Regional Assessment of Atlantic Salmon (*Salmo salar*) Smolt Resource Use and Body Size in Eastern Canada

By

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BSc Honours in Biology, University of New Brunswick, 2022

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

Master of Science

In the Graduate Academic Unit of Biology

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This thesis is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

December 2024

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ABSTRACT

Atlantic salmon (*Salmo salar*) are ecologically, economically, and culturally significant, particularly for many Indigenous Peoples, providing sustenance and holding spiritual, ceremonial, and relational importance. They play a vital role in linking freshwater and marine ecosystems through nutrient transport and trophic interactions. Populations across Eastern Canada are at risk, with many designated as endangered, threatened, or of special concern. This thesis examines resource use and body size relationships of smolts from multiple Eastern Canadian rivers, using archival samples (2000-2016) and new accidental mortalities (2022-2023) collected collaboratively. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes were analyzed to assess resource use prior to migration, across sites, and as a predictor of smolt body size. Tissue analyses revealed individual dietary specialization, with a decrease in $\delta^{15}N$ from long to short-term diets. Resource use was generally consistent across rivers within the same assigned population and was found to have a weak influence on body size.

ACKNOWLEDGEMENTS

I would like to begin by thanking my co-supervisors Dr. Charles Sacobie and Dr. Carole-Anne Gillis. To Charles, thank you for your unwavering support and guidance throughout my master's and honours. I deeply appreciate the opportunities you have provided me and the invaluable experience I have gained as part of your research lab. Your encouragement and reassurance throughout my project have meant so much to me. To Carole-Anne, thank you for your guidance and the practical experience you have shared with me. The hands-on learning opportunities and field trips helped me develop my skills and grow my passion for ecology. As a woman in STEM, you have been a tremendous inspiration to me, and I deeply value the knowledge I have gained through your mentorship.

I would like to thank Dr. Mike Power and Dr. Brian Dempson for providing an extensive dataset and for their guidance throughout my thesis. I would also like to thank Gespe'gewa'gi Institute of Natural Understanding, Miramichi Salmon Association, Nova Scotia Salmon Association, Department of Fisheries and Oceans Canada Newfoundland and Labrador, and Gulf region (DFO), the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (MELCCFP) Quebec for their responsiveness, for collecting smolt, and for contributing samples to my project. Additionally, I thank the Atlantic Salmon Research Joint Venture for helping with communication throughout this collaborative effort. This project would not have been possible without the continued support and collaboration of everyone involved.

I would also like to thank my supervisory committee member, Dr. Kurt Samways, for his support and for providing access to his lipid meter, as well as Dr.

Tillmann Benfey and Dr. Cindy Breau for allowing me to use their lab equipment. My gratitude extends to the technical staff of the Stable Isotopes in Nature Laboratory at UNB Fredericton for analyzing the stable isotope samples. I would also like to thank my funding agencies for this thesis, Mitacs and the New Brunswick Wildlife Trust fund. Additionally, I sincerely appreciate and thank my examining committee for their time and effort in reviewing this thesis.

I would like to thank the members of the Sahkupi Mawi lab, Nicole, Chandler, Hannah, Tanya, Emma, and Katie. Thank you for helping me navigate grad school, joining me for field trips, and assisting with data collection, sampling, and presentation practice. A special thank you to Hannah for helping me create and edit maps for my thesis. To the staff at the Grad House, thank you for listening and sharing stories about our projects. I am also grateful to my best friends Caroline, Alex, Hanna, Eric and Ryan. Thank you for being there for me when I needed help with sampling and presentations, and for listening to the details of my project.

Finally, I want to acknowledge my family: my sisters, Brett and Caitlin, who inspired me to pursue my master's. Thank you for passing down your love of science to me, and for supporting me throughout this journey. To my parents, Jeff and Cheryl, thank you for your love and encouragement throughout my years in school. I am truly grateful for all of the support I received during my master's, including your help with sampling invertebrates in the river!

