was accompanied by a considerable improvement in ocean thermal conditions, hypothesized to promote open ocean entry during conditions favorable to migration performance.

2.2 Introduction

The early phase of the marine migration of Atlantic salmon (*Salmo salar*) is considered a critical period in the species' life cycle as mortality can be high and have negative impacts on lifetime reproductive fitness (Thorstad et al. 2012). Fitness is enhanced by arrival at the marine environment when conditions are optimal for survival and growth, and the timing of the Atlantic salmon seaward migration is considered adaptive to prevailing ocean conditions such as temperature, food resources and predators (McCormick et al. 1998, Otero et al. 2014).

Although migration timing influences smolt to adult survival rates (Antonsson et al. 2010), the heterogeneous and stochastic nature of estuarine and coastal environments that migrating fish face generates variable selection regimes at differing spatial and temporal scales (e.g. Friedland et al. 1998, 2003).

During the transition from rivers through estuaries, predation appears to be a main natural contributor to smolt mortality (Hvidsten and Lund 1988, Jepsen et al. 2006, Thorstad et al. 2012). A nocturnal activity pattern is often observed and is considered adaptive in reducing visual predation (Thorpe and Morgan 1978, Thorpe et al. 1988, McCormick et al. 1998). Nocturnal movement appears to weaken in warmer conditions (Ibbotson et al. 2006, Haraldstad et al. 2017), which may reflect an increased ability of Atlantic salmon smolts to evade predation at higher temperatures (Thorpe et al. 1994). Survival and growth during the early marine phase are mediated by climate, as inferred by correlations between spring thermal habitat and annual spawning recruitment patterns (Friedland et al. 1999, 2003, 2012). The causal mechanisms, however, are poorly understood and there is no comprehensive understanding of how individuals may alter their migration behaviour to optimize fitness related traits when faced with biotic and abiotic variation in the marine environment (Byron and Burke 2014). Current and widespread population declines of Atlantic salmon (Chaput 2012, Mills et al. 2013, Soto et al. 2018) emphasize the need to improve our understanding of mortality mechanisms and substantial effort has been directed to quantifying internal and external influences on early marine survival to determine how both are linked to behaviour (Thorstad et al. 2012).

Acoustic telemetry studies (Heupel et al. 2006) applied to the early phases of migrating Atlantic salmon smolts generally demonstrate a high degree of variability in migratory patterns at the individual and population level (e.g. Lacroix 2008, Davidsen et al. 2009, Manel-la et al. 2011). Within populations, smolt migratory pathways can be complex, often displaying multiple directional changes and reversed movements (Lacroix et al. 2005, Davidsen et al. 2009, Halfyard et al. 2013). Residency times in coastal areas surrounding natal waters may vary from a few days to several weeks (e.g. Lacroix and McCurdy 1996, Thorstad et al. 2007, Davidsen et al. 2009, Dempson et al. 2011). Possible explanations for the variation include adaptive responses to the intensity of nearshore predation and arrival time at offshore areas (Manel-la et al. 2011), feeding (Dutil and Coutu 1988, Friedland et al. 1999, Rikardsen et al. 2004) and behavioural responses to physical environmental cues such as surface currents, salinity and temperature (Hedger et al. 2008, Martin et al. 2009, Moriarty et al. 2016). Temperature may influence migration rates in fish generally through the positive correlation with metabolic rate, overall activity and swimming performance (Booth et al. 1997, Clarke and Johnston 1999). Physical environmental variability may also influence migration speed through an effect on orientation behaviours (Mueller and Fagan 2008). While it is generally assumed that external sensory cues such as surface currents, temperature and salinity play an orientation role in determining Atlantic salmon migration routes (Byron and Burke 2014, Moriarty et al. 2016), the variable nature of estuarine and coastal environments may contribute to the observed natural variability in Atlantic salmon migratory behaviour through these habitats.

Owing to the implications for annual spawner returns, the migratory behaviour and survival of Atlantic salmon smolts have been particularly well studied, with correspondingly less effort

having been invested to understand the adult life stage (Drenner et al. 2012). Being iteroparous, post-spawned survivors (kelts) will re-enter the marine environment and some will return as repeat spawners (Mills 1989, Jonsson et al. 1990, Fleming 1998). In populations where the outmigration timing of smolts and kelts overlap, the migrating cohort consists of a mixture of fish which differ in experience, size, motivation, migration consequences, and potentially migration route. Prior experience may aid kelts with orienting towards migratory destinations (Hansen and Jonsson 1994). As compared to smolts, larger kelt sizes may have better locomotory ability (Bainbridge 1958), broader temperature tolerances (Handeland et al. 1998) and reduced risk of predation (Werner and Gilliam 1984, Eriksson 1994, Lorenzen 1996). Few studies, however, have investigated the role of ontogeny in shaping intra-population variability in movement patterns among Atlantic salmon monitored under the same environmental conditions. Thus, it is generally assumed that the behaviours of smolts and kelts are similar (Drenner et al. 2012).

Here, acoustic telemetry was used to compare the migrations of wild Atlantic salmon smolts and kelts in two Canadian (Newfoundland) populations from marine entry through coastal embayments, and in one case, beyond to the nearby coast. The objectives were to contrast: i) smolt and kelt residency and transit times, diel behaviours, and migration routes; and, ii) determine the influence of nearshore surface temperatures on observed movement patterns. The study expands on data from a previously published study of smolt migration in the Conne River (Dempson et al. 2011) to include data from concurrently tagged kelts and includes kelt and smolt data from a second population in the Campbellton River. We hypothesized that kelt migratory movements would be distinguished by faster, less nocturnal and more directed

movements, with differing migration routes, as compared to smolts. Moreover, given the importance of temperature for Atlantic salmon osmoregulation, locomotion, feeding, metabolism and growth (Elliott and Elliott 2010), we hypothesized that temperature would influence migratory decisions in the nearshore area. Specifically, we hypothesized increased temperatures would be associated with decreased nocturnal activity and increased seaward movements, with the effect of temperature on nocturnal activity and movement being more pronounced in smolts than kelts.

2.3 Methods

2.3.1 Study system

The study was conducted in two Newfoundland populations: Conne River, CR, (47.9 º N, 55.7 º W, drainage area: 602 km^2) located on the south coast drains southwest into the Bay D'Espoir (Area: 210 km^2, maximum depth:792 m) and Campbellton River, CBR, (49.2º N, 54.9 ºW, drainage area: 296 km^2) located on the northeast coast drains northeast into the Bay of Exploits (Area: 1100 km^2, maximum depth: 600 m)(Figure 2.1). The two embayments have relatively stable oceanographic environments with small tidal influences (1.5 and 2 m, respectively). The rivers are dominated by small salmon (< 63 cm fork length, L_F) maturing after one winter at sea (1SW) (2007-2016: CR 95-97% and CBR 89-94% of total salmon returns) (DFO 2014, 2017). Salmon 63 cm L_F or larger are almost exclusively repeat spawners at both Conne River and Campbellton River spending, on average, two months (consecutive) or 14 months (alternate) at sea between spawning events (Downton 2001, Dempson et al. 2004, DFO and MNRF 2008).

For the purpose of this study, the two rivers were divided into three regions: 1) the estuarine region denoting the transition zone from brackish to marine conditions, 2) the middle region and 3) the outer bay which encompasses the transition zone from the coastal embayment to the open ocean (Figure 2.1 a and b).

2.3.2 Salmon capture and tagging procedure

Downstream migrating smolts (Conne River: n = 62, Campbellton River: n = 28) and kelts (Conne River: n = 31, Campbellton River = 48) were intercepted at a counting fence operated by Fisheries and Oceans Canada. The fences were located approximately 2 km and 1 km upstream of the mouths of Conne River and Campbellton River, respectively. Smolts and kelts were removed from traps, anaesthetized, and measured for fork length (mm L_F) (Table 2.1) before a coded acoustic transmitter (VEMCO, Halifax, NS; Table 2.2) was surgically implanted in the peritoneal cavity following operating procedures described in Wagner et al. (2011). Tag size was chosen according to body length to minimize tag size relative to fish size. Tagged smolts were significantly larger in Campbellton River than in Conne River (GLM, $F_{1, 88} = 101.7$, $p < 0.001$), whereas no difference was found between kelts from the two systems (GLM, $F_{1, 76} = 4.03$, $p = 0.05$).

2.3.3 Acoustic receiver network

Acoustic receivers were deployed in the three regions of each study site and were suspended in the water column 5-50 m below the surface using sub-surface floats and bottom moorings. Receivers were also deployed along the northeast Newfoundland coast (Figure 2.1 c) as part of an Atlantic cod (*Gadus morhua*) monitoring program (Brattey 2013). The maximum axial length

of receiver coverage in Bay D'Espoir was 48 km and in Bay of Exploits was 30 km (Figure 2.1 a and b).

2.3.4 Characterization of the riverine and marine thermal habitat

Temperature recorders programmed to measure and store ambient temperature every hour were affixed to bottom moorings and sub surface floats and suspended in the water column approximately 3-4 m below the surface in the middle and outer regions of Bay d'Espoir (Vemco Ltd, Minilog Temperature Data Loggers) and in the estuary and outer regions of Bay of Exploits (Onset Ltd, HOBO Pendant Data Logger, UA-001-08). Temperature loggers were also placed in the two rivers at the sites of fish capture and release. The hourly measured temperatures were smoothed using a 24-hour centered moving average to reduce the short-term variability induced by cooling and warming associated with daylight and tidal fluctuations (Morak-Bozzo et al. 2016). The smoothed data were then averaged per day to produce a diel temporal resolution considered relevant to the movement data. The recorded sea surface temperatures (SST) in the two study systems fluctuated, but steadily increased during the study period (CR: 2.4°C to 9.71°C; CBR: 4.3°C to 13.7°C) (Figure 2.2). In the early part of the Campbellton River study, river and marine temperatures increased and declined by 8 °C in a period of two weeks, the latter being associated with strong northerly winds.

2.3.5 Data processing and statistical analyses

In all statistical analyses, a tag detection at a receiver station was used to represent the corresponding individual's location at that time. Because receivers with overlapping detection ranges have the potential to detect an individual simultaneously, receivers less than one km apart were grouped together and referred to as a receiver station. The grouping was based on

an average detection range of 400-500 metres as inferred by previous telemetry studies applying similar tag models in coastal areas (e.g. Lacroix et al. 2005, Hedger et al. 2008, 2009, Melnychuk 2012).

The two study systems (CR and CBR) were analysed separately to avoid the potential confounding effects induced by differences in acoustic array design and performance. Statistical analyses were conducted using R version 3.4.0 (R. Core team 2018). Model diagnostics were performed for each separate analysis to confirm normality of residuals, heterogeneity of variance, and the extent of temporal autocorrelations. Model selection was completed using the Akaike information criterion (AIC) (Anderson et al. 2001) and fixed effects were tested with a maximum likelihood ratio against simpler models by first removing the interaction and subsequent terms. If AIC values of the lowest AIC model and other candidate models differed by less than two AIC units, the principle of parsimony was invoked and the model with the fewest parameters was selected. All models tested converged.

Transit and residency times

To test the hypotheses that kelt nearshore migration was distinguished by faster movements through the embayments as compared to smolts, transit and residency times were compared between life stages. For each fish total nearshore residency was calculated as the number of days that elapsed between its first detection in the estuary and its last detection in the outer region, followed by an absence of detections for 10 or more consecutive days. The latter criterion was included to distinguish the seaward migration from the return migration of kelts as repeat spawners in the same summer following release. In addition to total nearshore

residency times, region specific residency was calculated to describe the proportion of the total residency time that each smolt and kelt spent in each region. Region specific residency was calculated as the time elapsed between first and last detection in a region before subsequent detection in an adjacent region. As some fish reversed their movements and returned to a region after being detected in an adjacent region, the region specific residency times were calculted as the sum of residency times per region. Transit times through the embayment were defined as the time that passed from when smolts and kelts were last detected in the estuary until first being detected in the outer region.

Smolt and kelt residency and transit times were compared in general linear models (GLM) where the statistical significance of life stage was evaluated. Following recommendations in Sokal and Rohlf (1973) model diagnostics were performed and appropriate transformations (e.g. logarithmic, cube root) or non-parametric tests (Welch's t-test) used when violations of model assumptions occurred. The proportions of smolts and kelts detected in the estuary, middle and outer regions were summarized, and if an individual was not detected in a region, residency in that region was not calculated for that fish.

Migration routes

Individual migration routes for CR fish were characterized based on the choice of one of the two passageways available to migrating salmon in the Bay d'Espoir (Lampidoes passage and Main passage, Figure 2.1 a), and for CBR fish on whether the majority of unique individual locations used by the fish to reach the outer region in the Bay of Exploits occurred on the western, middle or eastern section of the acoustic receiver array (Figure 2.1 b). In the Bay of

Exploits, a number of individual migratory pathways had few detections (N kelts = 5, N smolts = 0) or recorded a near equal number of detections in all sections (N kelts = 2, N smolts = 10) and could not be categorized. Spatial patterns in kelt and smolt migratory pathways were visualized by estimating and plotting the spatial kernel density distribution of smolt and kelt presence at acoustic receiver station locations (Wand 1994) applying a 500 · 100 m resolution and Silverman's (1986) rule of thumb for the selection of bandwith. The proportion of smolts and kelts using each migratory route were compared using Pearson's chi-squared tests with Yates' continuity correction.

Smolt and kelt migratory routes were further characterized as 'directed' if they involved less than three directional changes of more than 90° within 2 km. Significant proportions displaying directed movements were assessed using a binomial model with tag ID as a random intercept. To account for the potential bias due to receiver spacing on observed directional changes, the nearest neighbour distance between receiver stations was calculated and incorporated into the statistical model. The nearest neighbour distance was calculated with a 'cost distance analysis' using the shortestPath function in the r package 'gdistance' (van Etten 2018). The approach has been used succesfully to determine the shortest distance by water between differeing receiver stations in areas dominated by complex coastlines (Vollset et al. 2016).

Diel movement and temperature

The population level presence of diel movement was quantified by applying a generalized linear mixed model (GLMM), with tag ID as a random intercept (1|ID) and the binomial response day (y=1) or night (y=0) arrival at receiver stations within a region. The times of sunrise and sunset

were calculated for each day using the algorithm developed by the National Oceanic and Atmospheric Administration (NOAA) (https://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html). GLMM models that included temperature, day of the year (DOY), life stage, and the interactions between life stage and temperature and life stage and DOY were used to test for the statistical significance of temperature effects on diel movements and life stage specific migration responses. As temperature and DOY were highly correlated (Pearson's r: Conne River=0.9, Campbellton River=0.6), they were not used simultaneously in a model to avoid collinearly and related statistical problems with parameter estimation (Zuur et al. 2009). The continuous variables (temperature and DOY) were fitted both as linear predictors and as 2nd and 3rd order polynomial terms to explore potential non-linear effects on diel movement patterns. The full models considerd for each river data set were:

$$Y_{ij} = (\beta_0 + ID_{0j}) + Life\ stage_{ij} + Temp_{ij} + Life\ stage \cdot Temp_{ij} + Life\ stage \cdot Temp^2_{ij} + Life\ stage \cdot Temp^3_{ij} + e_{ij}$$

and

$$Y_{ij} = (\beta_0 + ID_{0j}) + Life\ stage_{ij} + DOY_{ij} + Life\ stage \cdot DOY_{ij} + Life\ stage \cdot DOY^2_{ij} + Life\ stage \cdot DOY^3_{ij} + e_{ij}$$

where Y_{ij} is the timing of arrival (day or night) at instance *i*, for individual fish *j,* ID denotes the tag ID of the j^{th} fish, lifestage distinguishes wheher the j^{th} fish is a smolt or a kelt, Temp is mean daily temepratrure (°C), and DOY is the calendar day of the year at which the i^{th} observation for the j^{th} fish was obtained.

Seaward movements and temperature

In addition to transit and residency times, we quantified migratory progression along a seaward vector from the time of estuary entry to the first detection in the outer region. This was done by calculating the daily change in the straight-line distance from the point of origin to individual locations, the former being represented by the river mouth in each study system. Accordingly, migratory progressions were characterized as: seaward – movement distances > 0, lack of seaward movement – distances = 0, and reverse seaward – movement distances < 0.

The individual detection time series were irregularly sampled in time, and the duration between successive relocations could occasionally span more than one day (CR smolts and kelts: 23 % and 1 % of relocations respectively, CBR smolts and kelts: 48 % and 24 % of relocations respectively). Interpolation was, therefore, applied to standardize the temporal resolution of seaward progression to one day. Here, two hour long time bins were used to interpolate the location sequences so that the position at time *t* was a function of the change in longitude and latitude between successive relocations divided by the number of hours that elapsed between them. To prevent interpolated positions from occurring on land, the shortestPath function in the r package 'gdistance' was used (van Etten 2018).

The analysis of migratory progression and the influence of temperature and life stage were assessed by evaluating the statistical support for each term and its potential interactions in explaining variation in seaward movements. Assessment was completed using two separate analyses. The first analysis assessed the probability that an increase in the linear straight-line distance from the origin (i.e. movement v. residency or landward movement) occurred during a day. The analysis was completed by applying a generalized linear model with the binomial response increase (y=1) or no increase (y=0) in movement along a seaward vector using tag ID

as a random intercept (1|ID). The second analysis assessed the magnitude of seaward movements as represented by the change in linear distance from the river mouth per day (CLD/day), excluding residency events and landward movements. The number of body lengths corresponding to CLD/day was used as the response variable to reduce the variation induced by size dependent mobility (Bainbridge 1958). CLD/day was further log transformed to achieve normally distributed residuals (Sokal and Rohlf 1973). A first order autoregressive term was nested under the random intercept of tag ID to account for temporal autocorrelation. Because investigations of model residuals suggested heterogeneity between levels in the life stage term of the Conne River population, a variance structure allowing for life stage specific differences in variance was added to that analysis (Zuur et al. 2009).

The two measures as described above represented the distance and direction of successive relocations during a day, but did not directly describe the swimming speed of tagged individuals. The presence of a temperature effect and potential differences in the responses of smolts and kelts were investigated by evaluating the model structure that best fitted the data from among a set of candidate models that included the predictors: Life stage, surface temperature, day of year (DOY), and the interaction between temperature and life stage and temperature and DOY. To avoid complications associated with colinearity, temperature and DOY were not included in the model simultaneously. Non-linear effects of temperature and DOY on the amplitude of seaward movements were evaluated by including candidate models with 2^{nd} and 3^{rd} order polynomial terms.

Because receiver spacing may have influenced the ability to detect movements and the direction and magnitude of detected movements, the effect of receiver spacing on the direction

and distance of seaward movements was evaluated by adding the nearest receiver station neighbor distance. To reduce model complexity and facilitate model selection, and because we considered that any effect of receiver spacing would be additive, interactions with this term were not included in the model. The resulting full models considered were:

$$Y_{ij} = (\beta_0 + ID_{0j}) + \text{Life stage}_{ij} + \text{Temp}_{ij} + \text{Life stage}\cdot\text{Temp}_{ij} + \text{Life stage}\cdot\text{Temp}^2_{ij} + \text{Life stage}\cdot\text{Temp}^3_{ij} + NND_{ij} + e_{ij}$$

and

$$Y_{ij} = (\beta_0 + ID_{0j}) + \text{Life stage}_{ij} + DOY_{ij} + \text{Life stage}\cdot DOY_{ij} + \text{Life stage}\cdot DOY^2_{ij} + \text{Life stage}\cdot DOY^3_{ij} + NND_{ij} + e_{ij}$$

where Y_{ij} is the binomial response (increase or no increase in seaward movement) or the continuous response CLD/day (log transformed) at instance i, for individual fish j , NND is the nearest neighbour distance of the receiver station of the most recent detection at instant i of individual fish j, and the remainder of the variables are as defined above.

2.4 Results

Of the 62 smolts released in Conne River in 2007, 41 (66%) were detected on estuarine region receivers, and 35 (56%) on receivers in the outer region. All except one of 31 tagged kelts (97 %) in Conne River were detected entering the estuary and outer region. In Campbellton River, over 90 % of smolts and kelts (26 and 46, respectively) were detected in the estuary, and more than 85 % on receivers in the outer region (25 and 41, respectively). Sixteen and five kelts from Campbellton and Conne River (16% and 33%), respectively, returned as consecutive repeat spawners in the summer following their release.

2.4.1 Estuarine and marine transit and residency times

In the two populations investigated, kelts moved significantly ($p < 0.05$) more quickly from the estuary to the outer region and displayed shorter residency times as compared to smolts. The exception being a lack of significant difference ($p > 0.05$) in residency times in the outer regions, and in the total nearshore residency times of Conne River smolts and kelts (Table 2.3 and Figure 2.3 a).

Smolts from Conne River spent on average 4.1 days in the estuary region before moving to an adjacent region. This was significantly longer than the estuary residency time of kelts that departed the estuaries after on average 2.6 days (GLM, square root transformed, $F_{1,63} = 4.7$, R^2-adjusted = 0.05, $p = 0.03$) (Figure 2.3 a). After departing the estuary, the mean transit time of smolts moving to the outer region was 8.1 days and this was significantly longer than for kelts who spent on average 1.8 days (GLM, log transformed, $F_{1,65} = 74.6$, R^2-adjusted = 0.53, $p < 0.01$) (Table 2.3). Combined with the earlier release times of Conne River kelts (Table 2.1), their shorter residency and transit times in the estuaries and middle region resulted in an approximately three-week earlier arrival in the outer region than smolts. Smolt and kelt arrival in the outer region was then followed by a prolonged residency that lasted approximately three weeks (Table 2.3 and Figure 2.3 a). The total residency time did not differ between smolts and kelts (GLM, $F_{1,63} = 0.86$, R^2-adjusted = -0.002, $p = 0.36$) who spent on average 35.9 and 32.1 days in the Bay d'Espoir (Figure 2.3 a). The majority of this time was spent in the outer region (49 % and 53 %, respectively).

The mean estuary region residency time of Campbellton River smolts was 11.1 days and differed significantly from that of the kelts which departed this region after an average of 3.6

days (GLM, cube root transformed, $F_{1,62} = 15.5$, R^2-adjusted = 0.19, $p < 0.01$) (Table 2.3 and Figure 2.3 b). Smolts then spent 19.4 days moving from the estuary to the outer region, which was significantly longer than that of kelts which spent on average 3.4 days (GLM, log transformed, $F_{1,55} = 24$, R^2-adjusted = 0.29, $p < 0.001$). Arrival in the outer region was not followed by a prolonged residency as smolts and kelts exited the region 3.6 and 1.5 days after entry, respectively (Figure 2.3). Total residency time in the Bay of Exploits was significantly longer (Welch's t-test, t = -6.1, df = 57, $p < 0.001$) by approximately two weeks for smolts (mean: 29.1 days) as compared to kelts (mean: 12.7). The majority of the total residency time of CBR smolts and kelts was spent in the estuarine region (40 % and 45 %, respectively; Figure 2.3 b).

2.4.2 Kelt and smolt migratory pathways

Few directional changes were observed in CR smolts and kelts while moving from the estuary to first entering the outer region. Only two (5 %) smolts and one (2.5 %) kelt that successfully entered the outer region showed multiple directional changes, and there was no statistical support for the hypothesis that directed movements varied by life stage, or as a result of differences in receiver spacing (Table 2.4). There was a significant life stage difference ($\chi^2 = 3.94$, $p < 0.05$) in use of migration route by kelts and smolts with 83 % of the former and 59 % of the latter opting to use the Lampidoes Passage.

Migration route could not be distinguished for five Campbellton River kelts that had missing detections in the middle and/or outer regions. Excluding these, a greater proportion of kelts (88 % of sample) displayed directed movements compared to smolts (68 %) as indicated by the most parsimonious model (Table 2.4, $\chi^2 = 4.38$, $p < 0.05$). The majority of smolts displaying

directed movements used the middle section of the Bay of Exploits (65 %), while the rest intersected with more than one section (23 %) or used the eastern (6 %) or western (6 %) sections of the bay (Fig. 4a). Compared to smolts, a greater proportion of kelts (45 %) used the eastern section of the bay ($\chi^2 = 13.33$, $p < 0.001$) to reach the outer region of the Bay of Exploits, with the majority of the remaining individuals using the middle (42 %) section of the bay (Figure 2.4 b).

Acoustic receivers deployed off the northeast coast of Newfoundland for a separate study detected smolts and kelts from Campbellton River after departing the Bay of Exploits. Five smolts (17%) were recorded on receivers located approximately 30 km west (n = 2) or east (n = 3) of the outer region of Bay of Exploits, and less than 10 km from the shore (Figure 2.4 a). Detections occurred between 6–13 July, on average 11.7 (± 3.42 SD) days after the last detection in the outer region of Bay of Exploits. Twenty-one kelts (51%) were recorded off the northeast coast between 2 June and 1 August. The first detection occurred on average 7.6 days (± 14.06 SD) after last being detected in the outer region. All kelt detections occurred east of the Bay of Exploits, the majority of which were located approximately 30 to 80 km northeast of Campbellton River in the nearshore area surrounding the islands of Twillingate and Fogo (Figure 2.1 c and Figure 2.4 b).

2.4.3 Diel behaviour and the influence of temperature

While present in the estuaries, river temperature had a significant and positive effect on the probability that a movement (i.e. arrival at a receiver station) occurred during daytime in Conne River salmon ($\chi^2 = 19.64$, $p < 0.01$) (Table 2.5), and the most parsimonious AIC ranked model

predicted a lower daytime probability of movement among smolts than kelts at all considered water temperatures ($\chi^2 = 19.87$, $p < 0.001$) (Table 2.5 and Figure 2.5 a).

Two models, one consisting of a positive effect of temperature and one consisting of a positive effect of DOY, where equally supported as the best AIC ranked models in explaining variation in diel movements of Campbellton River smolts and kelts while present in the estuaries (Table 2.5 and Figure 2.5 b). A life stage difference was not supported in the CBR population. A lack of difference in the diel movements of Campbellton River smolts and kelts was also evident as fish entered the middle and outer regions. Here, two models also received similar support based on the model selection criteria where one consisted of a positive effect of temperature and one consisted of a positive effect of DOY (Table 2.6 and Figure 2.5 d). In the Conne River population, the best ranked AIC model fit to data from the middle and outer regions supported a positive correlation between diurnal movements and DOY ($\chi^2 = 10.64$, $p < 0.001$), but with a lower probability of smolt movement during daytime than kelt movement ($\chi^2 = 4.33$, $p < 0.05$) (Table 2.6 and Figure 2.5 c).

2.4.4 Influence of nearshore temperatures on seaward movements

The best supported models describing the probability of seaward movement (i.e. an increase in the linear straight-line distance from the origin) in smolts and kelts from the two populations predicted an increase with time but not temperature (Table 2.7). In Conne River, the most parsimonious model fit to the amplitude of seaward movements consisted of year (DOY) and life stage and predicted a linear increase with time (Table 2.8 and Figure 2.6 a). In Campbellton River, SST measured in the outer region was the only explanatory variable included in the best supported model fit to the amplitude of seaward movements (Table 2.8). This model consisted

of a third order polynomial that predicted a peak in seaward movements at approximately 9 ºC (Figure 2.6).

2.5 Discussion

Kelt and smolt migratory routes and residency times were found to differ, whereas life stage contrasts in total nearshore residency and diel movements varied by population. Although the significance of temperature effects on the movements of smolts and kelts varied by population, when an effect was present nocturnal movements were found to decrease and seaward movements to increase with temperature as hypothesized.

Consistent with our expectations, smolts spent significantly more time moving from the estuaries to the outer regions than kelts, with the difference potentially reflecting the superior swimming abilities of the larger bodied adults. Because distance covered per tail beat is a direct and positive function of fish length (Bainbridge 1958), larger fish can cover more distance in less time using less energy (Schmidt-Nielsen 1972) compared to smaller fish when swimming at equal tail-beat frequencies. Previously reported transit and residency times of smolts and kelts tracked in coastal Gaspe, Quebec, however, found that despite their smaller size, smolts may travel at greater speeds than kelts (Hedger et al. 2008, Hedger et al. 2009). In the latter study, the longer period of coastal residency by kelts was explained by a prolonged residency in the delta likely because of a need to feed to improve somatic reserves for seaward migration (Hedger et al. 2009). Because Atlantic salmon spend up to 70 % of their somatic energy reserves during spawning, reserves for downstream migrating kelts can be critically low (Jonsson and Jonsson 1997). Thus, feeding early to increment reserves may increase migration performance

(e.g. Duijns et al. 2017) and subsequent returns for repeat spawning events (e.g. Haraldstad et al. 2018). In the Conne River population, the lack of life stage differences in outer region and total nearshore residency times further suggests that factors in addition to size-dependent mobility contribute to between-life stage variation in migration rates. Such factors could include the fitness benefit of feeding in the nearshore environment by kelts, provided appropriate prey sources are present. Other possible mechanisms may include responses to temporal characteristics of the habitat such as temperature, in cases where groups are monitored at different time periods. For example, the colder temperatures associated with the earlier release of Conne River kelts compared to smolts (Table 2.1 and Table 2.3), may have contributed to increased residency and transit times.

In general, Atlantic salmon smolts are considered to be nocturnal during the transition from fresh to sea water (Thorpe and Morgan 1978, Lefevre et al. 2012, Drenner et al., 2012, Haraldstad et al. 2017), a behaviour hypothesized to be an adaptive response to reduce exposure to and the efficacy of visual predators (Furey et al. 2016, Melnychuk and Welch 2018). Avoidance of daytime movement has been observed in Pacific salmon smolts migrating in a high-risk environment and to result in a near two-fold increase in survival to coastal areas compared to individuals migrating during daylight (Melnychuck and Welch 2018). In contrast to smolts, nocturnal movements in kelts is less prevalent in the literature (Hedger et al. 2009), with no diel pattern frequently reported (Hubley et al. 2008, Hedger et al. 2009, Halttunen et al. 2009; but see Scruton et al. 2007). The lack of tendency for nocturnal migration is likely a result of their large body size which precludes predation from many piscivorous fish and birds (Hedger

et al. 2009). The lower probability of nocturnal movements in Conne River kelts compared to the smolts is thus consistent with the literature.

Previous studies have found a decrease in nocturnal migration with temperature, suggesting an increasing ability of ectotherms to evade attacks from endotherm predators in warming conditions (e.g. Ibbotson et al 2006, Haraldstad et al. 2017). In warm conditions, the advantage of foraging in daylight probably outweighs the risk of predation (Thorpe et al. 1994). Consistent with this expectation, a significant and positive relationship was found between diurnal movements and thermal conditions in Conne River fish while present in the estuary, with smolts being more nocturnal in their movements than kelts across thermal conditions.

The contrast of nocturnal migration by Conne River smolts with diurnal migration by Campbellton River smolts and the differences between life stages in Conne River but not in Campbellton River points to population-specific variation in migration tactics. Contrasts may be related to geographical variation in predation risk, with the constraints imposed by the need to evade predation giving rise to adaptive or plastic responses in behaviour (Lima and Dill 1990, Skalski and Gilliam 2002). Melnychuck et al. (2018), for example, demonstrated that nocturnal migrations were not prevalent in rivers characterized by high turbidity/low visibility and hypothesized that this was due to the reduced foraging efficiency of visual predators and the associated predation risk for migrating smolts. The Conne River may, therefore, be a higher risk environment than the Campbellton River, an assumption supported by the lower reported river survival rates in the Conne River (Dempson et al. 2011). Population contrasts in diel timing may also have arisen in response to population differences in smolt size, as suggested by examples of size-dependent diel behaviours where smaller smolts display more nocturnal movement

than larger smolts (Ibbotson et al. 2011). Accordingly, the larger size of the Campbellton than Conne River smolts (Table 1) may have reduced their susceptibility to visual predators and the relative profit of nocturnal v. diurnal movements.

The reported presence and patterns of diel horizontal movements in coastal habitat for smolts appear to vary throughout the smolt literature, with examples of nocturnal (Hedger et al. 2008), diurnal (Lacroix and McCurdy 1996), or no clear diel patterns in seaward movements being present (Moore et al. 1998, Lacroix et al. 2004, Davidsen et al. 2009). In comparison, there are few descriptions of diel migration patterns for kelts (Hubley et al. 2008, Halttunen et al. 2009, Hedger et al. 2009). After leaving the estuary and transitioning to the middle and outer regions of the Bay d'Espoir and Bay of Exploits, smolts and kelts were primarily diurnal in their movements. Campbellton River salmon showing no apparent shift in diel behaviours associated with departing the estuaries, whereas both smolts and kelts from the Conne River population transitioned from a temperature dependent nocturnal migration to an increasingly diurnal migration in the middle and outer zones. Here, the effect of temperature on the diel movements of smolts and kelts could not be statistically disentangled from linear increases with time (day of the year) owing to interactions between the variables. Nevertheless, increases in diurnal movement may have been driven by warming sea surface temperatures given the implications of temperature for metabolic demand and the associations between growth and ration (Jobling 1981). For example, for migrating smolts an apparent temperature threshold (11-13 C°) has been proposed above which nocturnal movements switch to diurnal because of both the improved ability to avoid predators and the need to meet the higher metabolic demands imposed by temperature (Thorpe et al. 1994, Ibbotson et al. 2006, Haraldstad et al.

2017). Because the SST experienced by migrating smolts and kelts in the Bay d'Espoir and Bay of Exploits rarely exceeded the suggested threshold (Figure 2.2), it does not appear to explain the diurnal behaviours observed. Such thresholds may, on the other hand, be adaptive as a result of persistent environmental differences between the eastern and western Atlantic, with mean temperatures at all latitudes in coastal regions of the eastern Atlantic exceeding those of coastal regions in the western Atlantic (Chavarie et al. 2010). As the consistency in temperature differences meets the precondition for the development of geographic variation in biological characteristics (e.g. Conover & Schultz, 1995), our results may indicate behavioral adaptations to a colder local thermal regime. As such, the results of this study may be indicative of a behavioural shift associated with the transition from estuarine to marine habitats and a need to increase growth (Peyronnet et al. 2007, Friedland et al. 2009) and somatic reserves through increased foraging in daylight (Metcalfe et al. 1999).

Empirical data examining relationships between individual speed and direction of movements and marine temperature are limited for Atlantic salmon, but authors have proposed that such a relationship may exist (e.g. Dutil and Coutu 1998, Lefevre et al. 2012). Consistent with the expectation of temperature mediated seaward movements, the present study showed that the amplitude of movements along a seaward vector were correlated to SST in the Campbellton River population. The lack of a correlation in Conne River may reflect the comparatively more stable thermal environment in the Bay d'Espoir during the study period (Figure 2.2). However, in the Conne River there was a linear increase in seaward progression with time (Figure 2.6) and we cannot exclude that the increase was positively correlated with a similar temporal trend in SST or other temporally dependent characteristics of the environment. Photoperiod, for

example, is known to affect 'migratory restlessness' in migratory species such as birds, where increased daylength triggers increased activity and the expression of migratory behaviours (e.g. Eikenaar et al. 2014, Robart et al. 2018). Possible proximate explanations underlying temperature-mediated seaward movement may also include physiological and behavioural mechanisms. Temperature may affect seaward movements directly through its effect on activity levels and sustained swim speeds (Booth et al. 1997, Ojanguren and Brana 2000), while conditions below or above a thermal optimum may induce thermoregulatory behaviours (Reynolds and Casterlin 1979) through vertical and/or horizontal displacement. Active temperature selection by Atlantic salmon is supported by the relatively narrow range of temperatures occupied compared to availability in the habitat (Minke-Martin et al. 2015).

In conjunction with other external gradients such as salinity and current, temperature has been proposed as an orientation signal used by migrating salmon (Byron and Burke 2014, Moriarty et al. 2016). In Campbellton River, the seaward movements of smolts and kelts increased with temperature up to approximately 9°C, a threshold occurring in the upper range of previously reported estimates of average marine temperature use (3.9-9.7°C) in Atlantic salmon (Minke-Martin et al. 2015). Assuming these values represent a preferred thermal range, our results suggest smolts and kelts selectively migrate offshore under preferred temperature conditions. Alternatively, smolts and kelts tracked favorable temperatures through directional responses to local conditions (Willis 2011).

The lower between-individual variability in migratory passageways and more unidirectional movements of kelts compared to smolts from the Conne and Campbellton Rivers offered some support for the hypothesis that adults are more effective at orienting towards the open ocean.

Although orientation mechanisms during the marine phase of salmonid migrations are poorly understood (Byron and Burke 2014), it is often suggested that an innate spatiotemporal control of movement towards a goal outside the sensory environment (e.g. compass navigation), combined with directional responses to local environmental cues (e.g., salinity and water currents), are required (Dodson 1988, Lohmann et al. 2008, Byron and Burke 2014). Spatiotemporal control is thought to guide animals across extensive distances whereas directional controls are believed to act at a finer spatial scale, such as the coastal and riverine phase of salmon homing migrations. The accurate "homing" of stocked salmon to the rivers they left as smolts (Hansen and Jonsson 1994) suggested that juveniles learn cues associated with their home stream and use retained memories to guide their return as adults (Dittman and Quinn 1996, Nevitt and Dittman 1998). Being veteran migrants, kelts may similarly exploit memory to more efficiently orient themselves for seaward migration than smolts. As such, the association between the more eastern migratory route of Campbellton River kelts compared to smolts in the Bay of Exploits and the eastern/south-eastern direction of coastal movements by kelts following departure of the bay, may be indicative of an innate spatiotemporal control. However, because this study was not able to distinguish route-searching behaviour from other behaviours, or determine the environmental factors associated with spatial variation in migratory paths, our data cannot be interpreted as providing support for the memory hypothesis.

Directional changes in seaward movements may also reflect behavioural osmoregulation in response to spatiotemporal heterogeneity in salinity levels as smolts acclimate to seawater (Halfyard et al. 2013). The hypothesis, however, is not supported by more recent findings which

suggest that smolts develop sufficient seawater tolerance prior to seawater entry (Urke et al 2014a, 2014b). Surface currents may compromise unidirectional fish movements as a result of advection, although the literature suggests that smolt direction in the marine environment depends largely on the actual movement of the fish rather than on water currents (Thorstad et al. 2004, Økland et al. 2006, Martin et al. 2009). Nevertheless, the larger size of kelts suggests that their horizontal movements should be less constrained by surface currents than those of smolts because of their greater swimming capacity.

The total residency times of Conne River smolts and kelts and Campbellton River smolts appear high when compared to reports of Atlantic salmon migrations tracked in other coastal environments (e.g. Lacroix et al. 2004, Finstad et al. 2005, Davidsen et al. 2009, Halttunen et al. 2009, Hedger et al. 2009,). The high estimated survival of Conne River smolts to the outer region (Dempson et al. 2011) and the high proportions of Campbellton River smolts and kelts and Conne River kelts tracked to the open ocean, therefore, suggest that mortality was low and environmental conditions conducive to sustain prolonged residency in the nearshore areas. The considerable improvement in oceanic thermal conditions that would be associated with the prolonged nearshore residency thus indicates that such residency may promote open ocean entry during conditions favorable to migration performance, and potentially mitigate earlier mismatches in migration timing relative to ecosystem phenology (Cushing 1990, Satterthwaite et al. 2014). As such, our results indicate that coastal habitats may be important rearing or staging areas for migrating smolts and kelts in Newfoundland.

2.6 Conclusion

Identifying factors influencing marine movement patterns in Atlantic salmon is an important step in understanding underlying mechanisms and their linkage to survival. This study demonstrated that ontogeny and temperature influence the marine movement patterns of Atlantic salmon during the early migration phase. There were differences in smolt and kelt movement related to migration routes, migration rates and in diel movement, but the differences varied by population. When present, the differences were characterized by faster, more directed, and less nocturnal movements by kelts than smolts. Statistical support for a temperature effect on the movements of migrating smolts and kelts also varied by population. Identified temperature influences consisted of a positive correlation with seaward movements and a negative correlation with diel movements as hypothesized. We suggest that possible sources of the between-population variation may include temporal and physical contrasts in the biotic and abiotic environment shaping the constraints imposed by trade-offs such as those between the need to reduce predation risk and to increase growth and mass gain. Such contrasts may, therefore, result in population-specific variation in migration tactics.

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2.8 Tables

Table 2.1: Date of release, numbers and size (mean mm L_F ± SD) of smolts and kelts tagged in the Conne (CR) and Campbellton (CBR) Rivers.

	CR		CBR	
Life stage	Smolt	Kelt	Smolt	Kelt
n	62	31	28	48
Date of release	9-13 May	22-24 April	27-28 May	26-30 May
Mean ± SD L_F (cm)	17.7 ± 1.2	56.4 ± 6.7	23 ± 3.7	59 ± 5.4

Table 2.2: Numbers and specifications of acoustic tags fitted to smolts and kelts in the Conne (CR) and Campbellton (CBR) Rivers.

	CR		**CBR**					
Tag model	Smolt	Kelt	Smolt	Kelt	Nominal delay (s)	Exp. Battery life (days)	Power output (re 1 uPa @ 1m)	Weight in water (g)
V13TP-1H		30			60	300	149	6.5
V7-2L	62				45	70	136	0.75
V13-1H				11	70	676	152	6.5
V9-1H			2	33	60	102	151	2.1
V8-4H			26	4	60	61	147	0.9

Table 2.3 Transit and residency times (days) of Conne (CR) and Campbellton River (CBR) smolts and kelts, and SST (°C) during the day of smolt and kelt estuary, middle and outer region entry and the day of departure from the embayments. Mean values with an asterix denote smolt values that differed significantly from those of kelts (P<0.05). CI denotes confidence interval half length.

	CR						CBR					
	Smolt			Kelt			Smolt			Kelt		
Response	Mean	CI	Range	Mean	CI	Range	Mean	CI	Range	Mean	CI	Range
Estuary region residency	4.1*	1.08	0.01– 14.2	2.6	1.08	0.12 – 11.9	11.1*	3.50	1.2- 23.1	3.6	1.39	0.003 – 23.3
Middle region residency	7.1*	2.71	0.15 – 44.0	2.0	0.65	0.15 – 6.9	6.4*	3.69	0.02- 33.5	2.6	1.58	0.007 – 16.8
Outer region residency	19.7	3.37	0.01 – 34.9	20.6	6.45	0.13 – 54.2	3.6	2.90	0.02 - 34.2	1.5	1.31	0.001 – 16.1
Total nearshore residency	35.9	3.08	9.3– 53.9	32.1	8.42	1.0 – 73.3	29.1*	2.85	14.4 - 50.6	12.7	5.78	0.02 – 69.6
Transit estuary to outer region	8.1*	1.5	1.6 – 16.5	1.8	4.8	0.4 – 4.8	19.4*	3.17	5.3 - 29	3.4	1.2	0.65– 19.9
SST, estuary entry	5.7*	0.25	3.8 – 6.5	2.9	0.22	2.2 – 4.4	6.5*	0.79	3.5 - 10.9	5.0	0.44	2.2 – 8.3
SST, outer region entry	6.3*	0.20	4.9 – 7.5	3.7	0.32	2.5 – 6.3	10.5*	0.43	8.1 - 12.0	8.4	0.78	3.1 – 12.0
SST, departure of outer region	8.1*	0.37	5.9 – 9.6	5.6	0.85	2.5 – 9.7	11.9*	0.48	9.6 – 14.5	8.1	0.72	3.2 –12.8

Table 2.4: Ranking of the binomial models describing the probability that Conne (CR) and Campbellton River (CBR) smolts and kelts displayed directed or non-directed migratory routes, as a function of life stage and receiver station spacing. Asterix denotes the most parsimonious model, based on the number of parameters in cases where the lowest AIC model and other candidate models differed by less than two AIC units. (NND: distance to the nearest neighbour receiver station)

CR model	df	logLik	AIC	delta.AIC	CBR model	df	logLik	AIC	delta.AIC
y ~ NND	3	-198.34	402.68	0.00	y ~ Life.stage + NND	4	-129.45	266.91	0.00
*y ~ (1|ID)	2	-199.96	403.93	1.25	y ~ Life.stage · NND	5	-129.18	268.35	1.45
y ~ Life.stage + NND	4	-198.04	404.08	1.40	*y ~ Life.stage	3	-131.28	268.56	1.65
y ~ Life.stage · NND	5	-197.51	405.02	2.34	y ~ NND	3	-131.64	269.29	2.38
y ~ Life.stage	3	-199.61	405.21	2.53	y ~ (1|ID)	2	-133.92	271.84	4.93

Table 2.5: Ranking of the binomial mixed models describing diel movement of smolts and kelts from Conne (CR) and Campbellton Rivers (CBR) while present in the estuaries, based on the AIC criterion. Random effect held constant ((1ID)). Asterix denotes the most parsimonious model, based on the number of parameters in cases where the lowest AIC model and other candidate models differed by less than two AIC units.

CR model	df	logLik	AIC	delta AIC	CBR model	df	logLik	AIC	delta AIC
y ~ Life stage + poly(temp, 2)	5	-88.53	187.06	0.00	*y ~ DOY	3	-96.37	198.74	0.00
*y ~ Life stage + temp	4	-89.68	187.37	0.30	*y ~ temp	3	-96.37	198.74	0.00
y ~ Life stage · temp	5	-88.89	187.79	0.72	y ~ poly(DOY, 2)	4	-95.98	199.95	1.21
y ~ Life stage	3	-95.94	197.87	10.81	y ~ poly(temp, 2)	4	-96.24	200.49	1.75
y ~ Life stage + DOY	4	-95.77	199.55	12.48	y ~ Life stage + DOY	4	-96.29	200.58	1.84
y ~ Life stage + poly(DOY, 2)	5	-95.12	200.24	13.18	y ~ Life stage + temp	4	-96.35	200.70	1.97
y ~ poly(DOY, 2)	4	-96.31	200.61	13.55	y ~ Life stage	3	-97.74	201.48	2.74
y ~ Life stage ·DOY	5	-95.77	201.54	14.48	y ~ Life stage + poly(DOY, 2)	5	-95.95	201.90	3.16
y ~ DOY	3	-97.82	201.64	14.58	y ~ Life stage + poly(temp, 2)	5	-96.22	202.44	3.70
y ~ temp	3	-99.62	205.25	18.18	y ~ Life stage · DOY	5	-96.26	202.51	3.78
y ~ poly(temp, 2)	4	-99.58	207.17	20.10	y ~ Life stage · temp	5	-96.33	202.65	3.91
y ~ (1|ID)	3	-107.71	221.43	34.37	y ~ (1|ID)	3	-104.53	215.06	16.33

Table 2.6: Ranking of the binomial mixed models describing diel movement of smolts and kelts from Conne (CR) and Campbellton Rivers (CBR) while present in the outer and middle regions, based on the AIC criterion. Random effect held constant ((1ID)). Asterix denotes the most parsimonious model, based on the number of parameters, where the lowest AIC model and other candidate models differed by less than two AIC units.

CR model	df	logLik	AIC	delta AIC	CBR model	df	logLik	AIC	delta AIC
*y ~ Life stage + DOY	4	-595.82	1199.64	0.00	y ~ Life stage · poly(temp, 2)	7	-131.07	276.14	0.00
y ~ Life stage · poly(temp, 2)	7	-593.52	1201.05	1.40	*y ~ DOY	3	-135.63	277.25	1.11
y ~ Life stage · DOY	5	-595.70	1201.40	1.76	y ~ Temp	3	-135.96	277.92	1.79
y ~ Life stage + poly(DOY, 2)	5	-595.80	1201.60	1.96	y ~ Life stage	3	-135.97	277.94	1.80
y ~ DOY	3	-597.99	1201.98	2.33	y ~ poly(temp, 2)	4	-135.18	278.36	2.23
y ~ poly(DOY, 2)	4	-597.89	1203.78	4.14	y ~ Life stage + DOY	4	-135.52	279.04	2.91
y ~ Life stage + temp	4	-598.45	1204.90	5.25	y ~ poly(DOY, 2)	4	-135.62	279.24	3.10
y ~ Life stage · poly(DOY, 2)	7	-595.68	1205.37	5.72	y ~ Life stage + temp	4	-135.95	279.90	3.77
y ~ temp	3	-599.79	1205.58	5.94	y ~ Life stage + poly(temp, 2)	5	-135.14	280.28	4.14
y ~ Life stage · temp	5	-598.02	1206.05	6.40	y ~ Life stage + poly(DOY, 2)	5	-135.51	281.02	4.88
y ~ Life stage + poly(temp, 2)	5	-598.11	1206.22	6.58	y ~ Life stage · DOY	5	-135.52	281.03	4.90
y ~ poly(temp, 2)	4	-599.40	1206.79	7.15	y ~ Life stage · temp	5	-135.92	281.83	5.70
y ~ Life stage	3	-601.15	1208.29	8.65	y ~ Life stage · poly(DOY, 2)	7	-134.29	282.59	6.45
y ~ (1|ID)	3	-632.11	1270.21	70.57	y ~ (1|ID)	3	-144.13	294.27	18.13

Table 2.7: Fit statistics and most supported model fitted to the binomial seaward movement model (y=1: seaward movement, y=0: residency or landward movement) for Conne (CR) and Campbellton River (CBR) smolts and kelts based on the AIC criterion. Random effect held constant ((1ID)). (NND: distance to the nearest neighbour receiver station). Asterix denotes the most parsimonious model, based on the number of parameters in cases where the lowest AIC model and other candidate models differed by less than two AIC units.

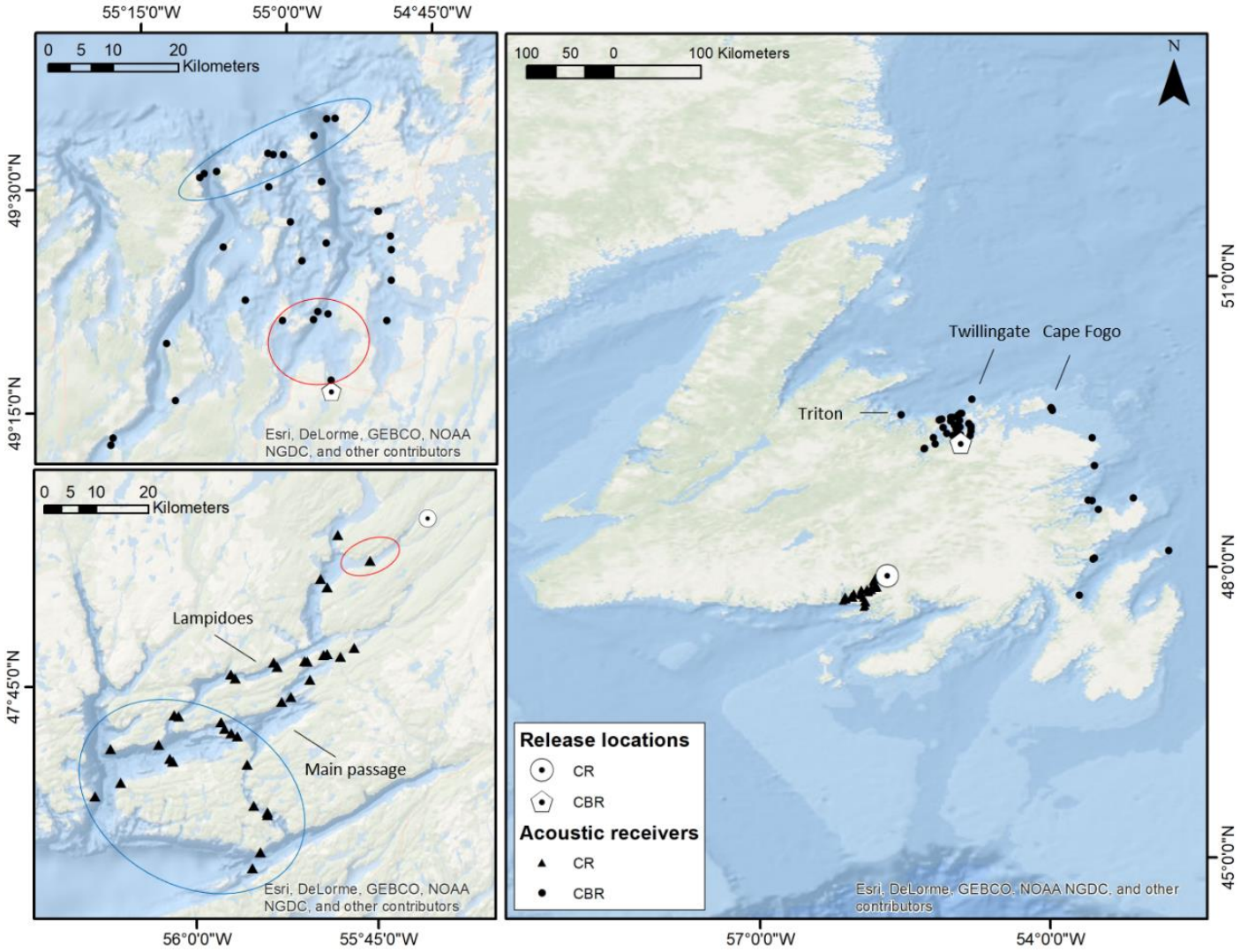
CR model	df	logLik	AIC	delta AIC	CBR model	df	logLik	AIC	delta AIC
*y ~ Life stage · DOY	5	-271.18	552.37	0.00	*y ~ NND + Life stage + DOY	5	-358.41	726.81	0.00
y ~ NND + Life stage · DOY	6	-270.60	553.20	0.83	y ~ NND + Life stage · DOY	6	-357.70	727.39	0.58
y ~ Life stage + DOY	4	-276.87	561.74	9.38	y ~ NND + DOY	4	-364.29	736.58	9.77
y ~ NND + Life stage + DOY	5	-276.81	563.62	11.25	y ~ Life stage · DOY	5	-373.45	756.89	30.08
y ~ DOY	3	-280.63	567.26	14.89	y ~ Life stage + DOY	4	-375.84	759.67	32.86
y ~ NND + DOY	4	-280.45	568.90	16.54	y ~ DOY	3	-378.23	762.47	35.65
y ~ Life stage · temp	5	-295.14	600.27	47.91	y ~ NND + Life stage + temp	5	-381.51	773.01	46.20
y ~ temp	3	-297.27	600.55	48.18	y ~ NND	3	-383.69	773.39	46.57
y ~ NND + Life stage · temp	6	-294.73	601.47	49.10	y ~ NND + Life stage	4	-383.07	774.14	47.32

y ~ Life stage + temp	4	-296.78	601.57	49.20	y ~ NND + Life stage · temp	6	-381.28	774.57	47.76
y ~ Life stage	3	-297.87	601.75	49.38	y ~ temp	3	-386.39	778.79	51.97
y ~ NND + Life stage + temp	5	-296.48	602.95	50.59	y ~ 1	2	-387.71	779.41	52.60
y ~ NND + Life stage	4	-297.65	603.30	50.93	y ~ Life stage + temp	4	-386.03	780.06	53.25
y ~ (1|ID)	2	-302.16	608.33	55.96	y ~ Life stage	3	-387.33	780.67	53.86
y ~ NND	3	-302.02	610.04	57.67	y ~ Life stage · temp	5	-385.59	781.19	54.38

Table 2.8: Fit statistics of the 10 most supported models fitted to the continuous response, magnitude of seaward movements per day (log transformed) for Conne (CR) and Campbellton Rivers (CBR) smolts and kelts based on the AIC criterion. Random effect held constant (1ID). (NND: distance to the nearest neighbour receiver station). Asterix denotes the most parsimonious model, based on the number of parameters in cases where the lowest AIC model and other candidate models differed by less than two AIC units.

CR model	df	logLik	AIC	delta AIC	CBR model	df	logLik	AIC	delta AIC
*y ~ Life stage + DOY	6	-578.90	1169.81	0.00	*y ~ poly(temp, 3)	7	-977.37	1968.74	0.00
y ~ NND + Life stage + DOY	7	-578.23	1170.47	0.66	y ~ Life stage + poly(temp, 3)	8	-977.14	1970.29	1.55
y ~ Life stage · DOY	7	-578.58	1171.15	1.34	y ~ poly(temp, 2)	6	-981.08	1974.15	5.41
y ~ NND + Life stage · DOY	8	-577.80	1171.59	1.78	y ~ Life stage + poly(temp, 2)	7	-980.85	1975.71	6.96
y ~ Life stage + poly(DOY, 2)	7	-578.86	1171.72	1.91	y ~ Life stage · poly(temp, 3)	11	-976.81	1975.62	6.88
y ~ Life stage + poly(DOY, 3)	8	-578.84	1173.67	3.86	y ~ temp	5	-983.14	1976.28	7.54
y ~ Life stage + temp	6	-580.94	1173.88	4.07	y ~ Life stage · poly(DOY, 3)	11	-977.13	1976.26	7.52
y ~ Life stage · poly(DOY, 3)	11	-575.73	1173.46	3.65	y ~ NND + Life stage*poly(DOY, 3)	12	-976.70	1977.40	8.65
y ~ Life stage · poly(DOY, 2)	9	-578.25	1174.49	4.68	y ~ NND + Life stage * poly(temp, 3)	12	-976.73	1977.46	8.72
y ~ NND + Life stage·poly(DOY, 3)	12	-575.26	1174.51	4.70	y ~ Life stage + temp	6	-983.00	1978.01	9.27
y ~ NND + Life stage·poly(DOY, 2)	10	-577.41	1174.83	5.02	y ~ Life stage · poly(temp, 2)	9	-980.82	1979.63	10.89
y ~ Life stage + poly(temp, 2)	7	-580.90	1175.80	5.99	y ~ Life stage · temp	7	-982.99	1979.98	11.24
y ~ Life stage · temp	7	-580.93	1175.85	6.05	y ~ NND + Life stage · temp	8	-982.77	1981.54	12.80
y ~ NND + Life stage * temp	8	-580.20	1176.41	6.60	y ~ NND + Life stage · poly(temp, 2)	10	-980.72	1981.43	12.69
y ~ poly(DOY, 2)	6	-582.35	1176.70	6.89	y ~ poly(DOY, 3)	7	-985.28	1984.57	15.83

2.9 Figures


55°15'0"W
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0 5 10 20
Kilometers
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Kilometers
Lampidoes
Main passage
47°45'0"N
Esri, DeLorme, GEBCO, NOAA NGDC, and other contributors
56°0'0"W
55°45'0"W
100 50 0 100 Kilometers
N
Twillingate
Cape Fogo
Triton
51°0'0"N
48°0'0"N
45°0'0"N
Release locations
CR
CBR
Acoustic receivers
CR
CBR
Esri, DeLorme, GEBCO, NOAA NGDC, and other contributors
57°0'0"W
54°0'0"W

Figure 2.1: Map of acoustic receivers and tagging and release locations in a) Bay d'Espoir and Conne River (CR) and b) Bay of Exploits and Campbellton River (CBR). The red circles denote the estuary regions and the blue circles denote the outer bay regions. c) Map showing the location of Conne River and Campbellton River in Newfoundland and the receiver locations along the Northeast Newfoundland coast where smolts and kelts from Campbellton River were recorded.

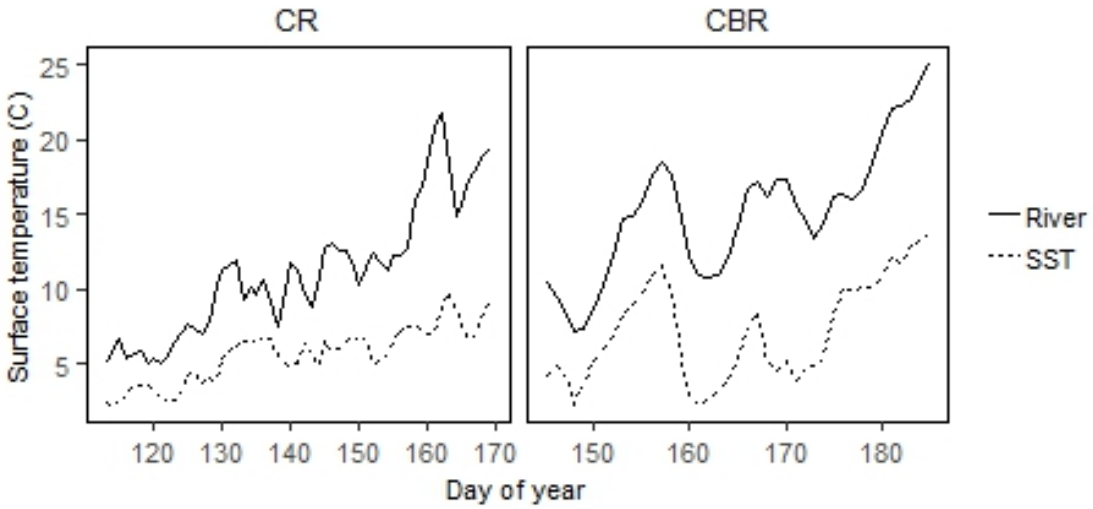

Figure 2.2 Mean daily surface temperatures (river [solid line] and SST [dotted line], °C) over day of year during the migration of Conne (CR) and Campbellton River (CBR) smolts and kelts.