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CHAPTER 1: INTRODUCTION

1.0 ATLANTIC SALMON (*Salmo salar*)

Life cycle

Atlantic salmon inhabit both freshwater and marine environments during their lifecycle and play a critical role in facilitating nutrient exchanges and trophic interactions between the two habitats. They spend their larval and juvenile life stages as embryos, alevin, fry, parr and smolt assimilating nutrients and growing in freshwater systems such as rivers and lakes. Parr undergo physiological changes and a downstream migration at age 2-5 years after which they transition to a marine habitat (Aas *et al.,* 2010). This transition process is referred to as smoltification or the smolt stage; a critical stage where the salmon must go through morphological, biochemical, and physiological changes to tolerate new habitats, diets, and stressors (Webb *et al*., 2007). Atlantic salmon spend their adult years in the Atlantic Ocean and mature in the marine environment, once sexually mature they migrate back to their natal rivers to spawn. An exception of this life cycle is seen in landlocked populations completing their entire life cycle in freshwater and in precocious parr that mature before migrating to the ocean. (Aas *et al*., 2010).

Habitat

Atlantic salmon are native to Eastern North America, Iceland, Greenland, and Northern European countries. The habitat use of juvenile Atlantic salmon is widely variable (Armstrong and Nislow, 2006) with young salmon preferring temperate to subarctic rivers with temperatures ranging from 7-22.5°C, that are well oxygenated, fast flowing and have variable habitats such as riffles and pools (DeGraaf and Bain, 1986;

Fry, 1971; Gibson, 1993). Parr typically inhabit water with large cobble or gravel substrate, low to moderate gradients, and low turbidity to facilitate an opportunistic feeding style (Fry, 1971; Gibson, 1993). There is an abundance of information on the habitats preferred by early juveniles; however, little is known about the specific habitat or feeding preferences of the smolt phase other than their habitat and feeding style typically depends on predator avoidance (Thorstad *et al*., 2012; Bardonnet and Baglinière, 2000).

Migration

The smoltification process occurs in the spring during the months of May and June, and the transition process takes the smolt six to ten weeks to complete (Webb *et al*., 2007; Thorstad *et al*., 2011; Harvey *et al*., 2020). Parr begin to smoltify once they become 10-15cm in size and specific environmental cues arise, such as the warmer temperatures during the spring (GoC, 2018; McCormick, 2012). Some morphological changes during the parr to smolt phase include an elongation of body shape and changes to the colouring, while physiological changes include a decrease in total lipids levels and redistribution of lipids in preparation for marine waters (Aas *et al*., 2010; Wedemeyer *et al*., 1980; McCormick and Saunders, 1987). Migration timing and multiple environmental factors such temperature, flow and the geomorphological conditions also play a role in the movement and survival during the migration (Thorstad *et al.,* 2012; Smith *et al*., 2002; Hinch *et al*., 2005).

Smolt feeding

Much of the energy required for the downstream migration is derived from the smolts' energy reserves and growth status (Thorstad *et al.,* 2012). While juvenile Atlantic salmon are generalist opportunistic feeders with a selection towards benthic macroinvertebrates, they consume prey based on availability and abundance in their habitats (Dixon *et al*., 2012; Thonney and Gibson, 1989; Johansen *et al*., 2011). A reduced food consumption is recognized in smolt (McCormick and Saunders, 1987; Stefansson *et al*., 2003), which could be related to predator avoidance during migration; however, smolts have been observed to feed in pools during the migration period (Thorstad *et al*., 2012; Aas *et al*., 2010). The effects of feeding during the out-migration and the survival of smolt have not been well studied, and more recent studies suggest smolts are exhibiting selective active feeding similar to their parr stage (Larsson *et al.,* 2011).

Smolt size at sea entry has been related to survival at sea with larger smolt having higher marine return rates compared to smaller smolts (Gregory *et al.,* 2019). Growth and size of smolt are influenced by multiple factors including resource use, environmental rearing conditions as well as genetics (Simmons *et al.,* 2022; Armstrong *et al.,* 2018; Withler *et al.,* 1987). Larger body size in fish is typically advantageous for smolt for predator avoidance, having more energy, and higher resilience in marine environments (Gregory *et al.*, 2019; Malerba *et al.,* 2018). Therefore, larger out-migrating smolt may help increase survival at sea and marine return rates.

Stable isotope analysis

Stable isotope analysis (SIA) is a tool used in trophic ecology to determine nutrient assimilation, trophic interactions, and dietary selections over a temporal period

(Peterson and Fry, 1987). Carbon (^{13}C) is often used in SIA to determine prey selection and energy pathway usage; it carries a low trophic fractionation between predators and prey and enables inference of prey items species are feeding on based on similarities (Post, 2002). Nitrogen (^{15}N) is typically paired with carbon and allows us to determine the trophic position of a species and carries a higher trophic fractionation of ~3.4‰ (Post, 2002). By examining the carbon and nitrogen content in consumers tissues we can determine ecological information on feeding habits, trophic position, and resource niche width (Peterson and Fry, 1987).

Tissues of consumers have different metabolic turnover rates (i.e., the rate at which stable isotope signatures are replaced) and through SIA one can examine the assimilated nutrients over a tissues turnover time period (Peterson and Fry, 1987). Fish tissues typically have a metabolic turnover rate ranging from a few days to months; juveniles, however, typically exhibit faster turnover rates as they are rapidly growing (Hesslein *et al.,* 1993). For example, fish muscle samples typically show a consumer's diet from around a 2–5-month period prior to sampling (Church *et al.,* 2009). Therefore, muscle tissues sampled during the Atlantic salmon smolt phase can provide a long-term perspective on the prey assimilated prior to and during the smolt phase. Tissues with slower metabolic turnovers such as the muscle and fin will give a longer snapshot into a species' diet whereas tissues with faster turnover rates such as the liver will give us a shorter timeframe (Church *et al*., 2009; Dvergedal *et al*., 2019).

Previous studies examining stable isotope variation among salmon have been conducted but typically focus on the adult life stage (i.e. Johnson and Schindler, 2012; Statterfield and Finney, 2002) or the shift from freshwater to saltwater which focuses on

the post-smolt phase (i.e. Hertz *et al.,* 2015; Dixon *et al*., 2012; Sinnatamby *et al*., 2009). These studies have found little variation when examining ^{13}C and ^{15}N ratios among smolts and over time; however, an expected trophic shift was observed while comparing the smolt to the one sea-winter (1SW) salmon (Dixon *et al*., 2012; Sinnatamby *et al*., 2009). With few studies conducted solely on the smolt phases prior to entering marine environments, this study focused on the smolt phase and comparing their stable isotope compositions prior to migration, regionally, as well as throughout the years.

1.1 SIGNIFICANCE OF STUDY

Atlantic salmon populations have been in decline for at least 30 years, and face many threats including freshwater habitat degradation, invasive species, and marine mortalities (COSEWIC, 2010). Freshwater habitat degradation has been one of the leading causes for decreased productivity and suitable conditions causing fewer smolts out-migrating each year. The growth of juvenile salmon can be hindered due to reduced habitat availability and stress caused by poor freshwater habitat conditions such as low pH and warmer waters (Netboy, 1968; Finstad *et al.,* 2011). It has been observed that the number of parr that smoltify in a given year is related to the number of eggs deposited during spawning, the parr's growth rate, as well as the physical characteristics of rearing habitats in lakes and rivers (Cunjak and Therrien, 1998; Thorstad *et al.,* 2012; Shearer, 1992). This suggests that a potential way to increase Atlantic salmon smolt populations is to focus management efforts on the health and productivity of their freshwater ecosystem (Hertz *et al.,* 2015).