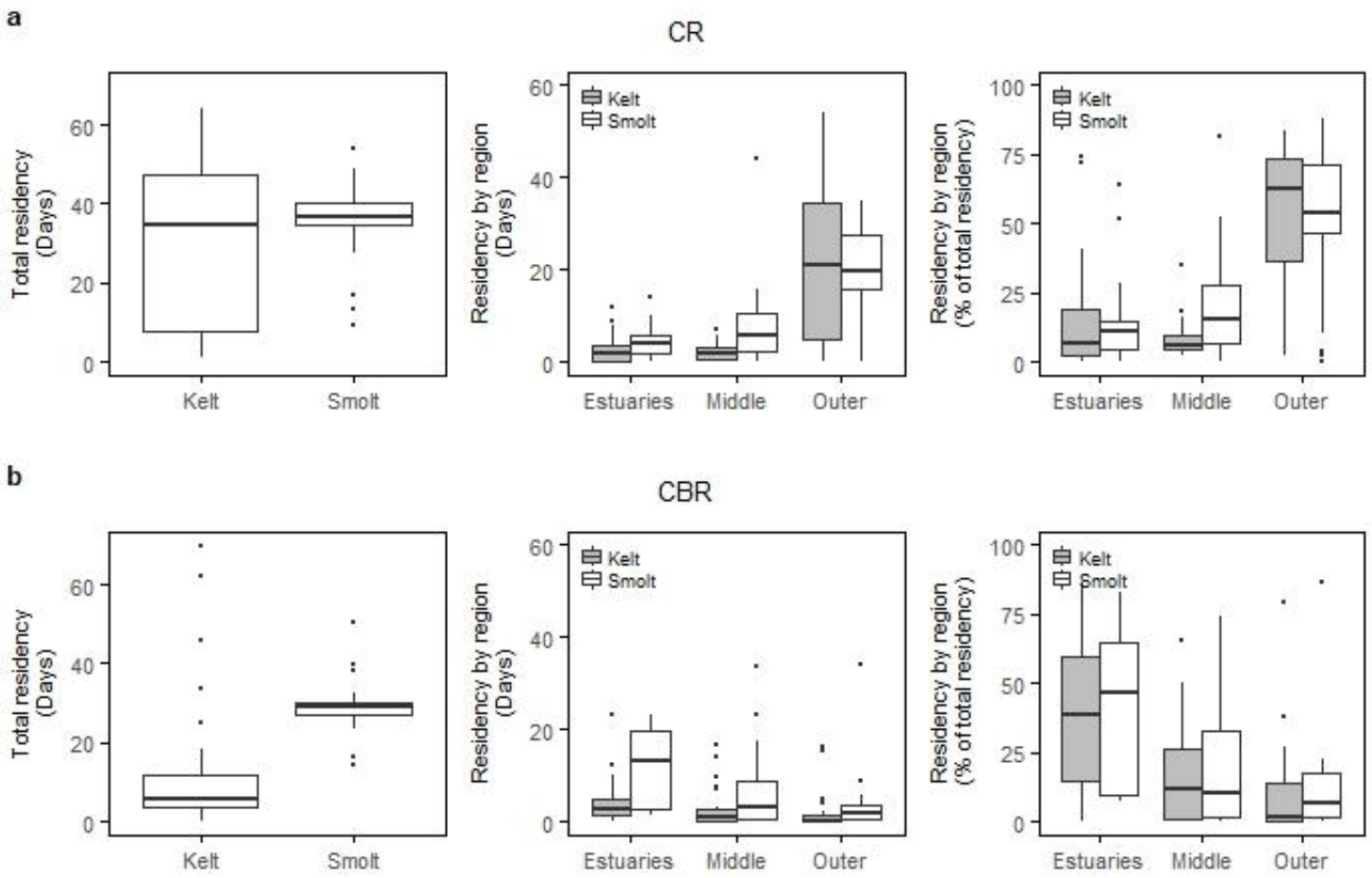

Figure 2.3 Box-and-whisker plots showing the median values (bold black lines), the interquartile ranges (boxes), and the 5th and 95th percentiles (whiskers) of total residency time, residency time by region and residency time by region expressed as percentage of total nearshore residency time of smolts and kelts from a) Conne (CR) and b) Campbellton (CBR) Rivers.

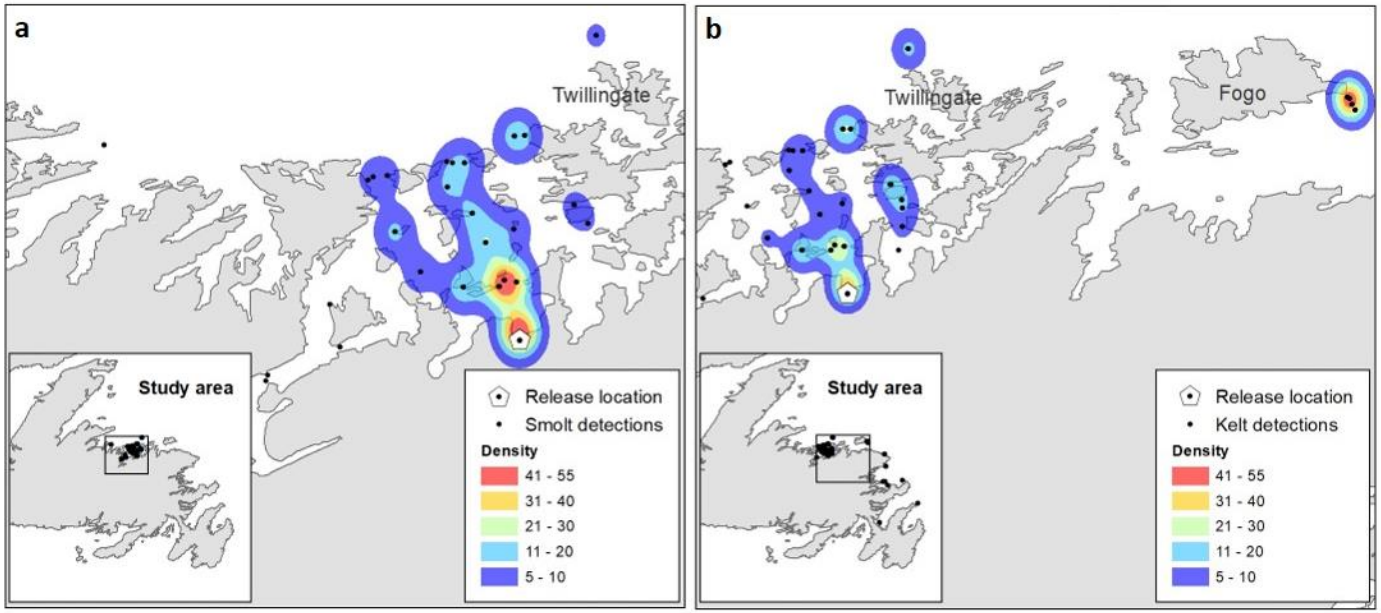

Figure 2.4 Two-dimensional kernel density estimates of the spatial distribution of Campbellton River smolt (a) and kelt (b) presence at acoustic receiver stations in the Bay of Exploits and its vicinity.

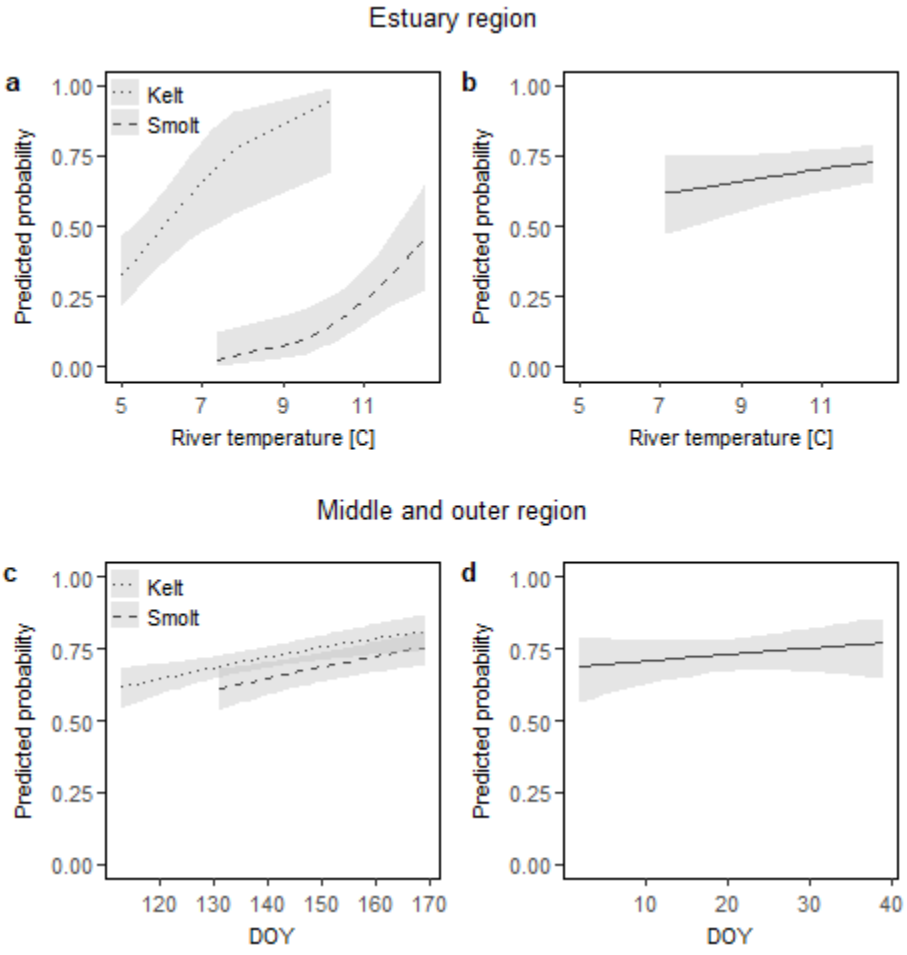

Figure 2.5 Predicted probabilities of daytime arrivals in the estuary region as a function of a) river temperature (°C) and life stage for Conne River salmon and as a function of b) river temperature (°C) for Campbellton River salmon. Predicted probabilities of daytime arrivals at receiver stations in the middle and outer regions as a function of DOY and life stage in c) Conne River salmon and d) DOY in Campbellton River salmon. Grey shaded area represents the 95 % confidence interval of predictions.

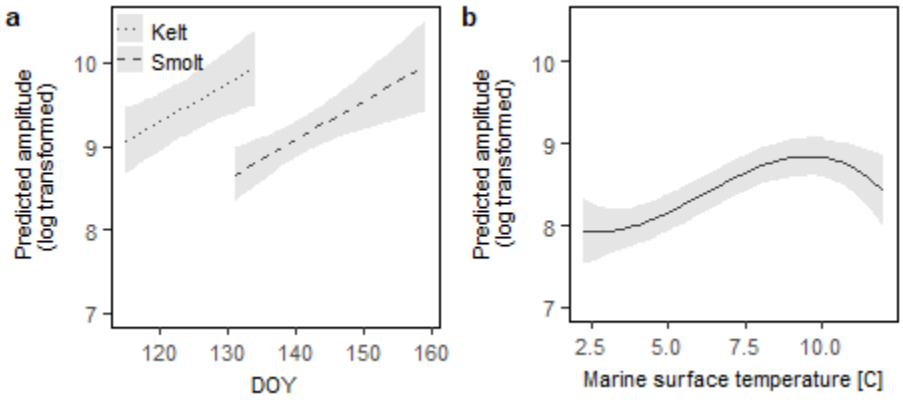

Figure 2.6 Predicted seaward migratory movements (body lengths · day^{-1}) as a function of a) day of year (DOY) and b) marine surface temperature (SST, °C) in Campbellton River smolts and kelts. Grey shaded area represents the 95 % confidence interval of predictions.

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3 Chapter 3: Influence of life history dependent migration strategies on Atlantic salmon diets

Co-authorship statement

This research was developed and carried out by Kristin Bøe, together with the supervisory assistance of Ian Fleming and Michael Power, and the guidance of Martha Robertson, Chris Parrish and Corey Morris. All authors discussed the results and contributed to the final manuscripts.

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3.1 Abstract

Migratory behaviour may vary according to the life history and demographic attributes of fish and lead to the spatial segregation of distinct population segments during the non-breeding season. In adult Atlantic salmon, spawning history differences are associated with intra-population variation in marine movements, but the degree of connectivity in spatial resource use among and within maiden and repeat spawning salmon is not well understood. We analysed muscle fatty acids, δ13C and δ15N of Atlantic salmon returning to spawn, and found significant differences among spawning histories. Maiden and alternate repeat spawning Atlantic salmon were differentiated from consecutive repeat spawners by fatty acid biomarkers associated with distinct biogeographic regions of the Labrador Sea, consistent with differential migration and divergent feeding locations. The presence and pattern of feeding contrasts among spawning history groups were further supported by dorsal muscle δ15N, which covaried with FA compositional values and distinguished consecutive repeat spawners from the two other groups. Because the degree of connectivity among population segments affects the ecological factors faced by such groups, an improved understanding of differential migration is necessary to better predict potential population responses to environmental change.

3.2 Introduction

Differential migration, defined as a migratory behavior associated with demographic attributes such as age, size and sex, is widespread in populations of migratory species such as birds and fishes (Cristol *et al.*, 1999; Secor, 2015). In most cases, the phenomenon results in the spatial

segregation of individuals during the non-breeding season, so that the ecological factors faced by population segments including prey availability and composition, may differ (e.g. Soto *et al.* 2018, Mathot *et al*., 2007). Differential migrations may, therefore, affect intra-population variation in fitness associated traits (Briedis and Bauer, 2018), as variation in the quality of the non-breeding area may influence the breeding success of migratory birds (Marra *et al.*, 1998; Norris *et al.*, 2004) or the parental quality of capital breeders relying on resources stored prior to breeding (Torniainen *et al.*, 2017). Thus differential migration may have important implications for population dynamics in the short and long term (Briedis and Bauer, 2018).

Atlantic salmon (*Salmo salar*) is a highly migratory salmonid that displays considerable phenotypic plasticity and variability in life history characteristics, of which some are associated with differential migration (Klemetsen *et al.*, 2003). For example, maiden spawners will spend one or more years at sea before returning to their natal rivers and spawning the first time, whereas consecutive repeat spawners breed annually, spending on average a few months at sea between spawning events while alternate repeat spawners breed biennially, spending more than a year at sea between spawning events prior to their river return (Klemetsen *et al.*, 2003). The brief residency at sea of consecutive spawners restricts their migration range, and they are believed to recondition in areas proximate to their natal river. In contrast, alternate spawners along with maiden fish are known to perform longer oceanic migrations (Reddin and Short, 1991; Lacroix, 2013; Strøm *et al.*, 2017). Thus, spawning history differences are associated with intra-population variation in the marine movements of adult Atlantic salmon.

Given the logistical difficulties in mapping long distance migrations undertaken by marine fish, detailed understanding of the migration phase of Atlantic salmon life history remains uncertain,

including aspects of dietary ecology and habitat use (Renkawitz *et al.*, 2015). The lack of detailed understanding of the migration patterns and feeding ecology of Atlantic salmon is particularly relevant as the juvenile to adult survival of Atlantic salmon has been in decline, possibly as a result of recent large-scale ecosystem and oceanographic changes in the North Atlantic (Beaugrand and Reid, 2012; Chaput, 2012; Mills *et al.*, 2013). Although there is a growing body of literature demonstrating links between the growth and survival of Atlantic salmon with ocean climate (i.e. Reddin and Friedland, 1993; Friedland *et al*., 2003), causal mechanisms remain poorly understood (Dempson *et al.*, 2010). Large scale changes in the North Atlantic pelagic food web (e.g. Buren *et al.*, 2014) coincident with changes in the oceanographic conditions may have influenced the availability and quality of Atlantic salmon food resources (Mills *et al.* 2013), and variation in Atlantic salmon recruitment may thus be under bottom-up control (Todd *et al.*, 2008; Rikardsen and Dempson, 2011).

Research surveys and tag-recaptures in the Northwest Atlantic indicate that post-smolts originating from North America enter feeding grounds in the main basin of the Labrador Sea by fall of the year they smoltified (Reddin and Short, 1991) and spring catches of fish maturing after one winter at sea (1SW) from the southern Labrador basin to the east of the Grand Banks further suggest that is where they overwinter (Reddin, 1985; Ritter, 1989; Sheehan *et al.*, 2012). The Labrador Sea may also be an important feeding and overwintering area for alternate repeat spawners, as suggested by electronic and conventional tag recapture data collected from the region (Ritter, 1989; Strøm *et al.*, 2017; Ó Maoileidigh *et al.,* 2018). Tag recaptures on the west coast of Greenland show that alternate repeat spawners also utilize this area together with North American origin non-maturing 1SW Atlantic salmon that feed here after their first winter

at sea (Ritter, 1989; Downton, 2001; Ó Maoileidigh *et al.,* 2018). Due to advancing sea ice and loss of preferred thermal habitat off the Newfoundland and Labrador Shelf and the coast of Greenland during winter, Atlantic salmon are believed to overwinter in the southern Labrador Sea/Grand Banks before completing the return migration (Reddin, 2006). Corroborative evidence for the overwintering habitats of maiden and alternate repeat spawning Atlantic salmon in the Labrador Sea is, however, lacking as limited research and commercial fishing has been conducted during the winter months (Reddin, 2006).

A notable example of a population that displays considerable life history variation in migration strategies is that of the Campbellton River (Downton, 2004; Reddin *et al.*, 2011). The Atlantic salmon of Campbellton River (Newfoundland, Canada) is a population where consecutive spawner returns are high (Downton, 2001) and whose migratory behaviors have been studied using acoustic (Chapter 2), archival telemetry (Reddin *et al.*,2011) and tag-recapture analyses (Downton, 2001, 2004). In contrast to maiden and alternate spawners returning to spawn in the same year, consecutive spawners overwinter in freshwater as post-spawners, followed by seaward migrations in spring and an approximately two-month long feeding migration before reconditioned individuals return in late summer (Downton, 2004; Reddin *et al.,* 2011). Acoustic detections of tagged individuals along the northeast coast of Newfoundland throughout the summer indicate residency in shelf waters (Chapter 2). The main geographic feeding area during the year of return for consecutive spawning salmon thus differs from that inferred for conspecific maiden (i.e. predominantly 1SW fish) and alternate repeat spawning fish that are presumed to feed in the central Labrador Sea (Reddin, 2006).

The putative feeding areas for Campbellton River life history types, the Newfoundland and Labrador (NL) Shelf and the central basin of the Labrador Sea, are characterized as distinct water masses as a result of strong contrasts in bathymetry and hydrographic properties associated with distinctive spring and summer phytoplankton community composition and production dynamics that are consistent across years (Head *et al.*, 2003; Fragoso *et al.*, 2016). Due to haline stratification driven by Arctic and ice melt water, phytoplankton blooms occur first (April to early May) on the NL Shelf, followed by the central Labrador Sea bloom (May to June) that is driven by thermal stratification (Frajka-Williams and Rhines, 2010; Harrison *et al.*, 2013; Fragoso *et al.*, 2017). The shelf blooms are dominated by chlorophytes and diatoms in contrast to central Labrador Sea blooms which are characterized by comparatively higher densities of dinoflagellates, prymnesiophytes and synechococcus cyanobacteria (Li *et al.*, 2006; Harrison *et al.*, 2013; Fragoso *et al.*, 2017). In addition to the distinct phytoplankton communities, there is also a strong and consistent pattern in the species composition of zooplankton that differentiates shelf and basin water masses, including considerably greater abundances of the large herbivore copepod *Calanus finmarchicus*, euphausiids and amphipods in the central Labrador Sea than on the shelf (Head *et al.*, 2003; Pepin *et al.*, 2011b).

Analyses performed in the northwest Atlantic have demonstrated that water fatty acid (FA) biomarker composition is a reliable predictor of phytoplankton community structure (Parrish, 1998; Reuss and Poulsen, 2002), and that FA signatures can be traced further up the food chain, including to fish (St John and Lund, 1996; Dwyer *et al.*, 2003; Dalsgaard and St John, 2004). The contrasting biogeographic properties between putative feeding areas thus facilitates the investigation of feeding area use by Atlantic salmon based on FA biomarkers. Based on what is

known about maiden, consecutive and alternate repeat spawner migration patterns on the east coast of Canada, we hypothesized that: i) the FA signatures of consecutive spawners would be consistent with the FA phytoplankton biomarkers for the NL Shelf region, whereas ii) maiden and alternate repeat spawning Atlantic salmon would exhibit similar FA compositions consistent with feeding in the central Labrador Sea, and iii) as a consequence of these spatial differences in feeding, muscle stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) would also differ among spawning history groups consistent with differences in FA compositional values. Furthermore, it was hypothesized that the stable isotope ratios would reflect size-dependent feeding given the potential for the ingestion of larger prey sizes as fish grow and gape size increases.

3.3 Methods

3.3.1 Atlantic salmon sample collection

Collection of Atlantic salmon tissue for stable isotope and fatty acid composition analyses was obtained from returning fish as they migrated up the Campbellton River (Figure 3.1) in summer 2016 to spawn. Upstream migrating individuals were intercepted at a Department of Fisheries and Oceans (DFO) operated fish counting facility, located approximately 500 m upstream of the river mouth. The spatial proximity of the facility to the ocean ensured that samples could be collected at the beginning of the freshwater spawning migration period, thus ensuring that lipid and FA composition were assessed prior to significant mobilization of somatic lipid reserves in response to fasting, which in Atlantic salmon is initiated at freshwater entry (Kadri *et al.*, 1995; Doucett *et al.*, 1999). All salmon were measured for fork length (L, cm), weighed (kg), and scales were collected for the determination of age and spawning history following standard guidelines for Atlantic salmon (ICES, 1992). Sex was genetically determined based on the method

described in Yano *et al.* (2013). Scale pattern analysis of sampled Atlantic salmon revealed 7 unique life history combinations with one sea-winter and consecutive repeat spawning being the dominant maturation and re-maturation strategies (Table 3.1).

All fish were anaesthetized prior to the collection of tissue and biological information and monitored post-sampling to ensure complete recovery before individuals were released back into the river. Muscle tissue was collected using a 4 mm diameter disposable biopsy punch (Milltex ®) inserted anterior to the dorsal fin and above the lateral line which removed approximately 50 mg of dorsal muscle. The sample from a subset of the biopsied fish (80 % of the individuals in each spawning history group) was split, with *ca* 10 mg used for stable isotope analyses and the remainder for FA analyses.

Lipid extraction, lipid class separation and fatty acid methyl ester (FAME) derivatization

Muscle tissues for lipid and FA composition analyses were frozen and stored at -20°C before being transferred to lab facilities where each sample was weighed (g). Samples were then stored in chloroform, under nitrogen, at -20°C prior to lipid extraction. Lipid content was determined using the procedure described in Parrish (1999). Muscle tissue was homogenized in a 2:1 mixture of chloroform:methanol and diluted with chloroform extracted water three times to separate the bottom, organic layer from the aqueous solution. The organic layer was removed using a double pipetting technique and weighed to calculate muscle lipid concentration ($g \cdot g$ wet $weight^{-1}$). Lipid class composition was determined by analysing

extracts using Chromarod-Iatroscan (Iatroscan Mark VI TLC-FID) thin layer chromatography/flame ionization (Parrish, 1999). The fatty acid profile of the sample was measured using fatty acid methyl ester (FAME) derivatization on a HP 6890 GC-FID (gas chromatography) following Hixson et al (2014). FAs were expressed using the shorthand notation A:Bn-X, where A represents the number of carbons, B is the number of double bonds, and X is the position of the first double bond relative to the terminal methyl group (CH_3).

Stable isotopes and elemental analysis

Atlantic salmon muscle tissues analysed for stable nitrogen and carbon isotope composition were dried at *ca* 50-60°C before being ground and homogenised. Analyses were performed on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer coupled to a Carlo Erba elemental analyzer with an analytical precision of ± 0.2‰ ($\delta^{13}C$) and ± 0.3‰ ($\delta^{15}N$) at the Environmental Isotope Laboratory, University of Waterloo. Measurement precision was determined by repeat analysis of laboratory working standards placed at the beginning, middle and end of each run that were cross-calibrated to International Atomic Energy Agency standards CH_6 for $\delta^{13}C$ and N^1 and N^2 for $\delta^{15}N$. Measurement consistency was further established by running duplicates of one in every ten samples. Isotope ratios were expressed in the conventional (δ) notation in parts per thousand (‰), after the following equation:

$$\delta\ ^{15}N \text{ or } \delta^{13}C = [(R_{sample}/ R_{standard})-1] \times 1000$$

Where R_{sample} is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N and δ denotes a difference in the ratio of the heavy versus the light isotope of the sample relative to the selected international standard ($R_{standard}$), Vienna Peedee Belemnite limestone for $\delta^{13}C$ and atmospheric air for $\delta^{15}N$

Total elemental C and N were measured as the percent composition of dry mass and used to calculate C:N ratios by mass. Because lipid synthesis discriminates against ^{13}C in favour of the lighter isotopes, analysing samples with varying lipid values may introduce error to $\delta^{13}C$ measurement. A general recommendation is, therefore, to lipid extract samples prior to isotope analysis, or to apply a mathematical correction only when the C:N ratio exceeds 3.5 (Post et al., 2007). Here we used an evidentiary approach (e.g. Fagan et al. 2011) to assess the need for lipid correction that involved determining muscle lipid content and testing for significant correlations between lipid content and C:N and $\delta^{13}C$, with significant relationships being considered as the necessary prerequisite for lipid correction (e.g. Post et al. 2007).

3.3.2 Statistical analyses

Data inspection and statistical analyses were performed in the statistical software R (www.r-project.org). In all analyses, model suitability was assessed by visual inspections of residuals for indications of violations of model assumptions. For one-way analysis of variance (ANOVA), Levene's test for homogeneity of variance among groups was run prior to analysis. Differences among means were determined by Tukey's test. When heteroscedasticity was identified, Welch's ANOVA (Moder, 2010) was used with Games-Howell post hoc test (Ruxton and Beauchamp, 2008). For permutational analysis of variance (PERMANOVA), the assumption of homogeneity of group variances and the need for data transformation was tested using a multivariate analogue of Levene's test (PERMDISP) (Anderson, 2006). Pairwise contrasts of the PERMANOVA results were applied to determine differences between FA profiles (Arbizu and Monteux, 2017) using Bonferroni adjusted p-values.

FA compositional variability among spawning histories and major FA sources of variation

Since dietary lipid composition may influence consumer compositional values (Tocher, 2010), significant differences in the amount and nature of lipids among spawning history groups were evaluated using one-way ANOVAs. PERMANOVA was applied to individual FA profiles to assess composition variability and significant differences in FA composition among spawning histories using the vegan package in R (Oksanen *et al.*, 2017) that employs Bray-Curtis coefficients. Only FAs with mean proportions > 0.2% were included in the analysis (33 out of 71). A similarity percentage analysis (SIMPER) using the same R package was used to quantify overall dissimilarities among spawning history groups and to identify those FAs contributing to observed contrasts among groups. One-way ANOVAs were used to evaluate significant differences among spawning history groups in the FAs contributing 70% of the cumulative percent dissimilarity among groups.

Principal coordinates analysis (PCO) based on Bray-Curtis dissimilarities was applied to describe FA composition and visualize dissimilarities among samples. For an objective test of the presence of distinct FA profiles associated with hypothesized feeding area differences, K-means clustering (Legendre and Legendre, 1998) was applied to individual scores on PCO axes 1 and 2 where the optimal number of clusters was determined using the gap statistic (Tibshirani *et al.*, 2001). As the first PCO axis (PCO1) represents the main axis of variation in the multivariate dataset, this was used to represent the 'FA biomarker spectrum' and applied as an 'index' of fatty acid composition (Pond and Ward, 2011).

FA biomarkers associated with shelf and central basin prey origin

Higher values of bacterial, dinoflagellate, prymnesiophyte and calanoid copepod biomarkers were used as indicators of Labrador Sea central basin energy sources, whereas higher values of diatom and chlorophyte biomarkers were interpreted as indicative of feeding in Newfoundland Shelf waters. We used Parrish et al.'s (2005) indicators of bacterial FAs: (*i*15:0 + *ai*15:0 +15:0 + 15:1 + *i*16:0 + *ai*16:0 + *i*17:0 + *ai*17:0 + 17:0 + 17:1), and followed Pepin et al.'s (2011a) indicators of diatoms (16:1n-7 + 16:4n-1 + 20:5n-3, 16:1n-7/16:0 and $\sum C_{16}/\sum C_{18}$), prymnesiophytes (18:1n-9 and 18:4n-3) and dinoflagellates (22:6n-3/20:5n-3), and Dalsgaard et al.'s (2003) biomarkers for chlorophytes (18:3n-3 + 18:2n-6) and 20:1n-9 and 22:1n-9 were used as indicators for calanoid copepods (Dalsgaard *et al.*, 2003). Furthermore, we included Parrish et al.'s (2005) measure of the biochemical status of diatoms, the ratio of (16:2n -4 + 16:3n-4 + 16:4n-3 + 16:4n-1) to (16:0 + 16:1n-7 + 16:1n-5 + 16:2n-4 +16:3n-4 +16:4n-3 + 16:4n-1) which provides an index of nutrient sufficiency in diatoms that has been shown to reach a peak shortly after the peak of the spring bloom. FA sums and ratios and individual FAs previously established as biomarkers for the specific phytoplankton, bacterial and zooplankton derived energy sources were compared among spawning history groups using one-way ANOVAs.

Stable isotope analysis and associations with the FA composition, spawning history and fish length

Significant relationships between lipid content and C:N, and lipid content and $\delta^{13}C$, and consequently the need for lipid correction were assessed using general linear models (GLM). The significance of differences in elemental and isotope values among spawning history groups

were evaluated using one-way ANOVAs. The consistency between FA composition and $\delta^{13}C$ and $\delta^{15}N$ was assessed by examining the statistical relationship between the two stable isotopes and the FA biomarker spectrum using general linear models.

Although inherent in the different spawning histories are length differences, with repeat spawners being larger than maiden spawners, the presence of size-dependent feeding versus feeding area differences in explaining variation in $\delta^{15}N$ was investigated using general linear models. The AIC criterion for small sample sizes (AICc) (Burnham et al. 2002) was used to determine the model structure that best explained variation in the data among candidate models consisting of the predictors: fish length, spawning history, and an additive model including both spawning history and fish length. If size dependent feeding is more important, we would expect a model with L as the only predictor to be most supported. Conversely, if feeding area differences have an effect, we expect more support for a model that only includes the spawning history term, or a model with both spawning history and length that suggests differences in size dependent feeding within respective feeding regions. In the latter case, we would expect similar sized fish with different spawning histories to have different $\delta^{15}N$ values consistent with the hypothesized differences in feeding location.

3.4 Results

3.4.1 Lipid and FA compositional variation among spawning histories

The mean lipid density of Atlantic salmon muscle tissue across spawning histories was 12.9 g · 100 g wet weight^{-1} (± 3.6 SD) and did not differ significantly among maiden, alternate repeat and consecutive repeat spawning fish (one-way ANOVA, $p > 0.05$) (Table 3.2). Depot lipids

(triacylglycerol, TAG) contributed most to the total lipids identified (mean 83.9% ± 5.4 SD), followed by acetone mobile polar lipids (AMPL, mean 6.0% ± 1.8 SD), and phospholipids (PL, mean 5.4%± 4.7 SD). One sample originating from an alternate repeat spawner had deviating compositional values (36.0% TAG and 50.8% PL), and was subsequently removed as an outlier. After this removal, the percentage of TAG, AMPL, and PL to the total lipids identified did not differ among spawning history groups (Table 3.2). In preliminary analyses, length corrected body mass was also compared between the three spawning groups, but significant differences were not found ($p > 0.05$).

The FAs occurring in highest abundance in maiden and repeat spawners were 18:1n-9 (mean: 17.6 %) 16:0 (13.1%), 20:1n-9 (11.1%), 22:1n-11 (10.7 %) and 22:6n-3 (9.0 %) (Table 3.2). There was no heterogeneity of dispersions among groups ($F_{2,69} = 0.71$, $p = 0.49$) (PERMDISP) and the FA data was therefore not transformed. PERMANOVA revealed that muscle fatty acid profiles were not the same among spawning history groups ($F_{2,69} = 76.7$, $p < 0.05$), with all pairwise comparisons being statistically significant. Similarity percentage analysis (SIMPER) identified 18:1n-9 (oleic acid), 20:5n-3 (EPA), 16:1n-7 (palmitoleic acid), 22:1n-11 (cetoleic acid), 20:1n-9 (eicosenoic acid) and 16:0 (palmitic acid) as providing the greatest contribution to differences between maiden and consecutive repeat spawners. The same FAs also provided the greatest contribution to differences between consecutive and alternate repeat spawners with the addition of 22:6n-3 (DHA), and 22:5n-3 (DPA) and 22:6n-3 for alternate repeat and maiden spawning fish. The overall dissimilarity among spawning history groups was lowest between maiden and alternate repeat spawners (7% dissimilarity), and the overall dissimilarity between

consecutive and alternate repeat spawners was lower (13%) than the overall dissimilarity between consecutive repeat spawners and maiden Atlantic salmon (16%).

K-means clustering applied to the first two PCO axes supported the finding that differences in FA compositional values between maiden and alternate repeat spawning salmon were small compared to comparisons with consecutive repeat spawners. Two distinct clusters were identified where group one consisted of consecutive spawners, and group two of maiden and alternate spawners. Two individuals were misclassified, one being an alternate spawner grouped with consecutive spawners, and the other a consecutive spawner grouped with maiden and alternate repeat spawners (Figure 3.2). On PCO1, which explained 83.6% of the variation, consecutive repeat spawners received positive scores, the exception being the identified outlier, and maiden and alternate repeat spawners received negative scores apart from one alternate repeat spawner identified as an outlier. Data inspection revealed that the biomarker and isotope values of these individuals more closely resembled mean values of the spawning history group they were assigned based on K-means clustering (data not shown). Due to the possibility that pre-assigned spawning histories based on scale reading could be in error, the outliers were removed from further analyses.

3.4.2 Variation in shelf and central Labrador Sea FA biomarkers among spawning histories

Significant differences in the shelf and central basin biomarkers were identified among the three spawning history groups (Table 3.3). Consecutive spawners had significantly higher values of the three diatom biomarkers, whereas values of the biomarker associated with chlorophytes were significantly lower in consecutive repeat compared to alternate repeat and maiden spawners (Figure 3.3). Significant differences between alternate repeat and maiden spawners in

their values of two of the diatom markers were also identified, with alternate repeat spawners having higher values than the former (Figure 3.3). Levels of the index of diatom biochemical status associated with the peak spring bloom were significantly higher in consecutive repeat compared to maiden and alternate repeat spawning salmon ($p < 0.05$; Table 3.3).

In contrast to the biomarkers associated with the shelf biogeographic region, levels of the biomarkers associated with the central Labrador Sea were significantly higher in maiden and alternate repeat spawners than consecutive repeat spawners, the exception being the prymnesiophyte biomarker no. 2 (Figure 3.4). No significant differences between maiden and alternate repeat spawners were reported for bacterial, copepod and dinoflagellate biomarkers, whereas alternate repeat spawners had lower values of the prymnesiophyte biomarker 1 (Figure 3.4).

3.4.3 Atlantic salmon stable isotope and elemental composition, and correlations with lipid density, FA profiles and fish size

The mean value of the C:N ratio of Atlantic salmon was 5.8 (± 1.6 SD) across all samples and did not differ among spawning histories (one-way ANOVA, $F_{2,52}$ =2.36, p = 0.10) or vary with fish length (GLM, $F_{1,53}$ = 0.27, p = 0.60). No significant relationships between % C, or between C:N, and measured lipid concentration were found (GLM, $F_{1,53}$ = 0.48, p = 0.49 and $F_{1,53}$ = 0.11, p = 0.74, respectively). Furthermore, no relationship was identified between $\delta^{13}C$ and lipid concentration among the samples investigated (GLM, $F_{1, 53}$ = 1.43, p = 0.24). In view of the lack of empirical support for significant relationships between lipid concentration and the C:N ratio and/or $\delta^{13}C$, lipid correction methods were not applied.

Values of $\delta^{13}C$ ranged from -24.2‰ to - 20. ‰ within the spawning history groups (mean -22.1 ± 0.9‰) and did not differ significantly among each other ($F_{1,52} = 1.99$, $p = 0.14$). Values of $\delta^{15}N$ ranged from 10.9‰ to 14.8‰ and a significant difference among spawning histories was detected (Welch's ANOVA, $F_{2,9.4} = 32.85$, $p < 0.01$). Pairwise comparisons demonstrated that consecutive repeat spawners had significantly higher $\delta^{15}N$ compared to maiden and alternate repeat spawners (Games-howell post hoc test, $p < 0.05$), whose mean values in turn did not differ.

Assessment of the relationship between $\delta^{13}C$ and the FA biomarker spectrum (PCO1 scores) indicated no significant association between the two (GLM, $F_{1,53} = 0.13$, $p = 0.71$), whereas positive values on the FA biomarker spectrum were associated with higher values of $\delta^{15}N$ as compared to samples with negative values on the FA biomarker spectrum (GLM, $F_{1,53} = 70.82$, $p < 0.01$) (Figure 3.5). Visual assessment of the relationship between the two groups derived by the cluster analysis and $\delta^{15}N$ values further indicated that the contrast in FA compositional values was associated with differences in $\delta^{15}N$ (Figure 3.5). Furthermore, a general linear model with $\delta^{15}N$ as response and FA-based cluster membership as predictor received more support (AICc 93.5) compared to a model with the spawning history term as predictor (AICc 95.9).

General linear models fit to $\delta^{15}N$ identified a significant effect of fish length, but the most AICc supported model suggested a significantly higher intercept for consecutive compared to alternate repeat and maiden spawners such that the latter two individual groups, which did not differ significantly, had lower values than consecutive repeat spawners at any size (Table 3.3 and Figure 3.6).

3.5 Discussion

Fatty acid derived estimates of dietary patterns of Campbellton River maiden, alternate and consecutive spawning Atlantic salmon indicated dietary contrasts related to the differential migrations associated with spawning history. Although differences in FA compositional values between maiden and alternate spawners were found, consistent with our expectations, the dissimilarity- and clustering analyses suggested a greater similarity in resource use by these groups as compared to consecutive spawners. The presence of spawning history dependent feeding patterns was further supported by dorsal muscle ratios of $\delta^{15}N$, which covaried significantly with FA compositional values and distinguished consecutive repeat spawners from the two other spawning groups. The positive correlation between $\delta^{15}N$ and fish size suggested a greater inclusion of higher trophic prey as fish grow, thus distinguishing maiden from alternate repeat spawners.

Ingested FAs undergo little degradation or modification during incorporation in consumer tissues (Iverson, 2009), and the FA composition of Atlantic salmon lipid depots, therefore, reflect that of the diet assimilated over time (e.g. Robin *et al*., 2003; Bell *et al.,* 2004). Information on dietary origin is possible because of the strong phylogenetic and trophic patterns that exist in FA synthetic capacity among organisms (Bell and Tocher, 2009), where unique FAs and FA compositions found in primary producers and some secondary producers are bioaccumulated through the food chain (Iverson, 2009, Dalsgaard 2003). Although slight modifications in consumer tissue levels may occur in response to selective oxidation and fat deposition that reflect the metabolic and structural role of ingested FAs (e.g. Menoyo *et al*., 2003; Budge *et al*., 2012), Atlantic salmon FAs reflect the food web origin of the major dietary

sources consumed. The FAs that differentiated among Atlantic salmon spawning histories in the current study consisted of FAs previously established as individual or components of biomarkers associated with phylogenetic groups that differentiate between the shelf and basin biogeographic regions of the Labrador Sea (Head *et al.*, 2003; Fragoso *et al.*, 2016) and, thereby, support the hypothesis of divergent feeding locations.

Previous studies on Atlantic salmon have demonstrated that the FA and stable isotope composition of somatic tissue after a dietary change equilibrates to that of the diet within a period of a few months, depending on the growth rate of the fish (Jobling, 2003; Jardine *et al.,* 2004; Trueman *et al.,* 2005; Budge *et al.,* 2011, Vander Zanden *et al.*, 2015). As such, the FA composition and $\delta^{15}N$ of maiden and alternate repeat spawners should represent signatures from food resources accumulated during the final growth season prior to their return (e.g. Dempson *et al.,* 2010). Despite the brief marine residency times of consecutive repeat spawners, the substantial increase in lipids and somatic mass that occurs from the time of freshwater emigration to subsequent return similarly suggests that FAs and stable isotopes represent a marine feeding signature (Jonsson *et al*., 1997). Consequently, the FA composition of maiden and alternate repeat spawners indicated a higher intake of basin energy sources during the marine residency period compared to consecutive spawners. Conversely, consecutive spawner tissue suggested higher dependency on energy derived from the continental shelf. The exception being the two outlier individuals with compositional and isotope values more closely resembling a different spawning history group than assigned based on scale-pattern analysis. An evidentiary approach thus suggests that misclassification during scale reading analysis may have occurred, likely due to poor quality scale material which can

obscure the successful characterization of spawning marks (White and Medcof, 1968). Alternatively, the migration patterns of these individuals deviated from their assigned spawning history groups.

In contrast to our expectations, the chlorophyte biomarker (18:3n-2 + 18:2n-6) indicative of shelf food sources was significantly higher in maiden and alternate repeat spawners compared to the consecutive spawning history group. Both terrestrial input and a higher abundance of chlorophytes should lead to increased ecosystem levels of 18:3n-3 (α-linolenic acid, ALA) and 18:2n-6 (linoleic acid, LA) near the coast of Newfoundland, as seen in the calanoid copepod *C. finmarchicus* (Pepin *et al*. 2011a). Yet the lower values of these FAs in consecutive repeat spawners may reflect interannual variation in chlorophyte blooms and/or terrestrial inputs, or alternatively, an ALA and LA source in the central Labrador Sea. Experimental work suggests that *synechococcus* bacteria may produce considerable amounts of LA and to a lesser degree ALA under certain conditions (Kenyon, 1972, Sahu *et al*., 2013), which would support the latter explanation.

Although fatty acid analysis indicated different food web origins of the energy assimilated by maiden, alternate and consecutive repeat spawners, the FA analyses applied are not able to identify differences in the specific prey items consumed (Budge *et al*., 2012). Previous work, however, demonstrates that variation in Atlantic salmon dietary patterns may be driven by differences in feeding location, prey availability, or a combination of both (Dempson *et al.*, 2010; Dixon *et al.*, 2012, 2017; Kelly *et al.*, 2018). Feeding differences related to prey availability in Atlantic salmon may occur in response to fish size, as the range of available prey sizes increase as fish grow (Jacobsen and Hansen, 2001; Rikardsen and Dempson, 2011; Jacobson *et*

al., 2018). An increased inclusion of higher trophic level prey in the diets of larger fish thus results in a predictable increase in consumer $\delta^{15}N$ (Post, 2002; Sweeting *et al.*, 2005), consistent with the significant increase in $\delta^{15}N$ with fish fork length identified in the current study. Size dependent feeding may thus explain the differences in FA composition between maiden and alternate repeat spawners, as has been found in other North Atlantic fish species sampled within a geographic region (Budge *et al*. 2002). When corrected for size, however, $\delta^{15}N$ values were still significantly higher in consecutive than alternate and maiden spawners (Table 5, Fig. 6) suggesting that fish size alone did not fully explain the variation in $\delta^{15}N$. As such, the correlation between $\delta^{15}N$ and the FA biomarker spectrum and the correlation between $\delta^{15}N$ and the cluster assigned spawning history groups, indicated that ingestion of larger-sized prey increased with fish size within respective feeding areas.

Contrasting $\delta^{15}N$ values among consumers feeding in different marine geographic regions may occur in response to the considerable spatial variation in $\delta^{13}C$ and $\delta^{15}N$ that is found at the base of the food chain in marine ecosystems (Jennings and Warr, 2003; Chouvelon *et al.*, 2012). Previous studies have noted an offshore-nearshore gradient in $\delta^{15}N$ and $\delta^{13}C$ across the Newfoundland and Labrador Shelf, with values of certain species becoming significantly higher in nearshore areas such as fjords and bays (Sherwood and Rose, 2005; Kelly *et al.*, 2018). Some of these species included those known to be preyed upon by Atlantic salmon such as capelin (*Mallotus villotus*), euphausiids and amphipods (Kelly *et al.,* 2018). Although the presence of a baseline difference in $\delta^{15}N$ and $\delta^{13}C$ between the shelf and basin biogeographic regions of the Labrador Sea has not been investigated, extensive inshore feeding by Atlantic salmon would undoubtedly result in increased $\delta^{15}N$ values compared to those feeding further offshore as has

been shown for fish feeding along the West Greenland coast (Hansen *et al.*, 2012; Dixon *et al.*, 2019). Nearshore residency by consecutive spawners, as indicated by acoustic telemetry (Chapter 2), may thus have contributed to the higher $\delta^{15}N$ as compared to the two other spawning history groups.

In addition to the potential effect of baseline differences among feeding locations on isotope signatures of consumers, dietary differences may also occur if prey availability differs among regions (Dixon *et al.*, 2017; Kelly *et al.*, 2018). Atlantic salmon are considered opportunist generalist predators (Jacobsen and Hansen, 2001; Rikardsen and Dempson, 2011), and the importance of different dietary items has been hypothesized to vary according to their abundance (Dixon et al. 2017). Differing availability of Atlantic salmon potential prey between putative feeding areas in the shelf and basin waters of the Labrador Sea likely exist based on contrasting hydrographic and physical features and associated zooplankton (Head *et al.*, 2003; Pepin *et al.*, 2011b) and forage fish assemblages (Gomes *et al.* 1995). Amphipod and euphausiid biomass, as well as that of the calanoid copepod *Calanus finmarchicus*, for example, appeared to be higher in the central Labrador Sea compared to shelf waters (Head *et al.*, 2003), indicating potentially abundant prey sources for Atlantic salmon feeding in the area. The importance of invertebrate prey to Atlantic salmon feeding in this region was noted by Sheehan *et al.* (2012) who found that the hyperiid amphipod *Themisto compressa* contributed 30 to 50% of ingested prey items by mass of post-smolt and adult Atlantic salmon in the central Labrador Sea. Being pronounced predators on copepods, hyperiid amphipods accumulate FAs derived from copepod wax esters such as 22:1n-11 and 20:1n-9 (Arts *et al.*, 2001; Mayzaud and Boutoute, 2015), and the higher levels of these FAs in maiden and alternate repeat spawners suggests

higher intake of copepod-based energy channels, possibly with hyperiid amphipods as the intermediary trophic level.

In contrast to the central Labrador Sea, shelf waters serve as nurseries or feeding grounds for numerous important forage fish such as sand lance (*Ammodytes dubius*), Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus*) and capelin (Parsons and Moores, 1974; Wheeler and Winters, 1984; Bundy *et al.*, 2000; Buren *et al.,* 2014), the latter being a key species on the Newfoundland Shelf providing an important energy pathway to higher consumers from the base of the food chain (Buren *et al.*, 2014). Capelin in the Labrador Sea undergo extensive migrations from offshore areas to coastal environments in spring (Maxner *et al.*, 2016), resulting in persistent staging and spawning aggregations on the northeast Newfoundland coast during summer (Davoren, 2013). These aggregations act as predator hotspots for breeding and overwintering seabirds and baleen whales (Davoren, 2013), and potentially for migrating Atlantic salmon. Occurring within the spatial (Davoren, 2013; Chapter 2) and temporal (Buren *et al*., 2014) range of the migration of Campbellton River consecutive spawners, these aggregations are potentially important in the diet of this life history group and would increase their $\delta^{15}N$ as compared to Atlantic salmon feeding on lower trophic level prey. The relative importance of baseline versus diet composition differences in explaining the contrasting $\delta^{15}N$ between spawning history groups could be resolved by further investigations of stable isotopes from putative food items collected in the two biogeographic regions.

Differences in feeding ecology related to differences in feeding locations and the biotic and abiotic properties of those locations (Pepin *et al.*, 2011b; Harrison *et al.*, 2013; Fragoso *et al.*, 2016), are likely to have potential consequences for group-specific adaptive landscapes

(Chapman *et al.*, 2012; Briedis and Bauer, 2018). For example, the significantly higher values of the FA biomarker associated with the spring phytoplankton peak bloom in consecutive repeat spawners suggested a higher reliance on energy sources derived from this seasonal event compared to the two other life history groups. This is perhaps not surprising considering the spring phytoplankton bloom occurs earlier on the northeast Newfoundland Shelf than in basin waters (Harrison *et al.*, 2013), providing more time for the energy derived from primary production to be transferred to higher consumers including Atlantic salmon (Odum, 1968; Heath, 1995). As such, it can be hypothesized that the timing of the consecutive repeat spawner migration in relation to the productivity cycle, is of particular importance for this life history group, allowing it to capitalize on the annual primary production peak through associated key energy conduits such as capelin, despite the brief consecutive repeat spawner residency time at sea.

The variable environmental conditions experienced by differentially migrating life history groups may carry over to affect body condition, parental quality and survival (Torniainen *et al.*, 2017; Briedis and Bauer, 2018) and potentially alter population dynamics. For example, FA composition of the fat depots available for reproductive effort in Atlantic salmon have direct bearing on egg lipids being strongly influenced by allocation from maternal lipids (Sargent, 1995; Tocher, 2010). Being important determinants of egg and offspring quality in fish (Brooks *et al.*, 1997; Sargent *et al.*, 1999; Tocher, 2010), the distinct FA compositional values among life history types may thus carry-over to demography as a result of the implications of FAs for egg quality and offspring survival and performance. Based on the observed sex ratios within repeat spawners in the current study (Table 3.1), and indications of higher post-spawning mortality in

Atlantic salmon males (Fleming, 1996) it is likely that repeat spawning salmon at Campbellton River are largely female and differential mortality rates may consequently alter the population sex ratio. In some rivers, the contribution of repeat spawners to the spawning population can be quite important (e.g. Campbellton, ~20%) (Downton, 2004) and are particularly valuable where conservation problems exist as repeat spawners may act as a buffer during periods of low smolt to adult survival. A more complete understanding of the variation in life history-dependent migratory characteristics as discussed here may inform our understanding of the spatial and temporal variation in Atlantic salmon survival and population dynamics in the North Atlantic.

3.6 Conclusion

Identifying spatial and food-web characteristics of the main energy sources assimilated is an important step in enhancing our overall understanding of the feeding, and movement ecology of Atlantic salmon at sea. The use of fatty acids together with stable isotopes is a relatively inexpensive method which can provide novel or corroboratory inferences on feeding area differences between or within Atlantic salmon populations. Analyses of Atlantic salmon fatty acid compositional values in the current study supported the association of spawning history-dependent migration strategies with divergent use of two distinct biogeographic regions of the Labrador Sea. Divergent feeding area use has potential consequences for the adaptive landscapes experienced by distinct population segments, which in turn may carry over to affect body condition, parental quality and survival. The identification of dietary food web origin and ultimately how this affects survival and growth is of particular importance given the large-scale

changes in the pelagic food web observed in the North Atlantic, which are believed to affect Atlantic salmon recruitment through bottom-up control.

3.7 Acknowledgements

We would like to thank L. Fudge, H. Lace, G. Hill and K. Deering for the assistance at the Campbellton River counting fence and the Department of Fisheries and Oceans for field assistance. All sampling procedures followed standards and guidance provided by the Canadian Council on Animal Care and were approved by the Northwest Atlantic Fisheries Centre and Memorial University animal care committee (MUN Animal Care protocol # 14-12-IF).

3.8 Tables

Table 3.1: Length (L_F, cm), weight (kg) and sex ratio of maiden, alternate repeat (AR) and consecutive repeat spawning (CR) Atlantic salmon sampled in Campbellton River during the summer of 2016. Detailed life histories are expressed using the shorthand notation ASW:Bx, where A represents the number of winters at sea prior to first spawning, B is the number of repeat spawning events and x is the type of repeat spawner strategy (AR or CR).

Spawning history		**N**	**L, cm mean ± SD**	**L, cm range**	**Weight, kg mean ± SD**	**% female**
Maiden salmon	Total	43	53.6 ± 4.2	46.9 - 68.4	1.7 ± 0.5	62.8
	1SW	*42*	*53.4 ± 4.0*	*46.9 - 68.4*	*1.7 ± 0.5*	*61.9*
	2SW	*1*	*63.2*		*2.9*	*100.0*
Alternate repeat spawning salmon	Total	6	67.9 ± 5.7	57.0 - 72.9	3.6 ± 0.7	100.0
	1SW:1AR	*6*				
Consecutive repeat spawning salmon	Total	23	63.2 ± 5.4	52.0 - 77.4	2.8 ± 0.7	95.0
	0SW:3CR	*2*	*53.6 ± 2.3*	*52.0 - 55.3*	*1.8 ± 0.3*	*100.0**
	1SW:1CR	*6*	*61.5 ± 4.2*	*57.0 - 68.1*	*2.6 ± 0.5*	*80.0**
	1SW:2CR	*11*	*63.8 ± 3.5*	*59.5 - 70.4*	*2.8 ± 0.4*	*100.0*
	1SW:3CR	*4*	*69.0 ± 5.6*	*65.2 - 77.4*	*3.6 ± 1.0*	*100.0*

*Information on sex not available for 1 individual

Table 3.2: Mean (± sd) muscle total lipids and composition of lipids (triacylglycerols: TAG, acetone mobile polar lipids: AMP, phospholipids: PL) and fatty acids (FAs with mean equal to or greater than 0.2 %) of 1SW maiden, alternate repeat and consecutive repeat spawning Atlantic salmon sampled in Campbellton River during the summer of 2016. Results of statistical comparisons are provided for total lipids and composition and for those FAs identified by SIMPER to have the greatest contribution to dissimilarities between spawning history groups where superscript letters denotes statistically significant differences among groups as determined by Tukey HSD or Games-Howell post hoc test.

	Maiden spawners			Alternate repeat spawners			Consecutive repeat spawners					
	n=43			n=6			n=23					
Lipid composition (% of total lipids)	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Test**	**F**	**p-value**
TAG	84.69	±	4.15	79.74	±	14.35	83.67	±	3.09	ANOVA	0.77	0.46
AMP	5.74	±	1.66	5.97	±	1.92	6.51	±	2.06	ANOVA	1.36	0.26
PL	5.19	±	3.47	9.16	±	13.50	4.83	±	1.50	Welch's ANOVA	0.75	0.49
Total lipids (g · 100 g wet mass)	13.2	±	2.84	14.67	±	5.52	13.20	±	3.63	ANOVA	1.59	0.21
FA composition (% of total FAs)	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**		**SD**			
14:0	3.62	±	0.40	3.92	±	0.27	4.76	±	0.28			
15:0	0.38	±	0.03	0.33	±	0.05	0.29	±	0.03			
16:0	12.47[a]	±	0.83	12.20[a]	±	1.54	14.43[b]	±	0.92	ANOVA	36.4	<0.05
*i*17:0	0.29	±	0.02	0.25	±	0.06	0.19	±	0.03			
phytanic	0.30	±	0.05	0.27	±	0.08	0.18	±	0.11			
16:1n-5	0.23	±	0.02	0.21	±	0.02	0.24	±	0.02			
16:1n-7	5.10[a]	±	0.53	6.44[a]	±	1.31	9.11[b]	±	1.17	ANOVA	164.5	<0.05

16:1n-9	0.26	±	0.04	0.21	±	0.02	0.20	±	0.03			
16:2n-4	0.17	±	0.03	0.22	±	0.06	0.42	±	0.08			
16:3n-3	0.31	±	0.02	0.22	±	0.08	0.09	±	0.05			
17:1	0.51	±	0.04	0.37	±	0.13	0.17	±	0.07			
18:0	2.53	±	0.35	2.56	±	0.40	2.70	±	0.21			
18:1n-5	0.44	±	0.04	0.47	±	0.06	0.58	±	0.06			
18:1n-7	3.43	±	0.37	3.41	±	0.29	3.94	±	0.40			
18:1n-9	19.68[a]	±	0.90	17.74[b]	±	1.40	13.61[c]	±	1.71	ANOVA	176.6	<0.05
18:2n-6	1.29	±	0.07	1.32	±	0.27	0.85	±	0.14			
18:3n-3	0.68	±	0.05	0.63	±	0.16	0.49	±	0.12			
18:4n-1	0.16	±	0.04	0.21	±	0.07	0.27	±	0.08			
18:4n-3	0.67	±	0.14	0.85	±	0.30	0.80	±	0.21			
20:1n-11	1.23	±	0.25	1.20	±	0.33	0.69	±	0.20			
20:1n-7	0.48	±	0.04	0.65	±	0.18	0.77	±	0.13			
20:1n-9	11.96[a]	±	0.99	11.83[a]	±	1.43	9.36[b]	±	1.38	ANOVA	38.8	<0.05
20:2n-6	0.39	±	0.03	0.36	±	0.08	0.22	±	0.06			
20:4n-3	1.41	±	0.12	1.43	±	0.42	0.92	±	0.22			
20:4n-6	0.44	±	0.07	0.39	±	0.08	0.30	±	0.04			
20:5n-3	4.03[a]	±	0.58	5.02[a]	±	0.68	8.14[b]	±	1.45	Welch's ANOVA	82.9	<0.05
21:5n-3	0.32	±	0.03	0.33	±	0.04	0.33	±	0.04			
22:1n-11	11.74[a]	±	1.47	10.49[a]	±	0.91	8.69[b]	±	1.42	ANOVA	34.7	<0.05
22:1n-7	0.18	±	0.04	0.19	±	0.04	0.25	±	0.05			
22:1n-9	1.50	±	0.15	1.55	±	0.21	1.28	±	0.16			
22:5n-3	2.11[a]	±	0.33	2.83[b]	±	0.36	2.89[b]	±	0.38	ANOVA	42.6	<0.05
22:6n-3	8.58[a]	±	0.91	8.90[ab]	±	0.93	9.86[b]	±	1.11	ANOVA	12.87	<0.05
24:1	0.83	±	0.07	0.77	±	0.07	0.79	±	0.10			

Table 3.3: Mean values of maiden (M), alternate repeat (AR) and consecutive repeat (CR) spawner FA biomarkers for the NL Shelf and central Labrador Sea and ANOVA results. Chlorophyte biomarker: 18:3n-2 + 18:2n-6, diatom biomarker 1: 16:1n-7 +16:1n-4 + 20:5n-3, diatom biomarker 2: 16:1n-7/16:0, diatom biomarker 3: ∑C16/∑C18, , bacterial biomarker: (i15:0 + ai15:0 + 15:0 + 15:1 + i16:0 + ai16:0 + i17:0 + ai17:0 + 17:0 + 17:1), copepod biomarker 1: 20:1n-9, copepod biomarker 2: 22:1n-9, dinoflagellate: 22:6n-3/20:5n-3, prymnesiophyte biomarker 1: 18:1n-9, prymnesiophyte biomarker 2: 18:4n-3, peak spring bloom biomarker: (16:2-n4 + 16:3n-4 + 16:4n-3 +16:4n-1) / (16:0 + 16:1n-7 + 16:1n-5 + 16:2n-4 +16:3n-4 +16:4n-3 + 16:4n-1).

			Mean				
Biomarker	Region	M	AR	CR	Test	F	p-value
Chlorophyte	Shelf	1.97	2.10	1.31	Welch's ANOVA	95.48	< 0.01
Diatom 1	Shelf	9.17	10.84	17.95	one-way ANOVA	764.10	< 0.01
Diatom 2	Shelf	0.41	0.50	0.64	one-way ANOVA	119.70	< 0.01
Diatom 3	Shelf	0.64	0.69	1.09	Welch's ANOVA	397.02	< 0.01
Bacterial	Basin	1.35	1.31	0.99	one-way ANOVA	105.99	< 0.01
Copepod 1	Basin	11.96	11.91	9.19	one-way ANOVA	50.41	< 0.01
Copepod 2	Basin	1.50	1.52	1.26	one-way ANOVA	20.25	< 0.01
Dinoflagellate	Basin	2.15	1.88	1.20	Welch's ANOVA	256.55	< 0.01
Prymnesiophyte 1	Basin	19.68	18.06	13.33	one-way ANOVA	313.20	< 0.01
Prymnesiophyte 2	Basin	0.67	0.92	0.81	one-way ANOVA	7.68	< 0.01
Peak spring bloom		0.02	0.02	0.04	Welch's ANOVA	100.63	< 0.01

Table 3.4 Parameter estimates of the "Best" AICc selected model for describing the relationship between fish fork length (L, cm) and muscle $\delta^{15}N$ for the maiden (M), alternate repeat (AR) and consecutive repeat (CR) spawner groups.

	Estimate	SE	t value	Pr(>|t|)
Intercept (M)	9.97	0.85	11.77	< 0.01
L (cm)	0.04	0.01	2.25	0.03
AR	0.09	0.36	0.28	0.78
CR	1.29	0.22	5.70	< 0.01

3.9 Figures

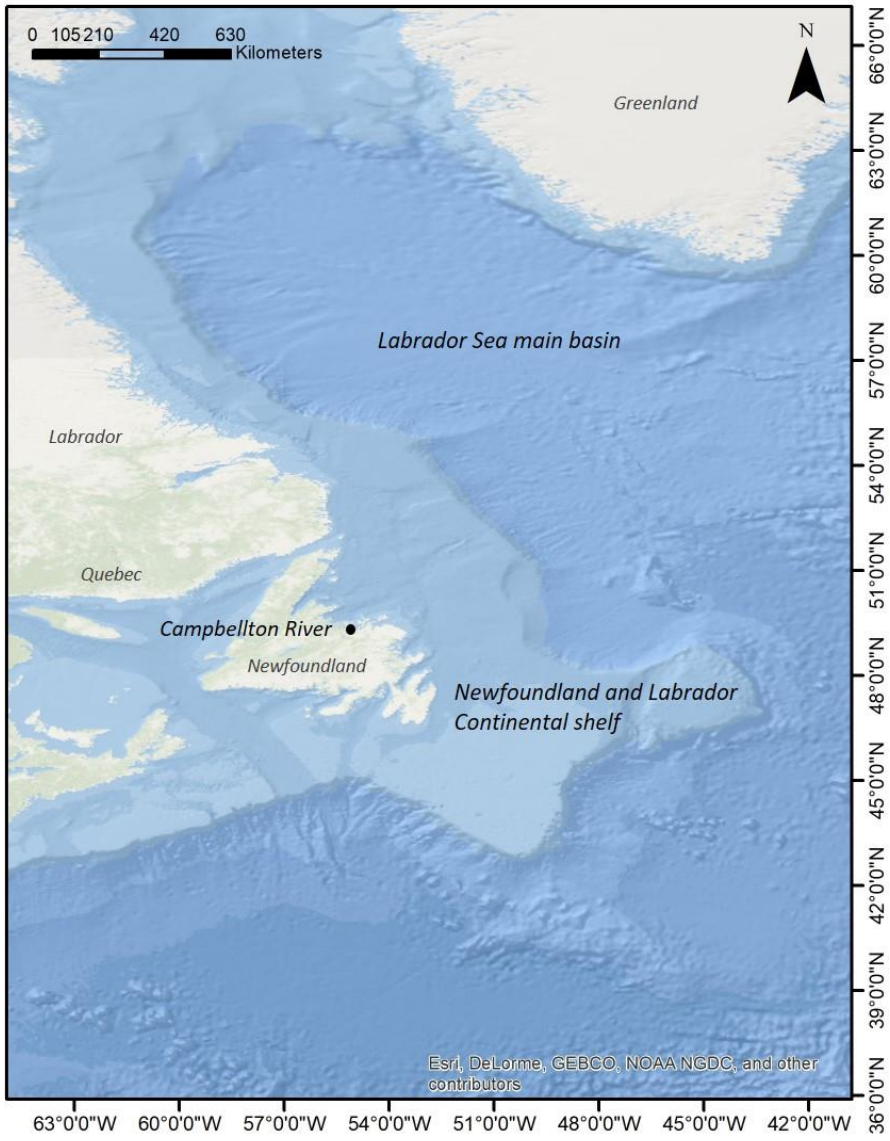

Figure 3.1 Location of Campbellton River on the east coast of Newfoundland, the Newfoundland and Labrador Continental Shelf and the Labrador Sea main basin.