Atlantic salmon are a significant species at least 49 Indigenous communities in Eastern Canada (i.e. Nova Scotia, New Brunswick, Prince Edward Island, Newfoundland and Labrador, Quebec, and Ontario) and are part of traditional diets since time immemorial and are a symbolic species in social and cultural ceremonies (Noble *et al.,* 2016). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has designated 16 populations of Atlantic salmon in Eastern Canada based on geographic boundaries with assigned conservation statuses (COSEWIC, 2015). Atlantic salmon are listed as a species of special concern, threatened or endangered 10 populations in Eastern Canada (COSEWIC, 2010). More recently, 19 designated units (DU) for Atlantic salmon have been identified through incorporating genetic and genomic data (Lehnert *et al.,* 2023; Bradbury *et al.,* 2021). In this thesis, the term "population" will refer to the COSEWIC designated populations, while "groups" will denote the newer genetically assigned designated unit populations. Across the 19 salmon groups, there are over 1000 rivers where salmon are present across Canada (Lehnert *et al.,* 2023; GoC, 2021). Studying this widespread species will allow us to gain information to help monitor and protect regionally different populations.

The inclusion of Indigenous methodologies into ecological studies encourages gaining as much information as possible from the death of animals. Conducting studies where non-invasive techniques can be used is vital for gaining information on species of concern without having to harm them (Grunow and Strauch, 2023). However, non-invasive techniques are not always available and occasionally during ecological sampling, accidental mortalities do occur. Moreover, some ecological studies that do sacrifice a subset of species may not use the entire organism for their study.

Opportunities to study and gain knowledge about a species of special concern using available data to provide information on a critical life stage of Atlantic salmon from various regions across Canada. This information can then be shared, discussed, and used to inform the development of co-management practices.

1.2 AIM OF RESEARCH

The goal of this study was to gain ecological information from a species at risk that had been euthanized for other studies. This project was a collaborative effort to obtain new information about Atlantic salmon smolts in Eastern Canada. Through collaboration, I obtained archived and new smolt samples from various regions in Eastern Canada and created a database to analyze their size and stable isotope composition. Examining trends over time, as well as between rivers, allowed me to examine if resource use, quantified by stable isotopes (i.e. energy source and trophic level), influenced smolt size prior to their entry into the sea.

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CHAPTER 2: ATLANTIC SALMON (*SALMO SALAR*) SMOLT BODY CONDITION ASSESSMENT OF THREE EASTERN CANADIAN RIVERS

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Funding: Mitacs, New Brunswick Wildlife Trust Fund

Contributions

Conceived and designed the project; MP, BD, EM, CG, CS. Performed sample preparation and provided data; MP, BD. Analysed data; EM. Helped with statistical analysis, MP BD. Wrote the paper EM. Commented, edited, and contributed to the text CG MP BD CS.

2.0 ABSTRACT

Atlantic salmon (*Salmo salar*) have been experiencing population declines in Eastern Canada for the past 30 years, primarily attributed to at-sea mortality. To identify factors contributing to smolt body size, which has been associated with variations in survival, we examined multiple predictors with multiple linear regressions with data from out-migrating smolt collected from 2000 to 2016 at three rivers: Conne River (NL), Trinité River (QC), and St. Jean River (QC). The predictors included river, sex, age, year, and resource use (i.e., $\delta^{15}N$ and $\delta^{13}C$). Our analysis revealed that resource use was a significant predictor body size models, with full models explaining 66.5% of the variation in fork length and 19.1% of variation for condition factor, with river being the strongest predictor. Furthermore, we observed no significant increasing or decreasing trends in body size across the 16-year period for any of the rivers studied. Notably, rivers located at higher latitudes in the Boreal Shield ecoregion (Conne and Trinité rivers) did exhibit the largest smolt sizes. This study identifies key predictors of increased smolt body size using a comprehensive long-term dataset, providing valuable insights into the dynamics of three major salmon rivers in Eastern Canada and indicates stable trends in smolt body size over time.