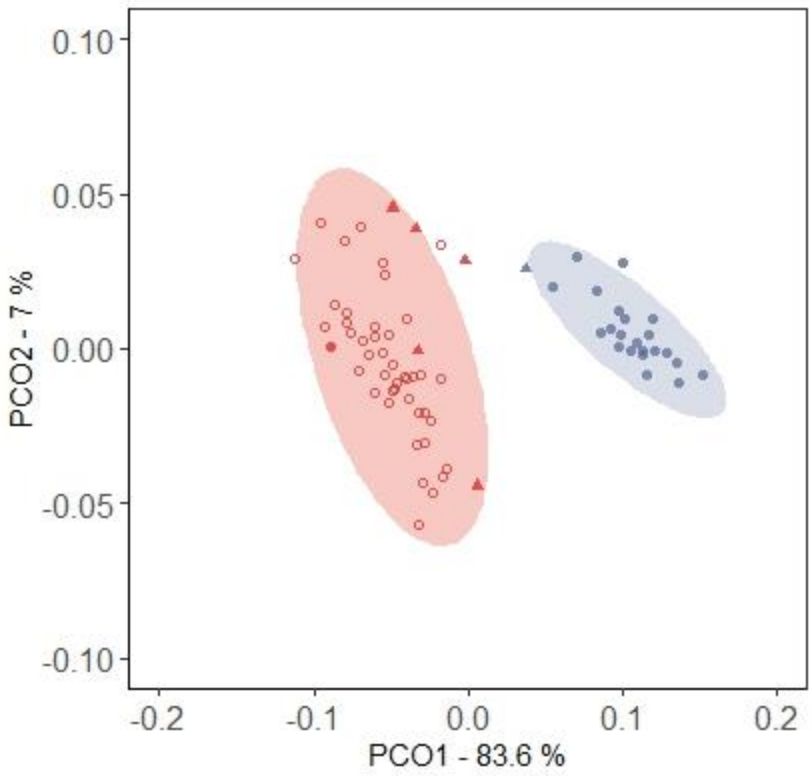

Figure 3.2 Principal coordinate analysis of FA composition (% of total FAs) for maiden (open circles), alternate repeat (triangle) and consecutive repeat (closed circles) spawning salmon score plot where colours indicate the 2 groups identified by K-means clustering of PCO scores**.**

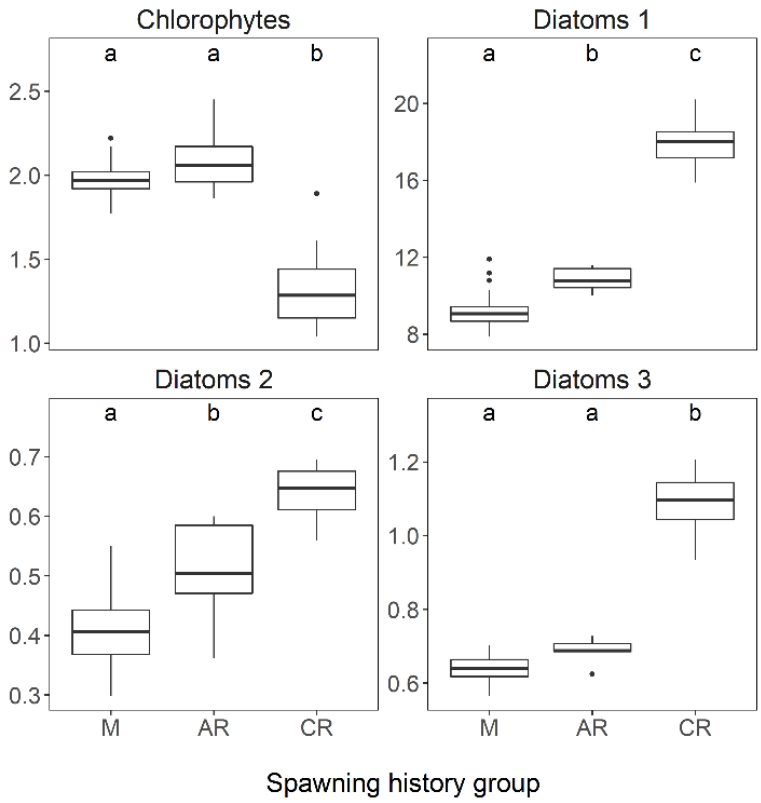

Figure 3.3 Boxplot of maiden (M), alternate repeat (AR) and consecutive repeat (CR) spawning Atlantic salmon values of biomarkers associated with the NL Shelf biogeographic region. Chlorophyte biomarker: (18:3n-2 + 18:2n-6), diatom biomarker 1: (16:1n-7 +16:4n-1 + 20:5n-3), diatom biomarker 2: 16:1n-7/16:0, diatom biomarker 3: $\sum C_{16}/\sum C_{18}$. The boxplot shows median values (black lines), the interquartile ranges (boxes), and the 5^{th} and 95^{th} percentiles (vertical lines). Letters denote significantly different mean values as identified by Tukey HSD or Games-Howell post-hoc tests.

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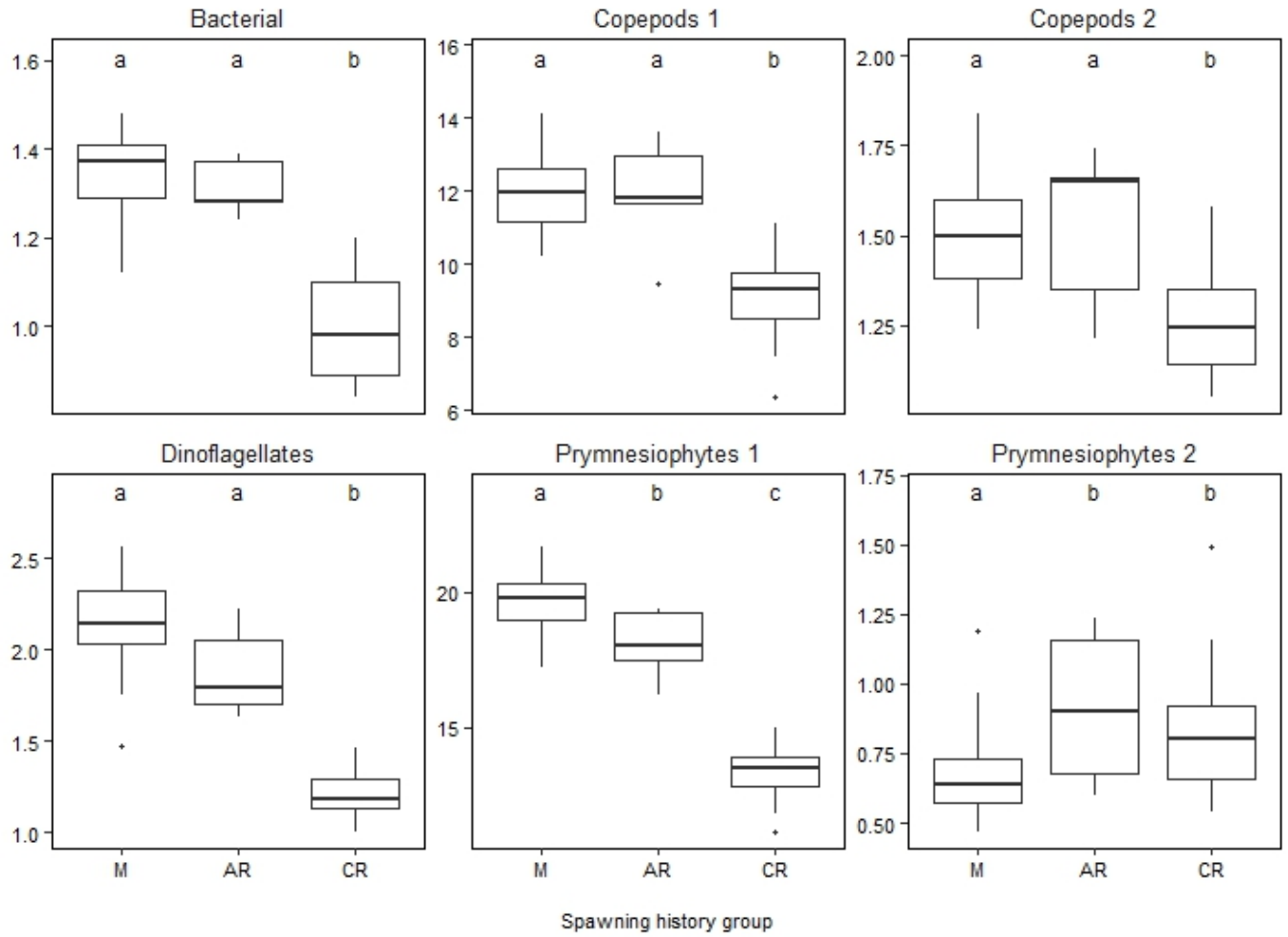

Figure 3.4 Boxplot of maiden (M), alternate repeat (AR) and consecutive repeat (CR) spawning Atlantic salmon values of biomarker associated with the central Labrador Sea biogeographic region. Bacterial biomarker: (i15:0 + ai15:0 + 15:0 + 15:1 + i16:0 + ai16:0 + i17:0 + ai17:0 + 17:0 + 17:1), copepod biomarker 1: 20:1n-9, copepod biomarker 2: 22:1n-9, dinoflagellates: 22:6n-3/20:5n-3,

prymensiophytes 1: 18:1n-9, prymnesiophytes 2: 18:4n-3. The boxplot shows median values (black lines), the interquartile ranges (boxes), and the 5^{th} and 95^{th} percentiles (vertical lines). Letters denote significantly different mean values as identified by Tukey HSD or Games-Howell post-hoc tests.

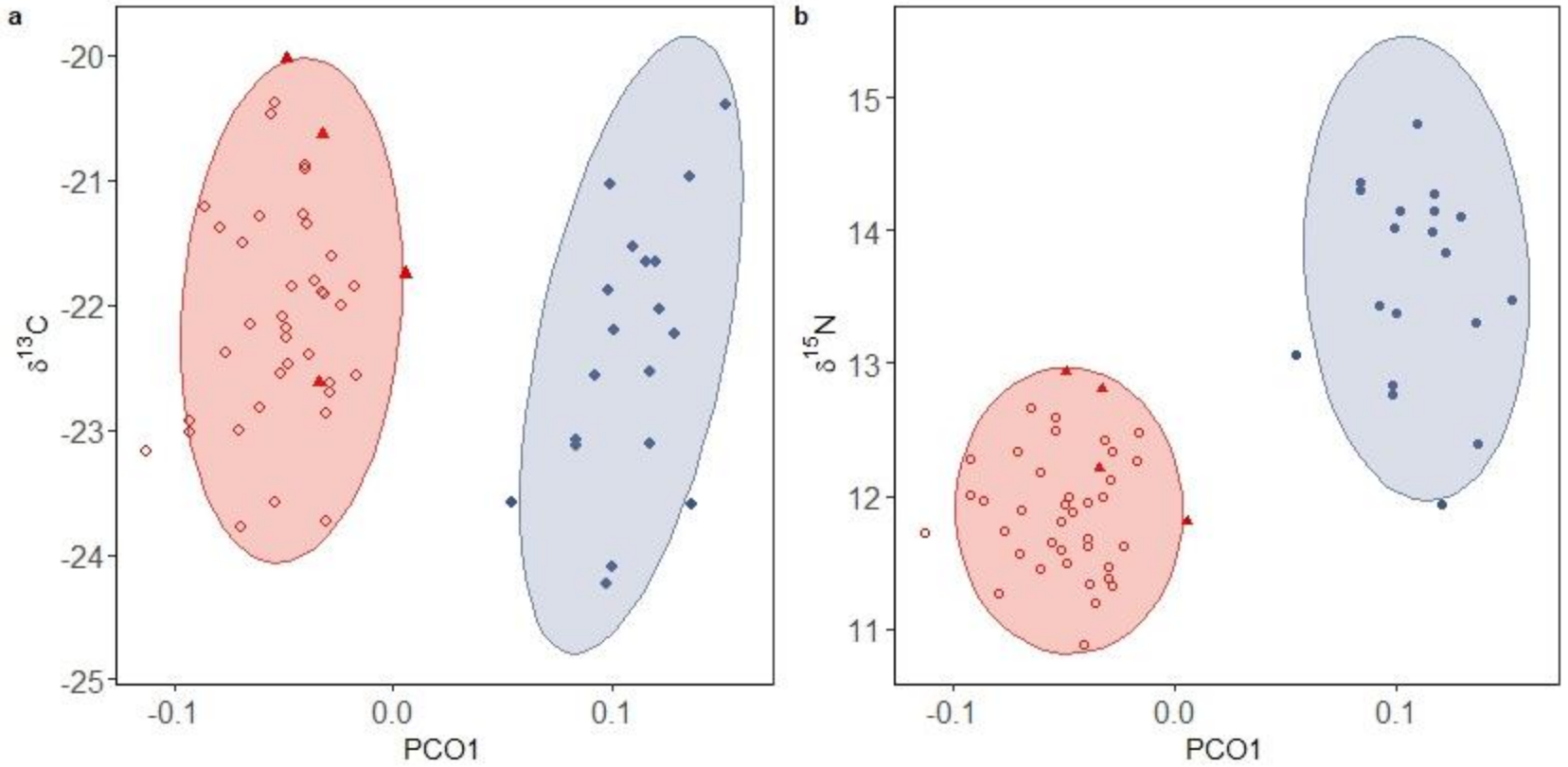

Figure 3.5 Scatter plot of individual scores on PCO1 (FA biomarker spectrum) v. ratios of a) $\delta^{13}C$ and b) $\delta^{15}N$ in maiden (open circles), alternate repeat (triangle) and consecutive repeat (closed circle) spawning Atlantic salmon. Colours indicate the 2 groups identified by K-means clustering.

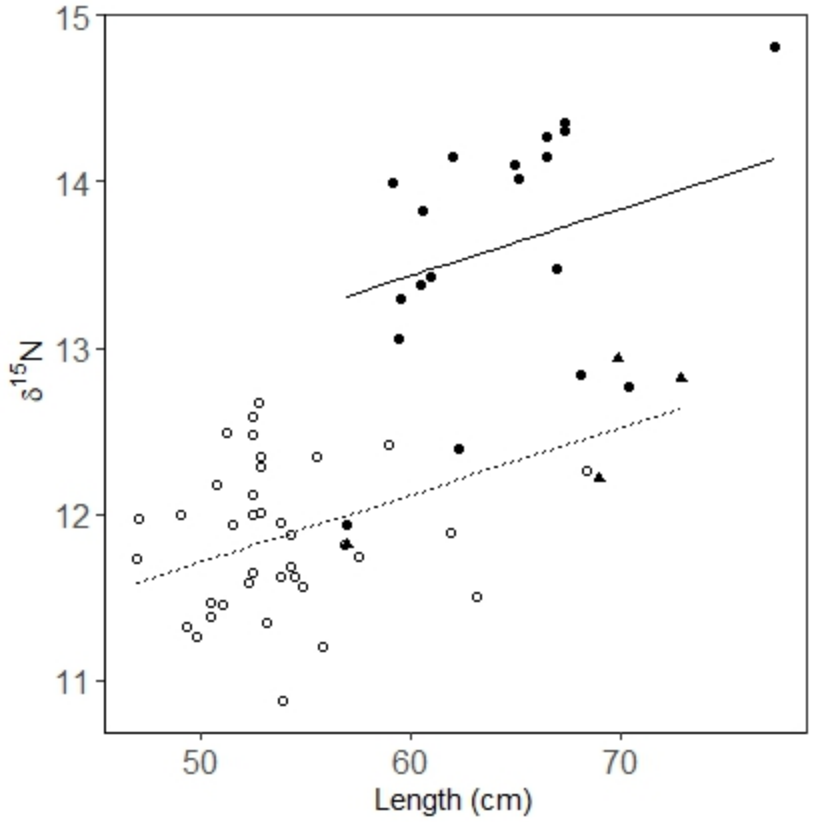

Figure 3.6 Scatter plot of individual fork length (cm) v. ratios of $\delta^{15}N$ in maiden (open circles), alternate repeat (triangle) and consecutive repeat (closed circle) spawning Atlantic salmon. The regression line represents the significant relationship between length and $\delta^{15}N$ values within consecutive repeat spawners (dotted line) and maiden and alternate repeat spawners (solid line) according to the most supported model based on the AICc criterion.

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4 Chapter 4: Life history contrasts in energetic and nutritional state and return probability of post-spawned Atlantic salmon

Co-authorship statement

This research was developed and carried out by Kristin Bøe, together with the supervisory assistance of Ian Fleming and Michael Power, and the guidance of Martha Robertson, Corey Morris and Brian Dempson. All authors discussed the results and contributed to the final manuscripts.

4.1 Abstract

Processes that occur in one part of the animal lifecycle may influence the success or performance in lifecycle-phases downstream. This phenomenon, often referred to as carry-over effects, is particularly relevant to species performing migrations that generally extend across habitats, seasons, and life stages. Atlantic salmon is an anadromous salmonid capable of spawning multiple times during its lifecycle, a life history which requires repeated migrations to restore depleted somatic reserves after reproduction. The prevalence of repeat spawning varies within and between populations, but a mechanistic and life history theoretic understanding of the factors contributing to repeat spawning is not well developed and the consequences for fitness related traits are not well understood. Using non-lethal tissue biopsies and mark-recapture analysis, we investigate somatic energy, lipid composition, and condition in post-spawned Atlantic salmon returning to sea and explore contrasts in post-spawner lipids and condition as a function of previous migration, and spawning history, length and sex. Furthermore, we explore linkages between spawning history and returns to repeat spawning. Dorsal lipid density was significantly higher in previously spawned Atlantic salmon and females compared to first-time spawned individuals and males. Fatty acid composition differed significantly among spawning histories. Return probabilities to annual spawning were consistent with lipid density patterns, as males and first time spawning kelts had lower return rates compared to females and consecutive repeat spawning individuals. To our knowledge, this is the first study to report the role of previous spawning history on the post breeding condition and energy density status of Atlantic salmon. We suggest that spawning history related contrasts in energetic and nutritional state in post-spawned Atlantic salmon may be a

carry-over effect of contrasts in the non-breeding habitat as affected by spawning-history-dependent migration strategies, or alternatively, may represent an adaptive response to increased survival and recovery potential to age.

4.2 Introduction

Iteroparity describes a life history characterized by the possibility of repeated breeding during the lifetime of an organism. The benefits of repeat breeding include selection for bet-hedging for juvenile survival in highly variable environments, and age-related increases in fecundity for organisms with indeterminate growth (Fleming and Reynolds, 2003). The production of gametes, competition for mates and accessing appropriate breeding habitat are, however, energetically expensive and require resources that otherwise could be provided to competing functions such as growth, survival and maintenance. As a consequence, current reproduction should negatively impact future longevity and reproduction (Williams, 1966b) and an optimal iteroparous life history should balance the benefits of current reproductive investment against the costs in terms of reduced future reproductive value (Williams, 1966a).

In general, the cost of reproduction results jointly from the energy invested in reproduction and the reduced survival associated with reproductive behaviours (Bell, 1980). Behavioural costs may include aggression related to competition for breeding sites and mates that lead to elevated risks of predation, injury, and mortality (Fleming, 1996; Loison *et al.*, 1999). Depleted energy stores resulting from the energetic demands of reproduction reduce capacities for maintenance and growth and may lead to reduced physiological performance that has negative effects on survival (Baird *et al.*, 1986; Lambert and Dutil, 2000; Bonnet *et al.*, 2002). Capital

breeders that rely on stored energy and somatic material for reproduction must recover depleted somatic reserves to undertake a new breeding event, and if unsuccessful may skip breeding (Rideout and Tomkiewicz, 2011; Haraldstad *et al.*, 2018) or succumb to mortality prior to the next breeding opportunity (Baron *et al.*, 2013)

Atlantic salmon (*Salmo salar*) is an iteroparous salmonid whose life history is closely tied to marine migratory movements. The anadromous life history is characterized by temporally predictable juvenile migrations from freshwater rearing habitat to more productive marine feeding habitat (Gross *et al.*, 1988), which increases growth and fecundity compared to non-migrants (Fleming, 1998). During the spawning migration and reproduction, muscle tissue provides the primary source of energy and nutrients, with lipids being the main energy source followed by proteins (Jonsson and Jonsson, 2003). The energetic investment in reproduction leaves residual somatic energy critically low and is believed to constrain post-spawning survival (Jonsson and Jonsson, 2003). In anadromous individuals the energetic demands and elevated predation risk of the spawning migration impose additional reproductive costs, and in response there is an apparent decrease in iteroparity associated with anadromy (Fleming, 1998).

The cost of reproduction in Atlantic salmon appears to differ between the sexes and among populations, leading to variation in the presence and patterns of repeat breeding. Anadromous males on average are less likely to repeat breed than females, which may be attributed to intense sexual selection leading to injury, infection and ultimately lower survival (Fleming, 1996; Jonsson and Jonsson, 2011). Size-dependent energy costs during reproduction also appear to be linked to variation in survival and post-breeding recovery potential among populations (Jonsson *et al.*, 1991, 1997; Jonsson and Jonsson, 2003). Smaller fish (generally fish

maturing after one winter at sea, referred to as 1SW salmon) use a lower relative amount of energy to spawn, and those that repeat spawn tend to do so annually, whereas larger fish spend relatively more energy and are more likely to repeat spawn biennially, implying they require more time to restore depleted somatic reserves (Jonsson and Jonsson, 2011). A mechanistic understanding of the physiological and energetic factors that affect post-breeding recovery in Atlantic salmon, including the importance of post-spawning energetic state in determining repeat spawning rates, is not well developed. Furthermore, as size and life history are correlated in Atlantic salmon (i.e. age at maturity and number of breeding events), the potential effects of life history versus the energetic size effects on post-spawner state and repeat breeding rates have not been disentangled.

Life history theory predicts that optimal reproductive effort increases with age, with the result that differential investment among life histories may produce contrasts in post-spawner state having implications for recovery and survival (i.e. terminal investment theory) (Gadgil and Bossert, 1970; Clutton-Brock, 1984; Berg *et al.*, 1998). Furthermore, life history may affect reproductive expenditures insofar as differences in ecological factors such as habitat quality or availability of resources, faced by each life history form may result in differences in the physical state of returning breeders (Marra *et al.*, 1998; Harrison *et al.*, 2011; Briedis and Bauer, 2018) with those that have accumulated the largest energy reserves likely to experience lower relative reproductive costs (Van Noordwijk and De Jong, 1986; Lambert and Dutil, 2000). Differences in the use of non-breeding habitat may similarly affect the quality of somatic reserves available to breeding individuals (Torniainen *et al.*, 2017). In Atlantic salmon, repeat spawning may be associated with the use of spatially separate feeding locations for maturing

(first time spawning) and re-maturing (repeat spawning) individuals that result in differences in the diet and fatty acid composition of somatic lipid reserves (Chapter 3). For capital breeders, such as Atlantic salmon, that ingest no food during spawning and overwintering, differences in fatty acid (FA) composition prior to spawning may thus affect residual compositional values of post-spawned individuals returning to sea. As the quantity of essential fatty acids such as EPA and DHA in lipid depots are important for growth, immunity and stress resistance in fish (Adams, 1999; Arts and Kohler, 2009; Parrish, 2009), FA differences have the potential to affect the performance and recovery of post spawned fish.

In the present chapter, we investigate somatic energy, lipid composition, and condition in post-spawned Atlantic salmon (kelts) returning to sea and explore contrasts in post-spawner lipids and condition as a function of previous migration and spawning history, length and sex. Further, we explore potential contrasts in the return probability for Atlantic salmon kelts with different migration/spawning histories. The study population, Campbellton River, has a somewhat unique life history, insofar as there is a high percentage of repeat breeders due to the high post-spawning survival at sea (Reddin *et al.*, 2011; Chapter 3). The high return rates facilitate identification of kelt reproductive fates and determination of how fate is linked to individual variation during river emigration. The majority of repeat spawners in this population breed annually, overwinter in freshwater and migrate seaward in spring for an approximately two-month feeding period before returning in late summer as reconditioned individuals that will spawn in fall (Downton and Reddin, 2004; Reddin *et al.*, 2011). Telemetry and dietary studies indicate different feeding locations for consecutive repeat and maiden spawners that result in differences in the composition of somatic lipid depots at freshwater return (Chapters 2 and 3).

Thus, based on previous work, we hypothesize that: i) previous spawning history and sex will be linked to kelt energy density, with consecutive spawners and males, respectively, having lower energy density than maiden spawners and females; ii) as a result of differences in feeding area use, kelt spawning history groups will differ significantly in FA composition; iii) body condition will be strategy dependent, with consecutive repeat spawner kelts being in poorer condition than first time spawners across the length spectrum; and iv) because of differences in energy density and body condition, return rates will vary among kelt spawning groups, with consecutive repeat spawners having lower return rates than first time spawners.

4.3 Methods

Atlantic salmon sample collection

Atlantic salmon kelt tissue for analysis of lipid density and composition was collected from fish as they migrated down Campbellton River (49.2º N, 54.9 ºW, drainage area: 296 km^2) in the spring of 2016. Kelts were intercepted at a Department of Fisheries and Oceans (DFO) operated fish counting facility, located approximately 500 m upstream of the river mouth. All kelts were measured for fork length (L_F, cm) and mass (kg), and scales were collected for age and spawning history determination following standard guidelines for Atlantic salmon (ICES, 1992). Sex was determined externally based on secondary sexual characteristics such as the presence of a residual kype (Fleming and Reynolds, 2003). To provide individual data on kelt reproductive fates, dorsal muscle tissue (approximately 50 mg) was non-lethally collected from anaesthetized fish (100 ppm clove oil – methanol solution) (Iversen *et al.*, 2009) using a 4 mm disposable biopsy punch inserted anterior to the dorsal fin and above the lateral line. Empirical data support the use of white muscle as an effective surrogate for total-body lipids in salmonids

(Penney and Moffitt, 2014). An external T-bar anchor tag with information on fish identification and return reward in the event of recapture in the fishery was fitted to each fish. Post-sampling monitoring was conducted to ensure complete recovery before individuals were released back into the river.

As the accuracy of the estimated return probability of kelts is a function of the numbers of fish tagged and the true return rate that year (Bhattacharyya and Johnson, 1977), we expanded the dataset with an additional 110 T-bar tagged kelts that were part of a DFO population monitoring program to increase the statistical certainty of estimated returns and correlations with biological variables. To comply with the DFO monitoring program, these kelts were only measured for length (L_F) and scales were collected for age and spawning history identification without the use of anesthetics. All upstream migrating Atlantic salmon entering the counting facility during the 2016 spawning migration were examined for an external tag and tagged fish were recaptured to record length, mass and tag identification information.

Lipid extraction, lipid class separation and fatty acid methyl ester (FAME) derivatization

Muscle tissues collected for lipid and FA composition analyses were frozen and stored at -20 °C and then transferred to the lab, where they were measured for mass (g) and placed in chloroform, under nitrogen, at -20 °C prior to lipid extraction. Sample lipid content was determined following procedures described in Parrish (1999). Muscle tissue was homogenized in a 2:1 mixture of chloroform:methanol and diluted with chloroform extracted water 3X to separate the organic layer from the aqueous solution. The organic layer was removed via double pipetting and measured for mass to calculate muscle lipid concentration ($g \cdot g$ wet $weight^{-1}$). Lipid class composition was determined by analysing extracts using Chromarod-

Iatroscan (Iatroscan Mark VI TLC-FID) thin layer chromatography/flame ionization (Parrish, 1999). The sample fatty acid profile was measured using fatty acid methyl ester (FAME) derivatization on a HP 6890 GC-FID (gas chromatography) as described in Hixson *et al* (2014) FAs were expressed using the shorthand notation A:Bn-X, where A represents the number of carbons, B is the number of double bonds, and X is the position of the first double bond relative to the terminal methyl group (CH_3).

To investigate the importance of spawning history and sex in explaining variation in muscle energy density (hypothesis i), general linear models and variable selection based on the AIC criterion (Anderson, Link, Johnson, & Burnham, 2001) were applied to individual lipid density values (g total lipids · 100 g wet mass^{-1}). To account for the possibility of size dependent energy-density, fish length was also included as a covariate. Single variable models including the three predictors were tested against additive and interaction models containing length and sex, length and spawning history, or spawning history and sex. If two models differed by less than 2 AIC units, the model where all model terms were statistically significant was deemed the most statistically and predictively adequate. Principal component analysis (PCA) was applied to individual FA compositional values to identify those FAs that accounted for the major sources of variation in the data and to describe and visualize the intercorrelation among samples. Only FAs > 0.2 % were included (24 out of 61 identified). The presence of distinct groupings in the data associated with spawning history was assessed by applying K-means clustering to individual scores on the first two principal components where the optimal number of clusters was determined based on minimizing the total within sum of squares (Legendre and Legendre, 2012). The presence of non-random distributions of spawning-history across clusters was then

examined using chi squared tests (Sokal and Rohlf, 1973) (hypothesis ii). Significant differences in individual scores on PC1 and PC2 among clusters were investigated in general linear models.

Previous work has demonstrated a relationship between the energetic status (in terms of lipid density) of salmonid fish and the relative contribution of storage (triacylglycerols, TAG), vs. structural (phospholipids, PL) lipids to total lipids (Næsje *et al.*, 2006). Because the FA composition of storage and structural lipids may differ due to selective incorporation of FAs during biosynthesis in response to their different functional roles (Leaver *et al.*, 2008; Arts, T. *et al.*, 2009), variation in lipid density may influence the FA composition of total lipids. The relationship between lipid density and percent contribution of TAG and PL to total lipids was, therefore, investigated using correlation analyses. The importance of lipid density in explaining FA compositional variability was then investigated by including lipid density as a covariate in models fit to the PC1 and PC2 scores, and the AIC criterion along with the significance of model terms was used to select the "best" approximating model.

Differences in kelt condition as a function of sex or spawning history were assessed by evaluating the best approximating model using the AIC criterion along with the statistical significance of these terms in a length vs. weight model (hypothesis iii). Here, the relationship between weight and length was described by the standard relationship for fish (Elliott, 1975) expressed in its logarithmic form as:

$$\ln (W_i) = \ln(a) + b \ln (L_i) + e_i$$

where a and b are the estimated intercept and slope coefficients and e_i is the multiplicative error term for the *i*th fish.

The return rate of kelts to consecutive repeat spawning was calculated as the proportion of tagged fish leaving the system in spring 2016 that returned during the upstream migration the same summer. A model that treated the return status of individual fish tagged during the 2016 downstream migration as a binary variable (1 = returned, 0 = not returned) was used to evaluate return rates and significant correlations with the biological variables under study. The effects of fish length (L_F), spawning history (maiden or consecutive), sex, were evaluated using the AIC criterion (Anderson *et al.*, 2001) (hypothesis iv). Due to the different treatment of kelts tagged and only measured for length and spawning history, and kelts subjected to biopsies, the two groups were analysed separately to assess the importance of fish length, spawning history and sex (biopsied fish), and fish length and spawning history (non-biopsied kelts) for kelt return probabilities.

Data inspection and statistical analyses were performed in the statistical software R (version 3.4.4, www.r-project.org). In all analyses, model suitability was assessed by visual inspections of residuals for indications of violations of model assumptions.

4.4 Results

A total of 24 first-time spawned (L_F range: 48.0 – 66.4 cm) and 45 consecutively repeat spawned (range: 51.2 – 73 cm) kelts were intercepted and sampled for lipids in the spring of 2016. Scale pattern analysis identified 7 unique life history combinations with 1 sea-winter maturation and consecutive spawning being the dominant maturation and re-maturation strategies, respectively (Table 4.1). Three skipped spawners (i.e. alternate repeat spawners) were identified but removed from subsequent analysis due to the low sample size. The sex-ratio was

biased towards females, and among repeat spawners the female contribution increased with the number of previous spawning events (Table 4.1).

Influence of spawning history and sex on post-spawning lipids and FAs

Kelt dorsal muscle was characterized by low muscle lipid density, but with considerable between-individual variability (mean ± SD, 0.84 g ·10 g wet mass$^{-1,}$ ± 0.43). The variability was largely due to 10 female consecutive spawners having values (1.8 g·10 g wet mass^{-1} ± 0.33) more than two standard deviations higher than the population mean (Table 4.2). GLMs fit to kelt dorsal muscle supported an influence of spawning history and sex on lipid density, predicting higher lipid content in consecutive spawners and females compared to first-time spawners and males. Model investigations, however, revealed non-normally distributed errors in response to the collective outliers distinguished by the 10 lipid-rich female consecutive spawners. Even when excluding these outliers, the sex and spawning history effect was still supported according to the AIC (Table 4.3). Although an interaction model could not be distinguished from an additive model by the AIC criterion, the interaction was not statistically significant ($F_{1, 54} = 0.2$, $p > 0.5$). The most statistically robust model was thus the additive model that predicted higher lipid densities in females and in consecutive repeat spawners (Figure 4.1).

Kelt dorsal FA composition was characterized by a high contribution (% of total FAs) of a few components, with 22:6n-3 (DHA), 16:0 (palmitic acid), 20:5n-3 (EPA) and 18:1n-9 (oleic acid) contributing the most to the total FAs identified (Table 4.2). PCA and K-means clustering showed three distinct clusters in individual compositional values (Figure 4.2), that were not randomly distributed in terms of spawning history groups ($\chi^2 = 45.11$, df = 2, $p < 0.001$). Cluster

1 consisted exclusively of consecutive repeat spawners, 10 of which were the previously identified lipid rich kelts, cluster 2 of first-time spawners, and cluster 3 of consecutive repeat spawning kelts. Cluster 2 also included 7 consecutive spawners of which 6 were males (Table 4.4 and Figure 4.2). Males were not randomly distributed across clusters within consecutive spawners ($\chi^2 = 19.42$, df = 2, $p < 0.001$), as the majority were assigned to cluster 2. No differences between sexes were found within first time spawners as all individuals were assigned to cluster no. 2 (Table 4.4).

PC1, which explained 53.7% of the variation in the data primarily distinguished between samples based on 22:6n-3, 16:0, and 20:5-n3 which received positive loadings, and 20:1-n7, 20:1-n9 and 22:1n-11 which received negative loadings (Figure 4.2). PC2 only explained 12.4% of the variation and was largely driven by variation in values of 22:5n-6, 20:4n-3 and 20:1n-7. All pairwise comparisons of cluster PC1 and PC2 scores were significant, with cluster 2 receiving higher scores on PC1 than cluster 3 and 1, respectively (Tukey's HSD, $p < 0.001$). In turn, cluster 3 received significantly higher scores on PC2 than cluster 1 and 2, respectively (Tukey's HSD, $p < 0.05$).

Correlation analysis showed that associations between total lipid density and lipid class composition in terms of % TAG and PL were strong (Figure 4.3). TAG increased with total lipids (Pearson's r = 0.94), whereas phospholipids decreased with increasing levels of total lipids (Pearson's r = -0.81) and TAG (Pearson's r = -0.88). Because of the strong correlation between TAG and PL, lipid density (g ·100 g wet mass^{-1}) was included in the models fit to PC1 and PC2 scores as a proxy for lipid composition. Model selection provided most support for an additive model containing both cluster and lipid density, indicating that FA composition was influenced

by lipid class composition, but that cluster scores remained significantly different from each other when variation in lipid density was accounted for (GLM, PC1: $F_{3,65} = 227.9$, $p < 0.001$, PC2: PC2: $F_{3,65} = 70.8$, $p < 0.001$) (Figure 4.4).

Influence of spawning history and sex on post-spawning body condition

Investigations of the length-weight relationship within maiden post-spawners revealed no differences between sexes (GLM, $\beta_{Sex,}$ $F_{1,23}$= 1.0, p = 0.33) and this was also the case for consecutive post-spawners (GLM, $\beta_{Sex,}$ $F_{1,42}$ = 1.17, p = 0.3). Model selection yielded most support for two models containing the spawning history term as the AIC criterion did not distinguish between them (Table 4.6). The interaction between length and spawning history was, however, not statistically significant (GLM, $\beta_{LF:Spawning\ history,}$ $F_{1,65}$= 1.31, p = 0.25) so that the most statistically robust model was additive, predicting a greater weight at length in consecutive spawners compared to first time spawners (GLM, $\beta_{Spawning\ history,}$ $F_{1,66}$= 13.25, $p < 0.001$) (Figure 4.5).

Linkage between spawning history and return rates

Of the 69 kelts sampled for dorsal tissue and released in spring 2016, 8 females and no males were successfully recaptured as consecutive spawners (11.6 % of biopsied post-spawners) during the upstream spawning migration the summer of the same year. The low number thus compromised the ability to assess correlates to return rates and model selection yielded no support for any of the factors and covariates tested, except sex. Thus, the most useful model predicted a higher return probability in females compared to males (Likelihood ratio test, $p < 0.05$).

Among the kelts assessed only for spawning history and length, 76 first time spawned (mean LF: 53.2 cm ± 3.1 SD, range: 46.5 to 65.5 cm) and 80 consecutively spawned (mean LF: 61.3 cm ± 6.5, range: 43.2 – 74.2 cm) kelts were identified. Of these, 20 (12.8 %) were recaptured as annual spawners during the upstream migration in 2016. The three best ranked models fit to return probabilities of kelts all included the spawning history term. The AIC criterion did not distinguish between the additive and interaction models (Table 4.6) that also included fish length, although the length term was not statistically significant (Likelihood ratio test, $p > 0.05$). The most statistically robust model was thus additive and predicted a decline in return probability with size, but with a higher return probability in consecutive than first-time spawned kelts (Likelihood ratio test, $p = 0.002$) (Figure 4.6).

4.5 Discussion

Previous spawning/migration history had an important influence on post-spawner nutritional state, as consecutive repeat spawners showed significantly higher lipid density and mass at any given length compared to first-time spawners. Although fatty acid compositional values were in part driven by variation in lipid density, consistent with our expectations significant differences between spawning histories were found. Sex also influenced post-spawner state, with males showing lower lipid densities compared to females in both first-time and consecutively spawned Atlantic salmon. Due to low return rates, the ability to statistically assess significant covariates to kelt return rates was compromised, but return probabilities were nevertheless consistent with patterns in lipid density, as males and first time spawning kelts had lower return probabilities compared to females and consecutive repeat spawning individuals.

The significant correlation between kelt spawning history and lipid density and length specific mass, along with the lack of support for fish fork length as an explanation of post-spawner energetic state, supported the hypothesis that previous life history affects post-reproductive state in Atlantic salmon. To our knowledge, this is the first study to report the role of previous spawning history on the post breeding condition and energy density status of Atlantic salmon. The direction of the difference observed contrasted with that anticipated by terminal investment theory which predicts a higher reproductive effort by older individuals in response to aging (Williams, 1966a; Clutton-Brock, 1984). We suggest two, non-mutually exclusive, explanations for the higher energy densities of repeat spawners related to individual quality prior to breeding and reproductive investment. First, differences in individual quality in terms of somatic depots at the onset of breeding may result in contrasts in residual energy following spawning and overwintering (Lambert and Dutil, 2000). Such quality differences prior to breeding have been observed in migratory capital breeders in response to differences in the quality of the non-breeding habitat (Marra *et al.*, 1998). As such, migration to distinct geographic regions of the Labrador Sea by first-time and consecutive repeat spawning Atlantic salmon (Chapter 2), may have resulted in contrasting foraging success related to environmental variation that affected the availability or quality of food resources in the two regions. Support for feeding area, or life history, -dependent energy status was, however, not found by a previous investigation conducted on Campbellton River Atlantic salmon which indicated no differences in the dorsal lipid concentration of maiden and consecutive repeat spawners upon the initiation of the freshwater spawning migration (Chapter 2).

Secondly, differences in post-spawning energetic state may occur if the relative energetic investments differ among spawning histories. Terminal investment theory predicts that if the chance of future reproduction declines with age, expenditure on current reproduction should increase (Gadgil and Bossert, 1970). In brown trout (*Salmo trutta*), Berg *et al.* (1998) noted repeat spawning females spent relatively more energy during reproduction, resulting in a lower post reproductive energetic state and subsequent survival than maiden spawners, a pattern consistent with terminal investment theory. If higher investment by the comparatively older consecutive Atlantic salmon spawners had occurred in the present study, we would have expected lower energy levels after reproduction compared to first-time spawners, given similar energetic states prior to spawning (Chapter 2). As such, terminal investment theory does not appear to explain the spawning history contrasts in the energetic states reported for kelts in the Campbellton River. Conversely, life history theory predicts that if expectations of future reproduction are high, individuals should restrain reproductive effort to maximize survival (Fisher and Blomberg, 2011).Thus, differences in reproductive investment leading to contrasts in post-spawning energetic state may represent an adaptive response to age related increases in survival (Schaffer, 1974).

The higher return rates of previously (and consecutively) spawned Atlantic salmon kelts compared to first-time spawners in 2016 may suggest an increase in the probability of annual spawning with age, potentially reflecting age-related improvement of survival and/or in the ability to recover depleted somatic reserves. Where such increases in breeding probability have previously been observed (and where the effect of age has been disentangled from the effects of size), two non-mutually explanations have been proposed: i) natural selection removes low

quality phenotypes so that the proportion of high quality individuals increases with age (Forslund and Part, 1995), and ii) breeding probability increases with age due to an improvement in competence as a result of experience (Tavecchia *et al.*, 2001; Curio, 2008). As annual spawning in Campbellton River is associated with a shift in migration strategy (Chapter 2), previously spawned kelts differ from first-time spawners insofar as they have successfully completed the migratory-spawning circuit at least once before. As such, consecutive spawned kelts are veteran migrants compared to novice first-time spawners. Although the importance of experience for performance and survival in the wild has long been recognized for hatchery-reared fish (e.g. Suboski and Templeton, 1989), and increasingly so for wild fish (e.g. Petitgas *et al.*, 2010; Morris *et al.*, 2014), a similar mechanism has, to our knowledge, not been identified within adult migratory salmonids in the wild. Due to the low sample size in the current study, however, these results should be corroborated by additional investigations including a larger sample of fish.

The lower energy density of male post-spawners compared to females in the current study is consistent with results reported by Jonsson and Jonsson (2003) and may be explained by sex-dependent breeding behaviours. Atlantic salmon males spend considerably more time on the spawning grounds than females and undertake frequent excursions both up and downstream in search of breeding opportunities (Fleming, 1996). Combined with intense aggression associated with mate competition and guarding behaviors, the higher behavioral investment in reproduction by males has been hypothesized to result in greater energetic expenditure (Jonsson and Jonsson, 2003). Provided energy reserves at the onset of reproduction do not differ between the sexes, as suggested by Jonsson and Jonsson (2003) for Atlantic salmon

smaller than ca. 60 cm in length, the lower post-spawner energy density in males as noted here is consistent with the greater male spawning energy expenditure hypothesis.

In addition to lipids, protein content to a lesser extent also contributes to the energy status of fish but was not measured in the current chapter. Previous work on the nutritional status of Atlantic salmon of different life stages, however, has demonstrated a positive relationship between somatic content of lipids and the somatic content of proteins (g · 100 g somatic wet mass^{-1}) (Jonsson and Jonsson, 2003) in fish with similar lipid contents to the kelts in the current chapter. As such, the higher lipid content of repeat spawned kelts should be associated with higher protein content according to the literature, suggesting that the results showing a higher energy content of repeat spawned kelts would not change had protein estimates been included.

In addition to the contrasts in energetic state between kelts of different spawning/migration histories, contrasts in FA composition were also suggested by the non-random distribution of spawning histories across FA-based clusters (Figure 4.2 and Table 4.4). Although variation in lipid density was associated with variation in the composition of lipid classes (Figure 4.3) and consequently FAs (Leaver *et al.*, 2008; Arts and Kohler, 2009), the significant differences in PC1 and PC2 scores among clusters when lipid density was accounted for supported the conjecture that spawning history contributes to the variation in FA compositional values in post-spawned Atlantic salmon. Thus, the hypothesis that differences in life history-dependent migration strategies may carry over to influence residual nutritional values of post-spawned Atlantic salmon in the spring was supported. In addition to spawning history contrasts, FA compositional values also differed among consecutively spawned kelts characterized by

significantly lower scores on PC axis 1 and 2, respectively, in kelts previously characterized as lipid rich compared to the population mean. The underlying reason behind this difference within consecutively spawned kelts is not clear but may result from variation in migratory patterns within the life history group. Due to the importance of fatty acids such as EPA and DHA for growth, immunity and stress resistance in fish (Adams, 1999; Arts and Kohler, 2009; Parrish, 2009), differences in FA compositional values linked to condition and underlying health (Arts and Kohler, 2009) will hold consequences for performance and the recovery of post spawned Atlantic salmon. Although this study did not identify the presence, or non-presence, of such a link, we believe that the relationship warrants further investigation.

4.6 Conclusion

In contrast to terminal investment theory predictions, our results showed higher lipid density in repeat spawned kelts as compared to first time spawners. We suggest that spawning history related contrasts in the energetic status of kelts may be a carry-over effect of contrasts in the non-breeding feeding habitats as affected by spawning-history dependent migration strategies, or alternatively, may represent an adaptive response to increased survival and recovery potential related to age. An improved understanding of the factors influencing the returns of post-spawned Atlantic salmon may aid conservation and management efforts to ensure their successful contribution to future spawning events. This may be particularly important in populations where low juvenile to adult recruitment has resulted in conservation concerns and the hope that repeat spawners may offset reduced spawning escapements due to low maiden returns (Bordeleau *et al.,* 2019). Furthermore, knowledge of the physiological and energetic

factors affecting post-breeding recovery in Atlantic salmon may also enable an improved understanding of life history trade-offs related to the reproductive life spans of the species.

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4.8 Tables

Table 4.1: Length (L_F cm), mass (kg) and sex ratio of first time spawned, alternate repeat (AR) spawned and consecutive repeat (CR) spawned Atlantic salmon kelts sampled during the downstream migration in spring of 2016. Detailed life histories are expressed using the shorthand notation ASW:Bx where A represents the number of winters at sea prior to first spawning, B is the number of repeat spawning events and x is the type of repeat spawning strategy.

Spawning history		N	L_F cm, mean (±SD)	Kg, mean (±SD)	N female	% female
First time spawning	Total	24	54.4 (3.8)	0.9 (0.3)	18	80.6
	1SW	23	53.2 (3.0)	0.9 (0.2)	17	80.0
	2SW	1	66.0 (na)	1.9 (na)	1	100.0
Alternate repeat	Total	3	69.5 (1.8)	2.3 (0.2)	2	66.7
	1SW:1AR	3				
Consecutive repeat	Total	45	62.2 (4.9)	1.7 (0.4)	37	77.1
	1SW:1CR	16	57.7 (3.9)	1.3 (0.3)	12	66.7
	1SW:2CR	23	63.8 (3.2)	1.9 (0.3)	19	79.2
	1SW:3CR	5	66.8 (3.1)	2.2 (0.2)	5	100.0
	1SW:1AR:2CR	1	73.0 (na)	2.6 (na)	1	100.0

Table 4.2: Mean and standard deviation (sd) of kelt dorsal muscle total lipids (g · 100 g wet mass^{-1}) and lipid (% of total lipids) and fatty acid (% of total fatty acids) composition in first-time spawned and consecutively repeat spawned Atlantic salmon kelts sampled during the downstream migration in spring, 2016.

	First time spawners (N=24)		Consecutive spawners (N=35)		Consecutive spawner outliers (N=10)	
	mean	sd	mean	sd	mean	sd
Acetone mobile polar lipids	5.9	2.6	3.8	1.7	4.8	3.7
Free fatty acids	13.3	4.4	9.2	2.9	6.8	2.9
Phospholipids	66.8	8.4	71.3	7.0	30.8	11.1
Sterols	11.6	2.8	8.3	1.9	3.1	1.3
Triacylglycerols	0.4	1.3	5.5	7.8	53.6	12.6
Total Lipids (*g · 100 g wet mass^{-1}*)	0.6	0.1	0.7	0.2	1.8	0.3
14:00	0.8	0.2	1.2	0.4	2.4	0.4
16:00	14.8	1.1	13.8	1.6	8.8	0.6
16:1n-7	1.1	0.2	2.2	0.8	5.1	0.9
16:1n-9	0.4	0.1	0.3	0.1	0.2	0.0
18:00	4.6	0.7	4.7	0.7	3.3	0.4
18:1n-5	0.2	0.0	0.3	0.1	0.4	0.1
18:1n-7	2.0	0.3	2.2	0.3	2.4	0.3
18:1n-9	6.8	1.2	7.6	1.5	14.4	2.4
18:2n-6	0.6	0.1	0.5	0.1	0.8	0.2
18:3n-3	0.2	0.0	0.2	0.1	0.2	0.1
18:4n-1	0.7	0.5	0.7	0.4	0.3	0.1
20:1n-11	0.6	0.3	0.6	0.3	1.5	0.3
20:1n-7	0.2	0.1	0.4	0.2	1.2	0.1
20:1n-9	3.2	1.2	4.7	2.5	15.7	2.1
20:2n-6	1.0	0.5	0.7	0.4	0.4	0.2
20:4n-3	0.9	0.2	0.7	0.1	0.7	0.1
20:4n-6	1.9	0.4	1.2	0.3	0.5	0.1
20:5n-3	13.2	2.3	12.6	2.1	5.9	1.1
22:1n-11	1.2	1.2	2.5	1.9	10.1	1.7
22:1n-9	0.4	0.3	0.6	0.4	2.1	0.3
22:5n-3	4.4	0.7	5.5	1.0	5.1	0.7
22:5n-6	0.4	0.1	0.2	0.1	0.1	0.0
22:6n-3	37.0	3.3	33.2	5.1	14.5	3.0
24:1	0.9	0.3	0.8	0.2	0.9	0.1

Table 4.3: AIC model selection table for general linear models fit to explain variation in kelt dorsal lipid density (g lipids · 100 g wet mass^{-1}). Collective outliers removed.

Model	df	logLik	AIC	dAIC	R^2
y ~ Spawning history + sex	4	23.8	-39.5	0.0	0.15
y ~ Spawning history * sex	5	23.9	-37.8	1.8	0.13
y ~ L + sex	4	22.3	-36.6	3.0	0.10
y ~ Spawning history	3	20.7	-35.5	4.0	0.07
y ~ Sex	3	20.6	-35.1	4.4	0.06
y ~ L * sex	5	22.3	-34.6	4.9	0.08
y ~ L	3	20.2	-34.5	5.1	0.05
y ~ L * spawning history	5	22.2	-34.3	5.2	0.08
y ~ L + spawning history	4	21.1	-34.2	5.4	0.06

Table 4.4: The distribution of male and female first-time and consecutive repeat spawning kelts among the three identified clusters based on FA composition.

	First-time spawners		Consecutive repeat spawners		
cluster	Female	Male	Female	Male	Total
1	0	0	12	1	13
2	8	6	1	6	31
3	0	0	22	3	25
Total	18	6	35	10	69

Table 4.5: AIC model selection table for general linear models fit to explain variation in kelt length (cm) to weight (kg) relationship

model	df	logLik	AIC	dAIC	R^2
ln(weight) ~ ln(L_F) + spawning history	4	58.2	-108.4	0.0	0.92
ln(weight) ~ ln(L_F) * spawning history	5	58.9	-107.8	0.6	0.92
ln(weight) ~ ln(L_F)	3	51.9	-97.8	10.6	0.91
ln(weight) ~ spawning history	3	-8.3	22.7	131.1	0.49

Table 4.6: AIC model selection table for general linear models fit to explain variation in return status of kelts assessed for spawning history and length (L_F, cm).

Model	df	logLik	AIC	dAIC
y ~ L_F + spawning history	3	-50.99	107.98	0.00
y ~ L_F * spawning history	4	-50.42	108.83	0.85
y ~ spawning history	2	-54.02	112.03	4.05
y ~ 1	1	-59.74	121.48	13.50
y ~ L_F	2	-59.73	123.47	15.49

4.9 Figures

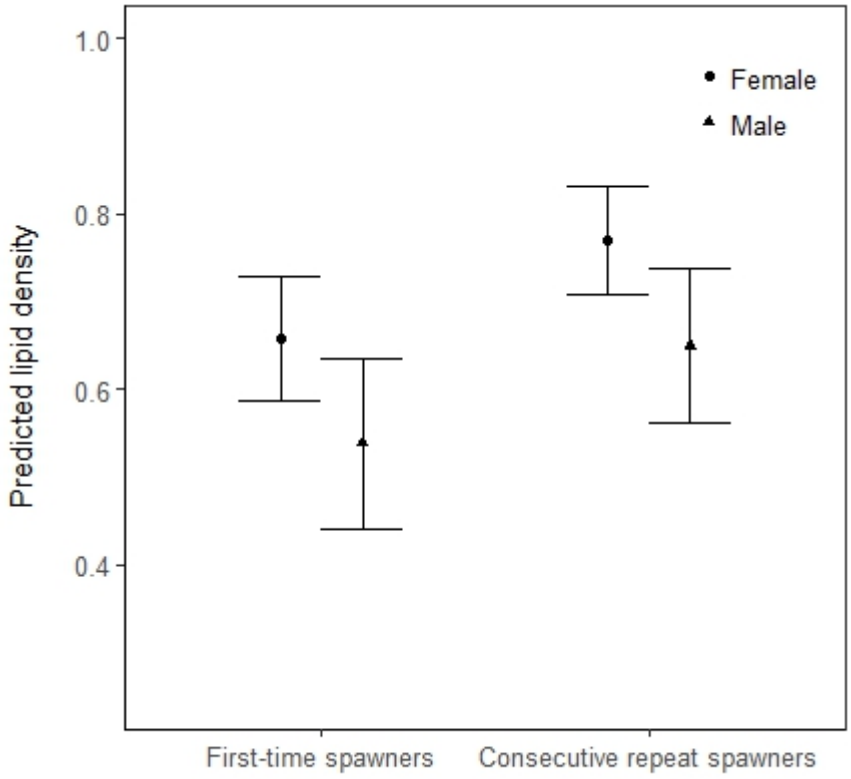

Figure 4.1: Predicted kelt dorsal muscle lipid density (g · 100 g wet mass^{-1}) as predicted by the most supported model fitted to kelts sampled for lipid density as a function of spawning history (consecutively spawned or first-time spawned) and sex at river emigration. The vertical bars represent the 95% prediction confidence intervals. Collective outliers removed.

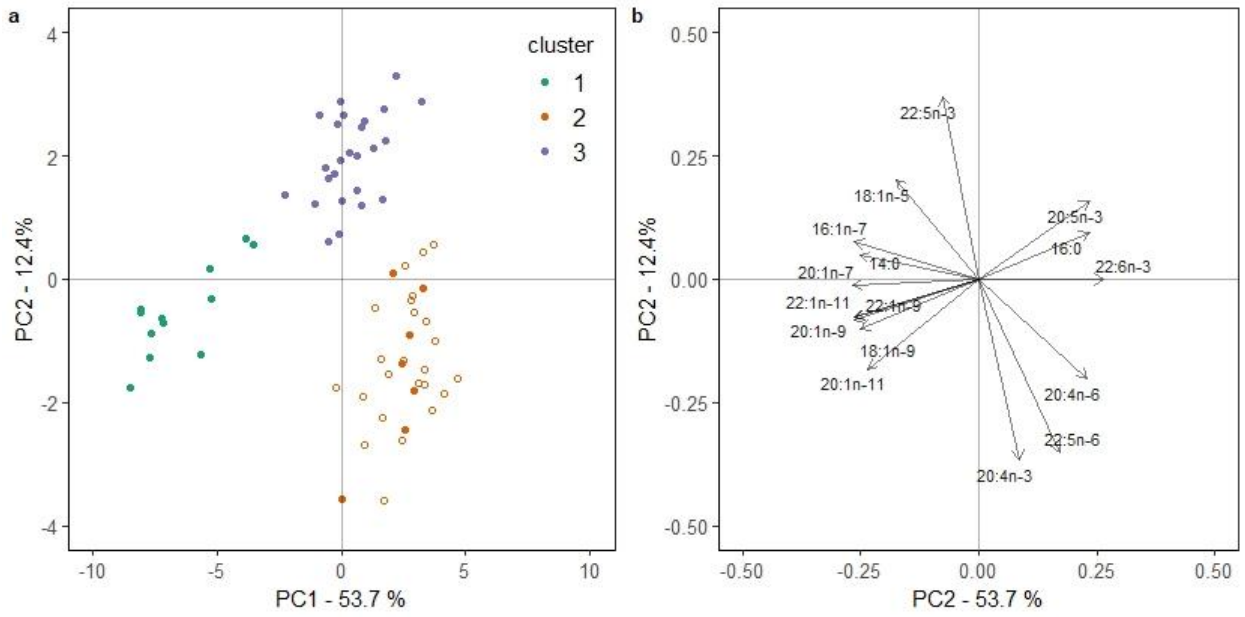

Figure 4.2: Principal component a) score plot of FA composition for first time spawned (open circles) and consecutively spawned (filled circles) kelts and assigned cluster membership based on cluster analysis, b) loading plot indicating the importance of each FA's contribution to distinguishing between the two first principal component axes. Only those FAs with the highest absolute loadings on the first two PC axes are displayed, following the procedures described by Olden and Poff (2003).

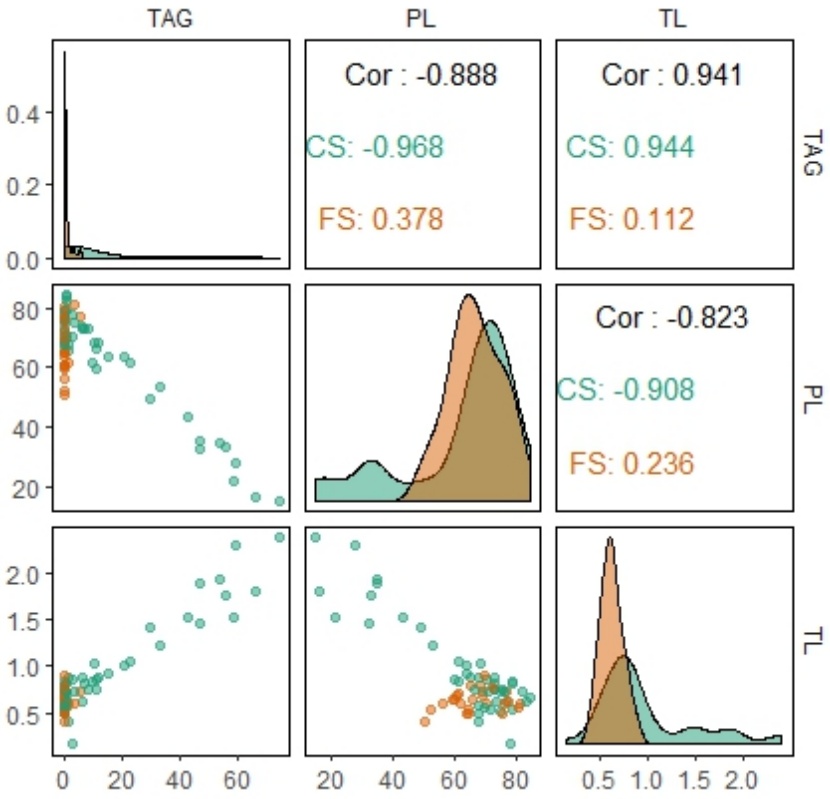

Figure 4.3: Scatterplot matrix of first time spawned (FS) and consecutively repeat spawned (CS) kelt dorsal muscle triacylglycerols (TAG, % of total lipids), phospholipids (PL, % of total lipids) and total lipids (TL, g · 100 g wet mass^{-1})

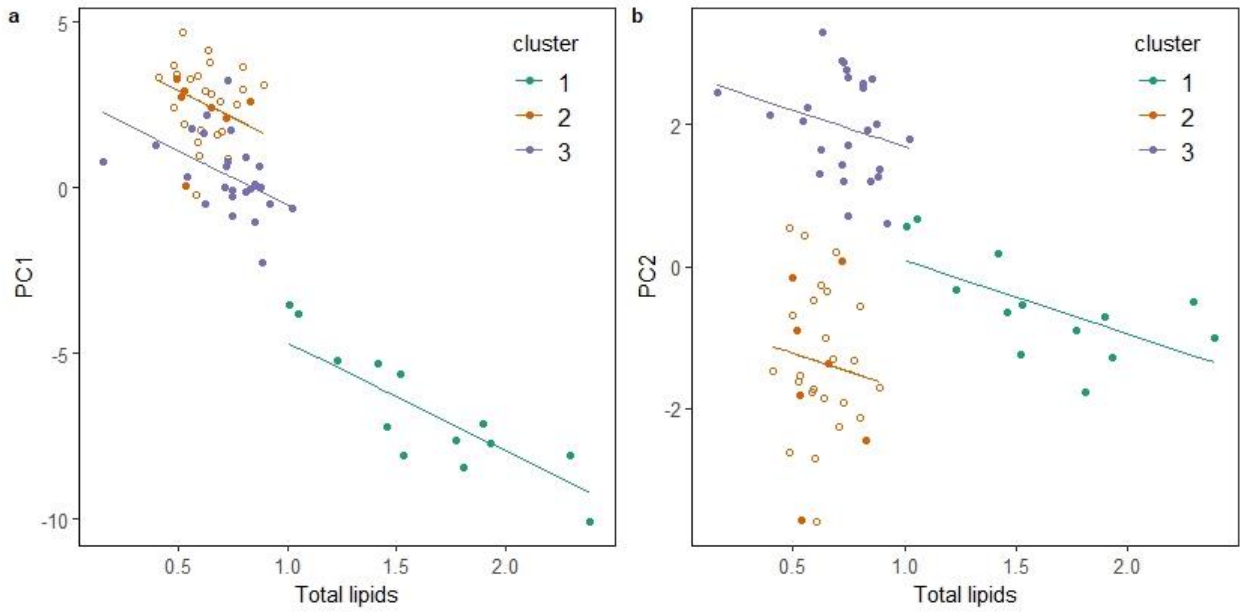

Figure 4.4: Scatter plot of kelt total lipids (g · 100 g total lipids^{-1}) and a) PC1 and b) PC2 scores. The regression lines represent the significant relationships according to the most supported models based on the AIC criterion.

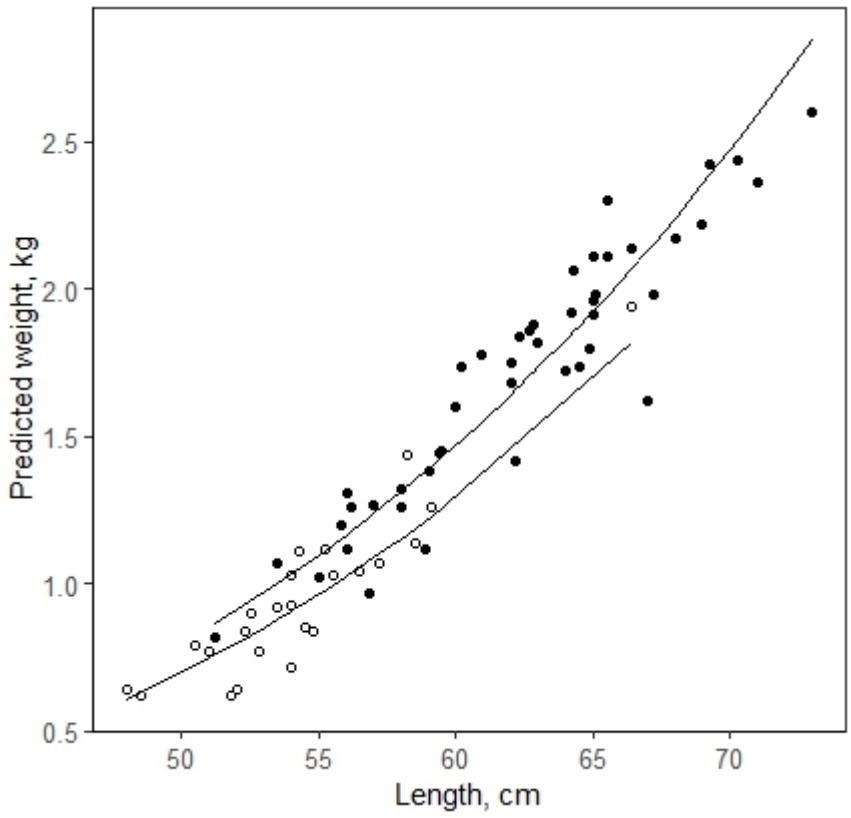

Figure 4.5: Scatter plot of kelt length (L_F) and weight of first-time spawned individuals (open circles) and consecutive spawned individuals (filled circles) sampled during the river emigration in spring, 2016. The regression lines represent the significant relationships according to the most supported model based on the AIC criterion.

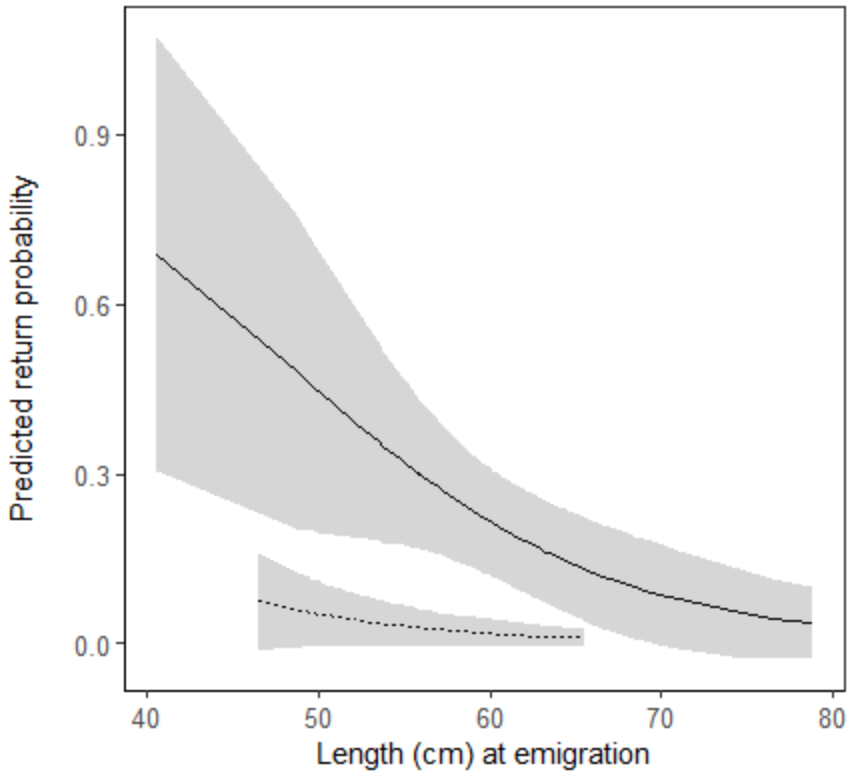

Figure 4.6: Predicted kelt return probability as predicted by the most supported models fitted to kelts sampled for length and spawning history as a function of spawning history (consecutively spawned kelt=solid line, first-time spawned kelt=dotted line) and length at river emigration. Shaded area represents the 95 % prediction confidence intervals.

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5 Chapter 5 – Conclusions

5.1 Summary

Atlantic salmon is an anadromous salmonid that performs extensive migrations over vast ocean distances. Due to logistical difficulties in monitoring fish movements across ocean basins, a detailed understanding of the marine phase of the lifecycle is limited. Current and widespread population declines attributed to reduced survival at sea, therefore, have emphasized the need to improve our understanding of proximate and ultimate factors shaping Atlantic salmon marine migratory dynamics and ultimately how these factors might influence fitness relevant traits such as growth and survival. The task is challenging due to the complex nature of migrations which occur over a variety of spatial and temporal scales, as well as across multiple levels of biological organization from individuals to populations and community structure. An improved understanding of migratory patterns, therefore, requires integrating knowledge across these levels as well as temporal and spatial scales. An outcome which is best achieved using a multidisciplinary approach.

The current thesis focused on the relationship between iteroparity and anadromy in Atlantic salmon of the northwest Atlantic. While the importance of repeat spawners in mitigating for short-term negative trends in juvenile to adult recruitment is receiving increasing attention (e.g. Bordeleau *et al.,* 2019), there has been little comparative work conducted on the iteroparous lifecycle in the freshwater or marine phase. Research presented in the current thesis addressed knowledge gaps related to the role of iteroparity in shaping migratory patterns in the nearshore and at-sea phase, as well as life history trade-offs and carry-over effects related to repeated migrations. By applying a multidisciplinary approach, the thesis was able to address different spatiotemporal scales (i.e. early coastal and open ocean migration) and life history stages

(juvenile, maturing, re-maturing and post-spawned) of the Atlantic salmon marine migrations to provide an integrated investigation of the patterns of anadromy in relation to iteroparity.

In chapter 2, detailed spatiotemporal patterns of migrations in the nearshore phase were investigated and compared between the juvenile (smolt) and adult (kelt) life stages in two populations from Newfoundland (Canada) using acoustic telemetry. The study hypothesized that due to contrasts in size, experience, and the motivation and consequences of migrations, smolts and kelts are exposed to different selective pressures and constraints which should result in contrasts in the migratory patterns observed. The study found that life stage, as well as temperature influenced the movement patterns of Atlantic salmon in the nearshore phase, but that the differences varied by population. Suggested sources of the between-population variation included responses to temporal and physical contrasts in the biotic and abiotic environment that shape the constraints imposed by trade-offs such as those between the need to reduce predation risk and increase growth and mass-gain. When present, differences in migratory movements were characterized by faster, more directed and less nocturnal movement by kelts as compared to smolts. Identified temperature influences consisted of a positive correlation with seaward movements, and a negative correlation with diel movements, as hypothesized. The temperature influence on seaward movements was suggested to offer a mechanism that may promote open ocean entry during conditions favorable to migration performance, potentially mitigating earlier mismatches in migration timing relative to ecosystem timing. The correlation with diel timing and temperature may in turn reflect an improved ability to avoid predation as well as a need to meet the higher metabolic demand imposed by temperature (Jobling, 1981; Thorpe *et al.*, 1994; Ibbotson *et al.*, 2006). In addition

to contrasts in migration speed and diel movements, smolts and kelts did not appear to use the same migration routes from the inner coastal embayment to the open ocean. Kelts from Campbellton River displayed a more easterly migration route compared to smolts, and a significant difference between life-stages in the choice of the two available passageways in the Bay d'Espoir was found in Atlantic salmon from Conne River. While it is generally assumed that external sensory cues, such as surface currents, temperature and salinity, play an orientation role in determining Atlantic salmon migration routes, exact mechanisms are poorly understood (Byron and Burke, 2014; Moriarty *et al.*, 2016), including the role of prior experience and innate spatiotemporal controls in guiding migrations (Dodson, 1988; Lohmann *et al.*, 2008). The lower between-individual variability in migratory direction in kelts offered some support for the hypothesis that adults are more effective at orienting towards the open ocean, either as a result of prior experience, or in terms of their responses to the external environment. As such, kelts may exploit memory to more efficiently orient themselves for seaward migration than smolts and/or be less influenced in swim direction by external environmental influences, e.g. directly by surface currents, or indirectly by osmoregulatory behaviours in response to thermal or salinity gradients.

The juvenile coastal migrations by Atlantic salmon constitute a critical life history phase (Thorstad *et al.*, 2012) but represent only a fraction (i.e. a few days to a few weeks) of the total marine residency time by maturing maiden and re-maturing alternate repeat spawning Atlantic salmon (Thorstad *et al.*, 2012). In contrast, the importance of the nearshore environment for consecutive repeat spawners is less well understood (Hubley *et al.*, 2008). In Chapter 2, detections of kelts from Campbellton River along the Newfoundland shelf throughout summer

indicated that the nearshore was the main feeding area of consecutive spawners from this population. Thus, the main geographic feeding area for consecutive repeat spawning salmon from Campbellton River differs from that inferred for conspecific maiden (predominantly 1SW) fish and alternate repeat spawning fish that are presumed to feed in the central Labrador Sea (Reddin, 2006). Locating main feeding areas of Atlantic salmon is important for identifying the biotic and abiotic factors to which migrating salmon are exposed, and ultimately for determining how these factors affect fitness relevant traits such as growth and survival. Contrasts in migratory movements associated with demographic attributes such as age, size, sex, or life history, may result in the spatial segregation of individuals during the non-breeding season, so that the ecological factors faced by population segments may differ. Such factors may consist of prey availability or composition (Cristol *et al.*, 1999; Mathot *et al.*, 2007), that influence fitness relevant traits. The third chapter, therefore, investigated the presence of life history dependent migratory patterns by maiden 1SW, alternate repeat, and consecutive repeat spawning Atlantic salmon from Campbellton River and influences of the divergent migrations on dietary patterns. Logistical constraints precludes a detailed investigation of feeding area use by multiple life history stages using telemetry, so this was done using a combination of non-lethal biopsies, FA and SI analyses. Significant differences in FA composition and ratios of $\delta^{15}N$ in dorsal muscle tissue among spawning-histories were identified, which supported the hypothesized divergent use of dietary sources among the different spawning history groups of Campbellton River Atlantic salmon. Dissimilarity and clustering analyses also demonstrated that the similarity in resource use was greater in maiden and alternate repeat spawners as compared to consecutive repeat spawners, suggesting a greater overlap in dietary

resources in maiden and alternate repeat spawners. The FAs that differentiated among the spawning histories consisted of FAs previously established as individual or components of biomarkers associated with phylogenetic prey groups that differentiate between the shelf and basin biogeographic regions of the Labrador Sea (Head *et al.*, 2003; Fragoso *et al.*, 2016), and thereby, supported the hypothesis of divergent feeding locations. As such, Chapter 3 suggested that migration route differences between life stages (juveniles and kelts) observed in the early coastal phase (Chapter 2) was also present after juveniles and adults entered the open ocean.

Although the study design employed in chapter 3 was not able to identify differences in the specific prey items consumed by different life history groups, potential differences in feeding ecology related to differences in feeding locations and the biotic and abiotic properties of those locations are likely to have potential consequences for group-specific adaptive landscapes (Chapman *et al.*, 2012; Briedis and Bauer, 2018). Thus, divergent migration patterns have the potential to influence population segment-specific fitness-associated traits such growth and survival. Differential growth and survival between population segments may carry over to affect population dynamics in the short, and/or long term. Although details regarding potential population consequences of the life history dependent migration strategies identified in chapter 3 are uncertain, the findings presented in the chapter may inform our understanding of the spatial and temporal variation in Atlantic salmon survival at sea. Furthermore, divergent migrations are believed to affect a populations' vulnerability to environmental perturbations such as adverse weather and food shortage, as only a part of a breeding population will experience these conditions at the same time (Taylor and Norris 2010, Briedis and Bauer 2018). As such, divergent migrations may increase population resilience to environmental change

equivalent to a portfolio effect (Schindler et al. 2010). One consequence of the divergent migrations that was identified, however, was the different FA composition of the fat depots available for reproductive effort in Atlantic salmon returning to breed. Being strongly influenced by allocation from maternal lipids, the FA composition of the fat depots available for reproductive effort in Atlantic salmon have direct bearing on egg lipids (Sargent *et al.*, 1999; Tocher, 2010). Due to the importance of egg lipids for determining egg and offspring quality and survival in fish (Brooks *et al.*, 1997; Sargent *et al.*, 1999), the distinct FA compositional differences among spawning history groups may carry-over to influence differences in demography.

The observation that processes occurring in one part of the animal lifecycle may influence the success or performance in lifecycle phases downstream is a cornerstone of behavioural ecology and life history theory (Williams, 1966), and a phenomenon referred to as 'carry-over' effects (Harrison *et al.*, 2011; O'Connor *et al.*, 2014). For example, producing gametes, competing for mates and accessing appropriate breeding habitat during reproduction, are energetically expensive actions which require resources that could otherwise be provided to competing functions such as growth, survival and maintenance. Current reproduction, therefore, should negatively impact future longevity and reproduction in the future (Williams, 1966). This is a central tenet of life history theory which predicts that trade-offs between current and future reproduction shape allocation strategies through the selection of optimal life-histories (Stearns, 1992). Another form of carry-over effect which has received comparatively less attention in the literature is the idea that effects in one season can influence individual performance in the next season (Harrison *et al.*, 2011). Being a phenomenon that extends across season, several studies

have investigated this type of carryover effect in the light of migrations (O'Connor *et al.*, 2014). In Chapter 4, carry over-effects extending across seasons and life-histories were investigated by contrasting the nutritional and energetic states of post-spawned kelts in relation to migration and spawning history. Life history theory predicts that optimal reproductive effort increases with age, with the result that differential investment among spawning histories may produce contrasts in post-spawner states that have implications for recovery and survival (i.e. terminal investment theory) (Gadgil and Bossert, 1970). Furthermore, due to the differences in the quality of somatic reserves of returning Atlantic salmon breeders (Chapter 3), Atlantic salmon with different spawning/migration histories may end up with different lipid and fatty acid composition after spawning as a result of being capital breeders that ingest no food during reproduction. Chapter 4 therefore hypothesized that previous spawning history would be linked to energy density and condition in post-spawned individuals, with repeat spawned kelts having lower energy density and condition than maiden spawners. It was also hypothesized that spawning-history dependent migration strategies would differ significantly in FA composition. The implications of potential energetic and nutritional contrasts for anadromy patterns was investigated by comparing marine return rates of post-spawners with different spawning histories. Chapter 4 thus assessed the importance of previous spawning and migration history for determining post-spawner energetic and nutritional state and investigated potential linkages with subsequent spawner returns using non-lethal biopsies and a mark-recapture assessment of individual fates. To assess the success of the non-lethal biopsy sampling technique, an evaluation of potential consequences in terms of growth and marine return rates was performed by comparing biopsied fish with a control group (Appendix 6.1).

The results in chapter 4 confirmed the hypothesis that previous migration/spawning history affects Atlantic salmon post-reproductive state in terms of energetic and nutritional state and somatic condition. In contrast to the predictions made by terminal investment theory, the results suggested a higher lipid density and body condition in repeat consecutively spawned kelts as compared to first time-spawners. Furthermore, the return probability for kelts as annual (consecutive) spawners depended on migration history, with consecutively spawned kelts having significantly higher return probabilities compared to maiden spawned kelts. As life history theory predicts that reproductive effort should be constrained if expectations of future reproduction are high (Fisher and Blomberg, 2011), the contrasting energy densities and return probabilities observed in Chapter 4 may be linked to age related increases in survival. Two alternative, non-mutually exclusive, explanations for increased return probability with age were suggested; i) natural selection removes low quality phenotypes so that the proportion of high quality individuals increases with age (i.e. increasing spawning events) (Forslund and Part, 1995), and ii) breeding probability increases with age due to an improvement in competence as a result of experience (Tavecchia *et al.*, 2001; Curio, 2008). In chapter 2, contrasts in migration capacity as suggested by the more 'efficient' orientation by kelts compared to smolts indicated an improvement in migratory competence as a result of experience (or alternatively as an indirect result of a larger size) and favours the second of the two suggested explanations. Interestingly, the contrast in lipid density identified between spawning histories was not identified in a similar analysis of spawner lipids of chapter 3. This also supported the notion that differential reproductive investment may be present.

In addition to energy density, contrasts in FA composition of kelts with different migration/spawning histories were identified, which supported the hypothesis that life history dependent migration strategies may also carry-over to affect the residual nutritional state of post-spawned Atlantic salmon. Due to low return rates, chapter 4 was not able to establish a direct link between nutritional state and return probability. Nevertheless, it was hypothesized that such a link may exist due to the importance of fatty acids such as EPA and DHA for growth, immunity, and stress resistance in fish (Adams, 1999; Arts and Kohler, 2009; Parrish, 2009). Thus among life history differences in FA compositional values may hold consequences for the performance and recovery of post-spawned Atlantic salmon. Evidence for potential links between nutritional status and return probability should be corroborated by additional investigations including a larger sample of fish.

5.2 Study significance

To my knowledge, this is the first study to provide comparative observations of nearshore marine migratory movements of smolts and kelts monitored under the same environmental circumstances. Chapter 2 thus improved knowledge on the role of life stage in shaping intrapopulation variation in migratory movements of Atlantic salmon during the nearshore phase. To my knowledge, chapter 3 was also the first study to use FA analysis to infer likely feeding areas of migrating Atlantic salmon and to use FA analysis to quantify contrasts in resource use among Atlantic salmon of different life histories. Thus Chapter 3 provided a useful example of how non-lethal biopsies coupled with FA analysis can provide a relatively non-invasive (Appendix 6.1), rapid and cost-effective manner to acquire information on marine habitat use of Atlantic salmon and other marine fish. The study also provided important

corroboratory evidence for the location of main feeding areas of maiden, alternate repeat, and consecutive repeat spawners in the Northwest Atlantic. Improved understanding of feeding area locations will facilitate the identification of potential biotic and abiotic factors affecting Atlantic salmon performance at sea, as well as improve the general understanding of the spatial and temporal variation in Atlantic salmon survival and population dynamics.

Chapter 4 was also, to my knowledge, the first study to identify an effect of previous spawning and migration history on current nutritional state of post-spawners, and a potential link between subsequent performance in terms of marine-return probability. Thus, Chapter 4 contributed to an improved understanding of factors influencing the return of post-spawned Atlantic salmon, as well as a better understanding of life history trade-offs related to the reproductive life span of the species. Potential management implications of the findings relate to the higher return probabilities of repeat spawned kelts compared to first time spawners and thus their potential importance for the productivity of populations.

The findings made by the three data chapters of this thesis demonstrates that the migratory patterns of different life stages and life histories associated with ontogeny and iteroparity in Atlantic salmon are not ecologically equivalent. These findings highlight the importance of considering life stage and life history when trying to understand movement data and migration dynamics of anadromous iteroparous salmonids as well as other migrating species. Divergent migration patterns have the potential to improve population resilience to environmental change, and thus may have conservation benefits, especially for populations subject to conservation concerns. This demonstrates that maintaining intrapopulation life-history diversity is important in migrating species. Furthermore, increased knowledge on the presence and

patterns of divergent migrations may inform conservation work by tailoring management efforts to the respective population segments.

Compared to the bird migration literature, however, divergent migration patterns in association with ontogeny or life-history has received less attention in fish, where the non-unitary nature of migrations within populations has received more focus, including the causes and consequences of such divergent migrations (e.g. Cristol *et al.,* 1999; Bell, 2005; Mathot *et al.,* 2007, Taylor and Norris, 2010). The lack of a similar research effort on marine migrations of teleosts (with the exception of partial migrations, see Chapman *et al.,* 2012) can likely be attributed to logistical constraints in mapping individual movements and their correlations with demography and individual states of fish at sea. Great research effort has instead been devoted to describing spatiotemporal patterns of migrations, and trying to understand the proximate mechanisms, including internal and external drivers of such patterns (e.g. Drenner *et al.,* 2012; Secor, 2016, section 1.5). This focus implicitly requires generalizations to be made in order to reach deductive inferences required to improve our basic understanding of fish movement. Furthermore, describing a species or population as a sum of its parts can vastly simplify both empirical data collection and theoretical models (Bolnick *et al.,* 2003). Adding layers of complexity by mapping and including variation associated with iteroparity and life-history in fish migration studies will, on the other hand, represent a more complete description of a biological system. Information on divergent migration patterns and potential consequences to individual fitness-related traits may also improve models of population dynamics by including the distinct properties of population components. This study demonstrated that an integrated approach, using multiple methodological approaches, can improve our understanding of how a

population is more than a sum of its parts. More recent methodological advancements in life-cycle tracers and telemetry methods means that researchers are now better equipped than ever before to combine methods to produce novel insights into the migratory ecology of anadromous salmonids.

5.3 Future directions

Although differences in migratory patterns of smolts and kelts were identified in chapter 2, the current study design was not able to identify the exact mechanisms underlying the patterns observed. Suggested mechanisms included size-dependent swimming ability, size-dependent predation risk, and contrasts in orientation abilities related to experience. Recent advances in modelling migratory capacities may be able to elucidate the importance of some of these mechanisms. Most notably, recent work examining the mechanisms of migration route in relation to safe passage of downstream-migrating fish in regulated rivers has provided methodological advances that may be used to improve knowledge of orientation mechanisms by Atlantic salmon during the early marine phase. By combining detailed migration data obtained by acoustic telemetry with numerical modelling of flow fields, Szabo *et al.* (2019) evaluated migration patterns and route choice in relation to governing flow patterns as revealed by computational fluid dynamics. A similar approach could be applied to the marine phase by using an acoustic receiver network allowing continuous positioning of individual location (e.g. Baktoft *et al.*, 2017) when combined with detailed descriptions of hydrographic properties including surface current, salinity and temperature gradients. Such descriptions exist for some larger Norwegian fjords in the shape of hydrographic numerical models having high temporal and spatial resolution (e.g. Sandvik *et al.*, 2016). Models could thus be combined with

migration data to infer the relative importance of, for example, surface flow or temperature on the directional choices of migrating smolts and kelts. While improving our understanding of the factors contributing to life-stage contrasts in migratory movements, this would also improve the overall understanding of the orientation mechanisms of Atlantic salmon during the marine migration phase.

Chapter 3 corroborated existing understanding of life history dependent differences in feeding areas used by Northwest Atlantic salmon as based on conventional tagging, fishery (Ritter, 1989; Reddin *et al.*, 2006), and telemetry data (Chapter 2). The chapter further identified potential contrasts in the trophic origin of the main dietary sources consumed. Although the results supported different food-web origins for the energy assimilated by spawning-history dependent migration strategies, the FA and SI analyses used in chapter 3 were not able to identify differences in the specific prey items consumed. Prey-specific feeding could be further investigated by collecting SI data from putative prey items from the two geographic regions and applying a stable isotope mixing model (e.g. Smith *et al.*, 2013) to estimate the relative contributions of various food resources to a consumer's diet. As previously mentioned in this chapter, the identification of feeding area is important for identifying the factors affecting the performance of Atlantic salmon at sea. This is, however, still difficult if distributional ranges are large, as is the case for the maiden 1SW and alternate repeat spawning Atlantic salmon in the central Labrador Sea. Conversely, the consecutive repeat spawner migration is limited in both space and time, which should facilitate the identification of ecological stressors and processes influencing repeat spawner growth and marine returns. For example, due to the presence of many commercially important fisheries on the Newfoundland shelf, estimates of the biomass

and distribution of prey in the region is available for some of the potentially important prey species of Atlantic salmon (e.g. DFO, 2018). If the relative importance of distinct prey items in the diet of consecutive repeat spawners were investigated using stable isotope methods as described above, biomass indices of identified important prey could be used to infer possible bottom-up controls on kelt return rates. As information on environmental conditions on the Newfoundland shelf is also routinely collected (e.g. Colbourne *et al.*, 2017), insights may also be gained on the importance of climate influences on kelt performance at sea.

Chapter 4 demonstrated a potential linkage between past migration and spawning history with subsequent performance in terms of marine return rates. Due to the low sample size, these findings should be replicated and corroborated with increased sample size studies. While the link between nutritional status (in terms of FA composition) and subsequent kelt growth and returns remains uncertain, it may be particularly relevant when considering that recent global warming trends are predicted to influence the fatty acid composition at the base of marine food chain (Colombo *et al.*, 2019). The ecological consequences of the FA differences observed among spawning history groups in terms of their effects on egg lipid composition and quality is also an interesting avenue of research that warrants attention due to potential for nutritional condition to influence demography as a result of the implications of FAs on egg and offspring quality and survival.

Lastly, Chapter 4 demonstrated how non-lethal biopsies can be coupled with mark-recapture techniques to assess linkages between energetic and nutritional state and kelt marine returns. An independent evaluation of the potential consequences of the biopsy treatment for fitness associated traits indicated no measurable effects of the treatment on growth and return rates

(Appendix 6.1), suggesting the method can be more widely applied to Atlantic salmon studies. The use of non-lethal biopsies can, for example, be coupled with other methods to improve our understanding of fish movement and its consequences for fish condition. If coupled with telemetry, for example, tissue biopsies could contribute to an improved understanding of how variation in movement patterns among individuals may be linked to fitness associated traits, such as energy status and growth.

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6 Appendix

A version of this manuscript was accepted in conservation physiology (10.1093/conphys/coz099) in 2019. This research was developed and carried out by Kristin Bøe, together with the supervisory assistance of Ian Fleming and Michael Power, and the guidance of Martha Robertson. All authors discussed the results and contributed to the final manuscripts.

6.1 Appendix – Evaluating the effect of dorsal muscle biopsies on adult Atlantic salmon growth and marine return

6.1.1 Abstract

Increasing conservation and animal welfare concerns have driven the development of non-lethal sampling of fish populations, with the use of muscle tissue biopsies now being routinely applied as a sampling method in the wild. Crucial to the success of non-lethal sampling, however, is an evaluation of the short- and long-term consequences of the treatment and ultimately the determination of how these may affect organism mortality and other fitness related traits. The current study evaluated the use of a dorsal muscle biopsies on post-spawned Atlantic salmon emigrating to sea and undertaking a ca 2-month long feeding migration before returning to spawn. Using mark-recapture, return rates and growth were compared between fish that were biopsied and externally tagged, and a control group tagged only with external tags. The biopsy treatment showed no lasting effects on fish as estimated from the two key fitness related parameters. Results, therefore, suggest the technique can be more widely applied to gather information on marine migrating Atlantic salmon and other anadromous

22 fishes that can be intercepted as they descend and ascend rivers during seasonal migrations.
23 Coupled with modern tagging technologies, the use of biopsies may facilitate an improved
24 understanding of movement and its consequences in terms of feeding patterns and growth.

6.1.2 Introduction

The use of aquatic biotelemetry methods has improved our understanding of the ecology of aquatic animals by facilitating detailed monitoring of their spatial and temporal movements at multiple scales (Hussey *et al.*, 2015). With the development of biological sensor tags, aquatic biotelemetry methods can now be used to provide detailed understanding of the physiological costs of movement having direct implications for animal conservation (Wilson *et al.*, 2015) or for an improved understanding of environmental risk (Hellström *et al.*, 2016). Central to any understanding of movement ecology is an understanding of its fitness consequences, with patterns of feeding and body condition being major determinants of movement patterns (Hussey *et al.*, 2015). For example, linked telemetry and dietary trace studies have demonstrated significant covariation between the behavioural and dietary ecology of fishes at ecologically relevant timescales, with the variation among individuals linked to habitat carrying capacity and population niche size (Harrison *et al.*, 2017). Particularly where threatened or endangered species are concerned, the ability to gain such information in an effective non-lethal manner is critical.

The importance of understanding and quantifying the consequences of movement and habitat use for fish has resulted in the development of multiple study approaches, including the use of contaminant concentrations and dietary tracers measured in fish tissue that are now among the cornerstones of environmental assessment and the ecological study of fish populations (Anderson *et al.*, 2017). A key methodology for both contaminant (Jardine *et al.*, 2006) and dietary studies (Newsome *et al.*, 2007) is the application of stable isotope analysis (SIA), with fatty acid analysis (FAA) being rapidly developed as a biomarker tool (Iverson, 2009). At macro-

scales, SIA can infer the spatial origin of an animal using intrinsic biological or biogeochemical markers that are particularly useful for studying animal movement because they do not require animal marking or recapture and yield time-integrated information that can be linked directly to a geographical region (Rubenstein and Hobson, 2004). FAA may in turn provide information on the spatial structure of foraging and specific or general ecological origin of energy assimilated during a feeding season (Budge *et al.*, 2006, Iverson, 2009). Although other non-lethal sampling methods such as the analysis of scales, mucus and fins are promising (e.g. Hutchingson and Trueman 2006, Church *et al.* 2009, Jardine *et al.* 2011), white muscle tissue is most commonly used in SIA-based contaminant studies and FAA- or SIA-based dietary studies (Anderson *et al.*, 2017) because of its relevance for human consumption, long-term assimilation of contaminants (e.g. Oliveira Ribiero *et al.* 1999), and extended dietary integration period (e.g. Hesslein et al. 1993; Pinnegar and Polunin, 1999). Due to increasing conservation and animal welfare concerns (e.g., Bennett *et al.*, 2016), the use of muscle tissue biopsies are routinely applied as a sampling method (e.g. Jardine *et al.*, 2011; Daly and Smale, 2013; Anderson *et al.*, 2017). Biopsies have several advantages over lethal samples, including facilitating the acquisition of a larger number of samples to generate more accurate statistical relationships (e.g. Baker *et al.*, 2004). Successive biopsies can also monitor individual levels of pollutants or dietary tracers over time, providing improved understanding of contaminant elimination rates (Walleghen *et al.*, 2014), and potentially the behavioral, environmental, and physiological mechanisms important for understanding organism movement. Finally, the application of biopsies may enable regulatory authorizations to permit sampling of populations and species where none were permitted before (Baker *et al.*, 2004).

The ultimate success of nonlethal methods, including biopsies, depends on ensuring high survival rates by minimizing the injury and mortality to sampled fish. Crucial, therefore, is an evaluation of the short- and long-term behavioral and physiological consequences of the treatment and ultimately the determination of how these may affect organism mortality and other fitness related traits. In that regard biopsies have a long history of assessment, with the bulk of studies on teleost fishes having concluded that there is generally low (<1%) mortality (Van Meter, 1995; Evans, 2008; Schielke and Post, 2010) and few, if any, long-term sublethal affects on fish (Tyus *et al.*, 1999; Smith *et al.*, 2018). Most studies, however, have been conducted under laboratory conditions (e.g. Crawford *et al.*, 1977; McAndrew, 1981; Tyus *et al.*, 1999) and are of short duration, i.e. hours (Henderson *et al.*, 2016), days (Mair, 1989) or a few weeks (Crawford *et al.*, 1977; Evans, 2008). Of the studies that have addressed the longer-term lethal and sublethal effects of teleost fish biopsies (e.g. Leitner and Isely, 1994; Tyus *et al.,* 1999; Ackerson *et al.*, 2014), few have investigated the procedural consequences under field conditions and under ecologically-relevant time-scales, such as feeding seasons, reproductive cycles and overwintering periods. Those that have (e.g. Baker *et al.*, 2004) only indirectly assessed mortality by determining the differences in recapture probability between biopsied and non-biopsied fish (e.g. northern pike *Esox lucius*) known to be resilient to capture and handling stress (Louison *et al.*, 2017). Given the noted variability of species' sensitivities to handling and capture, even among larger pelagic fishes (Mandelman and Skomal, 2009), there is a need for additional field-based assessments of the long-term consequences of biopsies, particularly for a species like Atlantic salmon, *Salmo salar*, known to exhibit environmental dependent sensitivities to handling (i.e. Kieffer *et al.*, 2002; Havn *et al.*, 2015).

The current study investigates the growth and return of post-spawned wild Atlantic salmon subject to a biopsy treatment prior to the initiation of the marine feeding season. Atlantic salmon is an anadromous salmonid subject to conservation concerns as many populations now are imperiled, largely due to reduced survival at sea (Parrish *et al.*, 1998; Chaput, 2012; Mills *et al.*, 2013). Knowledge gaps surrounding Atlantic salmon marine ecology and mortality sources have resulted in considerable research effort, including numerous studies that have applied SIA sampling (e.g. Dempson *et al.*, 2010; Kelly *et al.*, 2018) or telemetry methods (e.g. Hedger *et al.*, 2017; Strøm *et al.*, 2017; Lothian *et al.*, 2018) for improved understanding of marine feeding and movement ecology. Combining methods, knowing that biopsies held minimal consequences for long-term survival and growth would, therefore, enhance overall abilities to study wild Atlantic salmon. Therefore, the aims of this study were to determine: i) if the migratory return rate was lower in biopsied compared to non-biopsied Atlantic salmon kelts, and ii) if the growth of biopsied Atlantic salmon kelts that returned to spawn was lower compared to non-biopsied fish.

6.1.3 Methods

The study was conducted in a North American Atlantic salmon population from the Campbellton River, a medium sized river (drainage area: 296 km^2) located on the northeast coast of Newfoundland (Canada, 49.2º N, 54.9ºW). The river is dominated by small salmon (< 63 cm fork length, L_F) maturing after one winter at sea (1SW) (89-94% of total salmon returns), typically at 50-55 cm L_F (Downton and Reddin 2004). Salmon 63 cm L_F or larger are almost exclusively consecutive repeat spawners, which overwinter in freshwater as kelts and emigrate to sea in spring for an average feeding period of two months before reconditioned individuals

return to repeat spawn (Downton and Reddin 2004). The migration pattern of this life history contrasts to that of maiden 1SW and alternate repeat spawners which spend on average 14 months at sea (Klemetsen *et al*., 2003). A Department of Fisheries and Oceans (DFO) operated counting facility records on average 1500 (± 673 SD) downstream migrating post-spawned adults (kelts) annually (2002-2014), of which ca. 300 are tagged with external Floy T-bar anchor tags as part of a population monitoring program. This work has demonstrated that annual return rates of consecutive spawners to Campbellton River are variable, ranging from 9% to 40 % (Downton and Reddin, 2004).

Collection of Atlantic salmon tissue was obtained from kelts as they descended Campbellton River in spring 2016 during the seaward migration. Downstream migrating individuals were intercepted at the DFO operated fish counting facility located approximately 500 meters from the river mouth (Fig. 1). Kelts that underwent the biopsy treatment were moved from the trap to a sampling facility located c. 5 m from the counting facility and anaesthetized in an induction bath of clove oil. A blue external T-bar tag with information on fish identification and return reward in the event it was intercepted by the fishery was fitted to each fish before fork length (L_F) was measured. Three to five scales were then removed to expose a c. 0.5 x 0.5 cm area of skin anterior to the dorsal fin and above the lateral line before a 4 mm disposable biopsy punch (Milltex ®) was inserted, removing approximately 50 mg of dorsal muscle. Wounds were not disinfected as the use of topical antiseptics may disrupt the cutaneous mucus layer of the fish allowing easier penetration by pathogens (Wagner and Cooke, 2005). Following the biopsy procedure, post-sampling monitoring was completed for 30 min - 1 h to ensure complete recovery before individuals were released back into the river.

The control group consisted of downstream migrating fish intercepted at the counting facility, measured for fork length and fitted with a yellow external T-bar tag carrying fish identification and reward information. Being part of a DFO population assessment program, these fish were measured and tagged within the trap of the counting facility without anaesthesia to comply with the sampling protocol used in Campbellton River monitoring program. Tag identification and length were recorded and measured from kelts tagged in the monitoring program in previous years and intercepted at the counting facility during the 2016 downstream migration and were also included in the control group.

To account for potential carry-over effects from previous migration history (i.e. maiden versus repeat spawners) on kelt condition and return probabilities, only fish with known migration histories based on scale pattern analysis or mark-recapture history from the downstream migration in spring 2015 were included in analyses. Due to a low sample size of alternate repeat spawners, reflecting their overall low abundance in Campbellton River (Downton and Reddin, 2004), these were excluded from analysis. Potential biases due to the presence of kelts tagged prior to 2016 in the control group were investigated by comparing the growth and return rates to the control fish tagged in 2016.

Marine growth and return rates of the treatment and control groups were assessed by the size and number of externally tagged fish intercepted at the counting facility during the upstream migration in summer 2016. Because the recreational fishery in Campbellton River only occurs upstream of the counting facility (Downton et al. 2001), the proportion of fish intercepted during the upstream migration was considered an accurate representation of the marine return rate. Although this rate cannot directly be interpreted as survival because it is confounded by

repeat spawning strategy (consecutive vs. alternate spawning), the numerical dominance of consecutive compared to alternate spawners in the population suggests that the rate can be considered an approximation of it.

All upstream migrating salmon entering the trap were examined for an external tag via a video monitoring system (Panasonic, Color CCTV Camera WV-CP294, USA, Newark) installed to count all returning Atlantic salmon. When a tagged fish was identified, the fish was removed and measured for length (cm), mass (kg) and tag identification information was collected. Scales were also collected for the determination of spawning history and repeat spawning strategy following standardized international guidelines for Atlantic salmon (ICES, 1992).

6.1.3.1 Analyses

Data inspection and statistical analyses were performed in the statistical software R (version 3.4.4, www.r-project.org). In all analyses, model suitability was assessed by visual inspections of residuals for indications of violations of model assumptions, e.g. normality, variance homogeneity (Sokal and Rohlf, 1973).

The return rate of kelts to consecutive repeat spawning was calculated as the proportion of tagged fish in the control and treatment groups leaving the system in spring 2016 that returned during the upstream migration the same summer. The effect of the biopsy treatment on the return rate was assessed by testing the statistical significance of the variable in a generalized linear model that treated the return status of individual fish tagged during the 2016 downstream migration as a binomial response variable (e.g. returned, did not return) using a logit link function. As previous work has suggested an association between Atlantic salmon size

and repeat spawning rates (Jonsson *et al.*, 1991, 1997), we also investigated the potential effects of fish length and possible interactions with treatment on the return probability of kelts by including the continuous variable fork length ($L_{F,}$ cm) at release. Migration timing may also be correlated to migration success in Atlantic salmon (Birnie-Gauvin *et al.*, 2019), and to account for potential migration timing effects we included the day of migration (DOM) as a covariate. To investigate possible effects of prior spawning history on return probabilities of kelts, the factorial term for migration history (MH) distinguishing between first-time and consecutively spawned kelts was included. To reduce model complexity and collinearity arising from the correlation of kelt length and spawning history, the two terms were tested separately. The full statistical models considered were:

$$Y_{ijk} = \text{Treatment}_{i} + L_{Fi} + DOM_i + \text{Treatment}_{ijk} \times L_{Fi} + \text{Treatment}_{ijk} \times DOM_i + L_{Fi} \times DOM_i$$

and

$$Y_{ijk} = \text{Treatment}_{i} + MH_{ijk} + DOM_i + \text{Treatment}_{ijk} \times MH_{ijk\,i} + \text{Treatment}_{ijk} \times DOM_i + MH_{ijk} \times DOM_i$$

where Y_{ijk} is the return status of individual fish *i* exposed to treatment *j* (control, biopsy) of spawning history *k* (first-time spawned, consecutively spawned), L_{Fi} is the fork length of fish *i* at release, and DOM_i is the day of migration of fish *i* (the day that an individual kelt was intercepted at the counting facility). A step by step approach of model simplification from the full model using backward elimination was followed, with the Akaike information criterion (AIC)

and Akaike weights (w_i) used to select the "best" model (Burnham *et al.*, 2002; Anderson, 2008) and the significance of estimators tested with likelihood ratio tests (LRT) (Sokal and Rohlf, 1995). The w_i term can be interpreted as the probability that model *i* is the best approximating model for the data within the candidate set of models considered (Anderson 2008).

Growth was assessed as the difference between final (recapture) and initial (release) length ($L_{f,}$ cm) and compared between the biopsied and control groups using a general linear model. Because growth in fish depends in part on the size of the fish (Eberhardt and Ricker, 1977), and may be influenced by time spent at sea (Jonsson and Jonsson, 2014), fish length and at sea residency were included as covariates. The interactions considered were Treatment x ln(FL), Treatment x Residency, and ln(FL) x Residency and the full model was as follows:

$$Ln(y_{ijk}) = Treatment_{ijk} + ln(L_{Fi}) + Residency_i + Treatment_{ijk} x\ ln(L_{Fi} + Treatment_{ijk} x\ Residency_i + ln(L_{Fi}) \times Residency_i + e_i$$

where y_{ijk} is the growth in cm of individual fish *i* treatment$_j$, L_{Fi} is as defined above and $Residency_i$ is the number of days between the capture and release on the downstream migration and the recapture during the upstream migration. e_i represents the normally distributed error term. As the ratio n/k did not exceed 40 (Burnham *et al.*, 2002), model simplication from the full model was followed using the Akaike information criterion for small sample sizes (AICc) and Akaike weights, with the significance of estimators tested with F-tests.

6.1.4 Results

In total, 78 biopsied (mean ± SD $L_{F:}$ 58.7 ± 6.1 cm, range: 48.0 – 73.0 cm) and 156 control kelts ($L_{F:}$ 57.4 ± 6.6 cm, range: 40.6 to 78.8 cm) with known migration history were intercepted

between May 8th and June 17th during the downstream migration in spring 2016. Scale pattern analysis and mark-recapture history determined that 51% and 60% of the individuals consisted of first timed spawned kelts in the treatment and control groups, respectively, the rest being consecutive repeat spawners (Table 6.1). No significant difference in size was found between the two groups ($F_{1,232}$ = 2.1, p = 0.15). No mortalities were recorded in the treatment or control group during downstream tagging and all fish were deemed sufficiently recovered prior to release.

Kelt returns

In total, 30 externally tagged kelts from the treatment (n=10, 12.8 %) and control (n=20, 12.8%) groups returned to the DFO counting facility between 5 July and 30 August 2016.

Among the models fit to return status of biopsied and control kelts, the AIC criterion provided most support for an interaction between migration history (MH) and day of migration (DOM; Table 6.2), predicting a positive effect of DOM on return probability in consecutive spawners and a negative effect in first-time spawners (Figure 6.3). Although the AIC criterion did not distinguish between this model and a model also containing the treatment term, the Akaike information weights (w_i) indicated there was a less than 14 % chance of the model including treatment being correct (Table 6.2). Furthermore, the treatment effect as estimated was not significant (Table 6.3).

A comparison of return rates between control kelts tagged prior to, or during, spring 2016 revealed a significantly higher return probability in the former group (binomial model, χ^2 = 11.56, p < 0.001). When the analysis was limited to consecutive spawners, no difference was

found (binomial model, $\chi^2 = 2.01$, $p = 0.16$). The lack of difference in return rates in consecutive spawners suggested that the significant difference in the full dataset was due to the fact that fish tagged prior to 2016 consisted exclusively of consecutive spawners with a higher proportion of first-time spawners among those tagged in 2016.

Kelt growth upon return as repeat spawners

Kelts grew on average 3.13 cm (± 0.94 SD) during the period of marine residency which on average lasted 65 days (± 19.2 SD), and this corresponded to a 5.53 % increase in length (± 2.13 SD). The AICc criterion for log transformed growth did not distinguish between the four best ranked models, of which one included the treatment term (Table 6.4). The treatment term was, however, not statistically significant and there was a 13 % or less chance the model was correct based on w_i, compared to the highest ranked model with a w_i of 32 % (Table 6.4 & Table 6.5). The best supported AICc model for log transformed growth depended on the interaction between fish fork length at release and the duration of the marine residency (Table 6.4 & Table 6.5), with steeper declines in growth observed as a function of larger size as the duration of the sea residency increased (Fig. 6.3). Marine residency did not differ between biopsied and control kelts ($F_{1,28} = 0.86$, $p = 0.36$). No difference in growth was found between kelts tagged prior to, or during, spring 2016 ($F_{1,18} = 1.07$, $p = 0.31$).

6.1.5 Discussion

Data obtained from migrating kelts in the Campbellton River showed no effect of biopsies on marine return rates as measured by the number of individuals tagged and re-captured at a counting fence operated in the lower reaches of the river. Return rate, however, did appear to

depend on the interaction between migration history (MH) and day of migration (DOM), with there being higher return probabilities for consecutive spawners than first-time spawners, except during the early portion of the migration period. Although the return rates measured in the current study are a product of both survival and repeat spawning strategy, the low numbers of alternate repeat spawners found in Campbellton River in general suggests that return rates to annual spawning closely approximate survival. Although growth did depend on the length of the fish and the time it spent in the marine environment, no growth-related effects were observed as a function of the biopsy treatment. Combined, the results indicate that biopsies lead to no measurable long-term effects on key fitness parameters such as survival and growth and that use of biopsies in studies will not bias data in any meaningful way.

Consistent with shorter term survival studies on a wide variety of species, survivorship (as measured by return rates) associated with biopsies were high, with values of >99% not being uncommon for other teleost species (Schielke and Post, 2010) even when fish were subjected to multiple biopsy events (Tyus *et al.*, 1999). While the data suggest the acute responses of biopsied fish are minimal, rates do vary among species and may be affected by environmental and other factors. For example, Mair (1989) noted that the technique may be size-limited, being safe only for fish of a standard length greater than 120 mm. Size clearly favours biopsy use, with the practice being especially pronounced in elasmobranch studies (e.g. Heupel and Simpfendorfer, 2010; Hammerschlag and Sulikowski, 2011) where long-term effects are known to be minimal and short-term injury or haemorrhaging issues have largely been addressed (Meyer *et al.*, 2018). Warmer waters may also pose issues for biopsy use, with reported

mortalities for tested coral reef species (*Plectropomus maculatus* and *Lutjanus carponotatus)* as high as 3% (Evans, 2008), possibly as a result of the higher likelihood of infection when biopsy procedures are used in more pathogen rich environments (Jardine *et al.*, 2011). The degree of invasiveness also appears to correlate with mortality, with muscle only biopsies having much lower mortality than combined muscle and liver biopsies where survival was reduced to 90% after 11 weeks (Leitner and Isely, 1994). With suitable care and handling of fish during the biopsy procedure, results reported here and elsewhere in the literature indicate the use of biopsies have minimal to no mortality implications for larger, cold water fish.

Growth has similarly been assessed for multiple species with results generally pointing to no, or minimal, impact. Six week follow-up studies of biopsied smallmouth bass (*Micropterus dolomieu*) showed slightly negative but statistically non-significant effects on growth (Ackerson *et al.*, 2014), whereas long-term studies with rainbow trout (*Oncorhynchus mykiss*), razorback sucker (*Xyrauchen texanus*) and bonytail chub (*Gila elegans*) showed no differences in condition or growth between biopsied and non-biopsied fish held in a hatchery facility (Tyus *et al.*, 1999). The number of studies reporting a lack of short- and long-term responses in growth is encouraging as growth is typically more responsive to long-term chronic stresses and, as a consequence, has typically been used in assessing the chronic effects of stress regimes on fish (e.g., Adams 2002). Those studies having investigated long-term growth effects of biopsied fish, however, have done so on fish held in captivity (Tyus *et al.*, 1999), and as a consequence, have not accounted for potential synergistic effects of natural stressors such as predation, harvesting and food availability. Thus the first reported absence of a long-term effect for growth in the

wild, as found here, provides stronger evidence for the minimal effects of biopsies in field studies.

Although previous work on short-term influences of biopsy treatment hints at differences in responses among species (e.g., Tyus *et al.*, 1999; Henderson *et al.*, 2016), there is no direct account of this in the literature. As such, there is a need for more research on the phylogenetic factors that may contribute to variability in responses among species non-lethally biopsied for muscle or other tissue and on how environmental factors such as temperature may affect healing and the likelihood of infection. Before combining telemetry and biopsy methods on biopsy-tolerant species, direct testing of possible interaction effects should also be performed, particularly as the few studies that have conducted multiple muscle tissue or multi-tissue biopsies suggest contradictory effects. For example, Leitner and Islaye (1994) report an increase in mortalities whereas other multiple tissue biopsy (Tyus *et al.*, 1999) and repeat biopsy studies (Hamilton *et al.*, 2002) report minimal interaction or repeat sampling effects. Further, given the paucity of assessments of long-term effects, trials focusing on growth and survival endpoints need to be completed. Of the biopsy studies summarized in Ackerson et al. (2014) less than half (42%) commented on survival over any time period and only 14% reported on growth-related effects. In the current study, the lack of measurable responses to a biopsy treatment over a fitness-relevant time scale (i.e. a feeding season) suggests that combining methods is feasible for post-spawned Atlantic salmon migrating to sea. This is promising considering that combining methods could enhance our overall understanding of Atlantic salmon marine ecology, including relationships between movement and feeding patterns and their

consequences for fitness in terms of body condition and growth. The latter is particularly relevant given the established linkage between climate and ecosystem processes which is believed to affect the abundance and productivity of Atlantic salmon populations (Mills *et al.*, 2013).

6.1.6 Tables

Table 6.1: Summary information regarding numbers of sampled Atlantic salmon kelts in the treatment and control groups (numbers of returning fish in brackets), fork length (L_F), Julian day of migration (DOM) and marine residency time (days).

Treatment	First time spawners	Consecutive spawners	Total	L_F (mean ± SD)		L_F range	DOM (mean ± SD)		DOM range	Marine residency (mean +SD)		Residency range
Biopsy	47 (3)	31 (7)	78 (10)	58.7	6.1	48.0 - 73.0	138.1	2.5	133 - 141	69.7	21.0	50 -107
Control	80 (3)	76 (17)	156 (20)	57.4	6.6	40.6 - 78.8	143.1	9.0	129 - 169	62.7	18.4	41 - 102

Table 6.2: AIC model selection table containing the ten best ranked binomial models fit to explain Atlantic salmon kelt return probability to Campbellton River. MH= migration history, DOM=Julian day of migration, Treatment= biopsy or control.

Model	df	logLik	AIC	dAIC	weight
y ~ MH + DOM + MH x DOM	4	-81.5	171.0	0.0	0.370
y ~ Treatment + MH + DOM + MH x DOM	5	-81.5	172.9	-2.0	0.139
y ~ MH	2	-84.7	173.4	-2.4	0.114
y ~ MH + DOM + Treatment + DOM x Treatment + MH x DOM	6	-81.0	174.1	-3.1	0.079
y ~ MH + DOM + Treatment + MH x DOM + MH x Treatment	6	-81.1	174.2	-3.2	0.073
y ~ MH + Treatment	3	-84.6	175.3	-4.3	0.043
y ~ MH + Treatment + MH x Treatment	4	-83.7	175.3	-4.3	0.043
y ~ MH + DOM	3	-84.7	175.3	-4.3	0.042
y ~ MH + DOM + Treatment + MH x Treatment + DOM x Treatment	7	-81.0	175.9	-4.9	0.031
y ~ MH + DOM x Treatment	5	-83.6	177.3	-6.3	0.016

Table 6.3: Parameter estimates (logit scale) and likelihood ratio test statistics for the two most supported models fitted for explaining return probabilities of tagged Atlantic salmon kelts to Campbellton River. MH= migration history, DOM=Julian day of migration, Treatment= biopsy or control.

	Parameter estimates			Likelihood ratio test statistics			
Model	Term	Coefficient	SE	Effect	df	X^2	p
y ~ MH + DOM + MH x DOM	Intercept	-5.89	3.84	DOM	1	0.08	0.77
	DOM	0.03	0.03	MH	1	9.81	0.00
	MH	28.03	13.84	DOM x MH	1	6.34	0.01
	DOM x MH	-0.21	0.10				
Y ~ Treatment + MH + DOM + MH x DOM	Intercept	-5.72	3.90	Treatment	1	0.00	1.00
	Treatment	0.09	0.44	DOM	1	0.09	0.76
	DOM	0.03	0.03	MH	1	9.85	0.00
	MH	27.75	13.75	DOM x MH	1	6.33	0.01
	DOM x MH	-0.21	0.10				

Table 6.4: AICc model selection table containing the ten best ranked models fit to explain Campbellton River Atlantic salmon kelt growth (cm) during the marine migration period where Treatment= biopsy or control and residency defines length or the marine residency period.

Model	df	logLik	AICc	dAICc
$\ln(y) \sim \ln(L_F)$ + Residency + $\ln(L_F)$ x Residency	5	1.1	10.4	0.0
$\ln(y) \sim \ln(L_F)$	3	-2.2	11.3	1.0
$\ln(y) \sim \ln(L_F)$ + Residency	4	-1.3	12.1	1.7
$\ln(y)$ ~ Treatment + $\ln(L_F)$ + Residency + $\ln(L_F)$ x Residency	6	1.7	12.2	1.8
$\ln(y) \sim \ln(L_F)$ + Treatment	4	-1.7	13.0	2.6
$\ln(y) \sim \ln(L_F)$ + Residency + Treatment + $\ln(L_F)$ x Residency + Residency x Treatment	7	2.1	14.9	4.5
$\ln(y) \sim \ln(L_F)$ + Treatment + $\ln(L_F)$ + $\ln(L_F)$ x Treatment	5	-1.4	15.4	5.0
$\ln(y) \sim \ln(L_F)$ + Residency + Treatment + $\ln(L_F)$ x Residency + $\ln(L_F)$ x Treatment	7	1.8	15.6	5.2
$\ln(y)$ ~ Residency + $\ln(L_F)$ + Treatment + $\ln(L_F)$ x Treatment	6	-0.1	15.9	5.5
$\ln(y) \sim \ln(L_F)$ + Residency + Treatment + Residency x Treatment	6	-0.3	16.3	5.9

Table 6.5: Parameter estimates and F test statistics for the two most supported models fitted to marine growth (cm) of Atlantic salmon kelts that returned to Campbellton River where Treatment= biopsy or control and residency defines length or the marine residency period.

	Parameter estimates			F - test			
Model	Term	Coefficient	SE	Effect	df	F	p
$\ln(y) \sim \ln(L_F) +$ residency + $\ln(L_F)$ x residency	Intercept	-5.32	6.25	$\ln(L_F)$	1	14.59	0.00
	$\ln(L_F)$	1.53	1.54	Residency	1	2.00	0.17
	Residency	0.22	0.10	$\ln(L_F)$ x Residency	1	4.33	0.05
	$\ln(L_F)$ x Residency	-0.05	0.03				
$\ln(y) \sim \ln(L_F)$	Intercept	7.47	1.79	$\ln(L_F)$	1	12.64	0.00
	$\ln(L_F)$	-1.57	0.44				
$\ln(y) \sim \ln(L_F) +$ residency	Intercept			$\ln(L_F)$	1		
		7.21	1.78			12.99	0.00
	$\ln(L_F)$	-1.56	0.44	residency	1	1.78	0.19
	residency	$3.43e^{-3}$	$2.57e^{-3}$				
$\ln(y) \sim$ Treatment + $\ln(L_F)$ + residency + $\ln(L_F)$ x residency	Intercept (biopsy)			Treatment (control)	1	4.41	0.05
		-5.18	6.24				
	Treatment (control)	0.11	0.10	$\ln(L_F)$	1	11.32	0.00
	$\ln(L_F)$	1.47	1.54	Residency	1	2.68	0.11
	Residency	0.20	0.10	$\ln(L_F)$ x Residency	1	3.76	0.06
	$\ln(L_F)$ x Residency	-0.05	0.03				

6.1.7 Figures

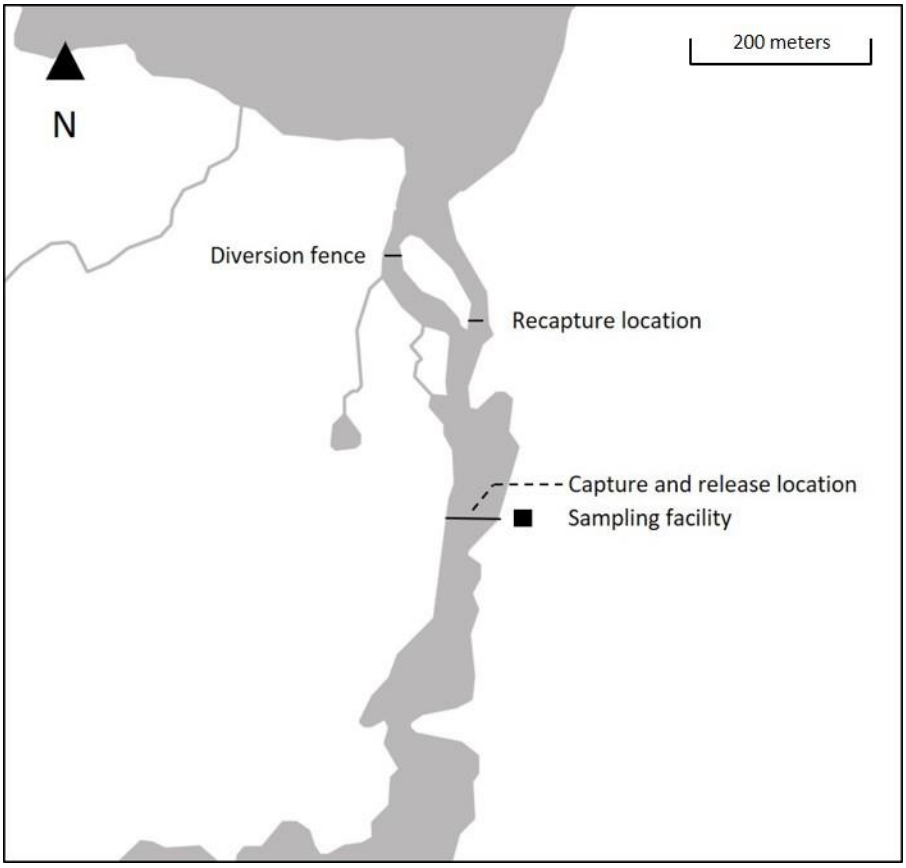

Figure 6.1: Map of the Campbellton River study site with location of capture and release, sampling, and recapture of Atlantic salmon kelts.

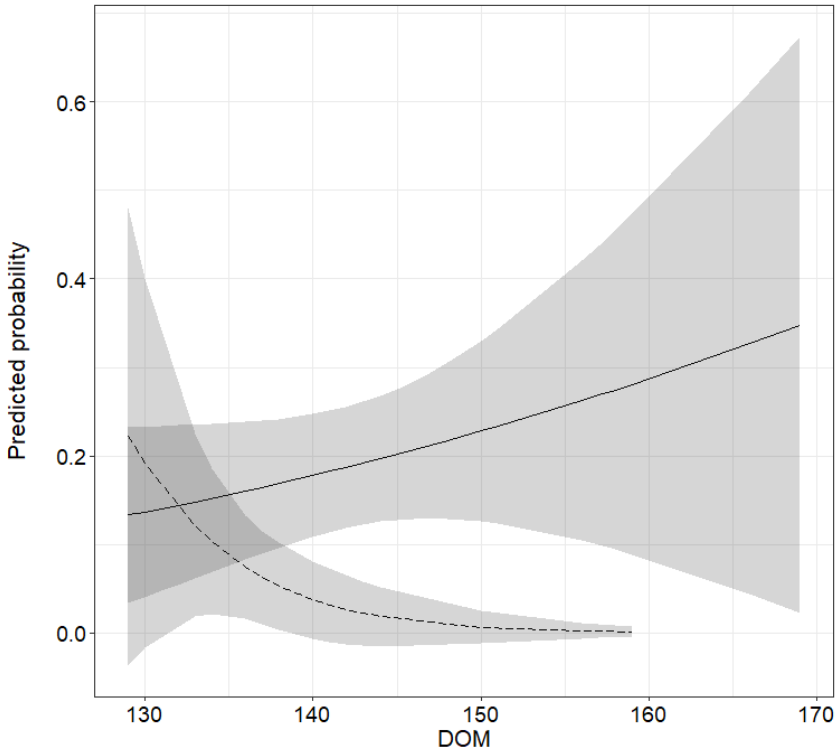

Figure 6.2: Predicted probability of an Atlantic salmon kelt returning as a consecutive spawner to Campbellton River in the summer of 2016 plotted as a function of day of migration (DOM) and migration history (solid line: consecutive spawners, dashed line: first time spawners). Shaded area represents the 95 % prediction confidence intervals.

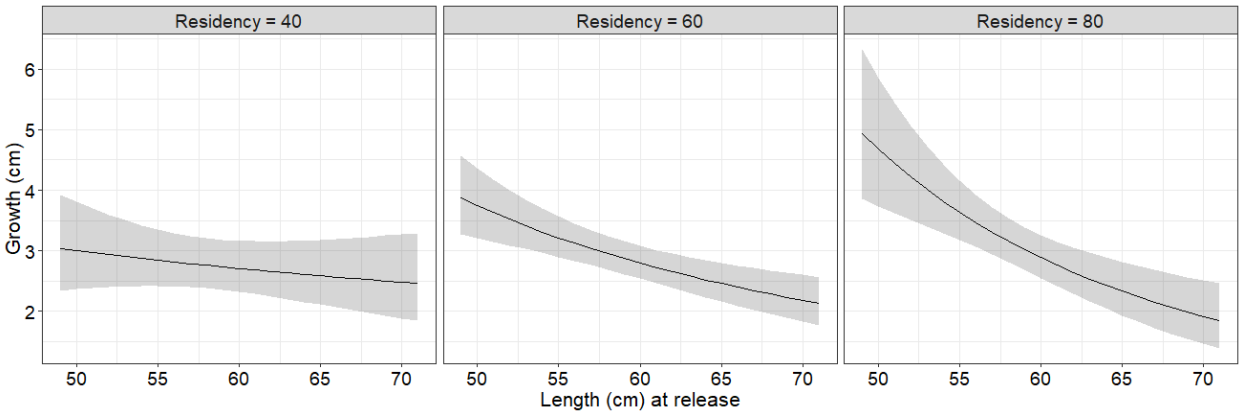

Figure 6.3: Predicted growth (length, cm) of Atlantic salmon kelts that return to Campbellton River plotted as a function of fork length at release and marine residency (days). Shaded area represents the 95 % prediction confidence intervals.

6.1.8 References

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