2.1 INTRODUCTION

Atlantic salmon (*Salmo salar*) inhabit freshwater and marine environments during their lifecycle and play a key role in facilitating nutrient exchanges and trophic interactions between the two habitats (Jonsson and Jonsson, 2003). They spend their juvenile years in freshwater environments, then undergo a downstream migration and transition to a marine habitat (Aas *et al*., 2010). The transition process is called

smoltification and is a critical life phase where the salmon must undergo physiological changes to tolerate saltwater habitats (Webb *et al*., 2007). Some of these changes include an elongated body shape and a redistribution of lipids (Wedemeyer *et al*., 1980; McCormick and Saunders, 1987) and all facilitate better survival at sea by increasing swimming and predation avoidance abilities (McCormick, 2012).

Many Atlantic salmon populations in Eastern Canada have been in decline at least 30 years and face multiple threats some of which include freshwater habitat degradation, invasive species, predation, marine mortalities, aquaculture and climate change (Armstrong *et al.,* 2018; Dadswell *et al.,* 2022; Dempson *et al.,* 2024). Marine return rates depend on the survival of out-migrating smolts which in turn depends critically on the condition and energy status of the smolt at migration (Simmons *et al.,* 2022; Thorstad *et al.,* 2012; Chaput, 2012). The growth of juvenile salmon may be hindered by habitat availability and the stresses caused by poor freshwater habitat conditions (Finstad *et al.,* 2011). In addition, increased at-sea mortalities have been a significant cause of lower salmon populations (Dadswell *et al.,* 2022; Simmons *et al.,* 2022; Armstrong *et al.,* 2018; Friedland, 1998).

Factors that contribute to the freshwater growth of Atlantic salmon include resource use (i.e., food availability and prey diversity), environmental rearing conditions as well as individual genetics (Simmons *et al.,* 2022; Armstrong *et al.,* 2018; Withler *et al.,* 1987). Thus, fork length and condition factor are often used as health indicators for many fish species, with condition factor being strongly correlated to energetic state in Atlantic salmon (Persson *et al.,* 2018). Studies examining the length of smolts and subsequent marine return rates have found evidence that larger smolts will yield higher

marine return rates (Gregory *et al.,* 2019; Armstrong *et al.,* 2018) and the hypothesis of "bigger is better" is supported for out-migrating smolt (Gregory *et al.,* 2018). Other biological characteristics such as age, sex, and prey abundance can also affect smolt sizes (Armstrong *et al.,* 2018). Smolt length generally increases with age, with older migrating smolts exhibiting slower growth rates compared to younger smolts (Russel *et al.,* 2012; Swansburg *et al.,* 2002; Okland *et al.,* 1993). Slower growth in freshwater environments yields longer smolts and has been observed to correlate with increased growth rates at sea (Einum *et al.,* 2002).

Condition factor can provide information about the energy reserve status of juvenile salmonids and is strongly correlated to the energetic state in Atlantic salmon (Persson *et al.,* 2018). There is increasing evidence to suggest that the environmental conditions in freshwater affect both the physiology and energy reserves of smolt in ways that have consequences for subsequent performance, including survival (McCormick *et al.,* 2009; Russel *et al.,* 2012). Thus, although the evidence is limited, links between condition factor and survival have been demonstrated empirically for the Ellida River in Iceland (Antonsson *et al.,* 2010) and the River Conon in Scotland (Armstrong *et al.,* 2018). Therefore, examining trends in length and condition factor among different rivers may provide insight on how smolt characteristics, and the freshwater conditions they depend upon are linked to survival during migration and at sea.

Resource use can be examined using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes and is often used in ecology to assess differences and shifts in diet over time and to infer the relative importance of dietary items (Boecklen *et al.,*2011). In stable isotope analysis (SIA), $\delta^{13}C$ is used to determine resource use and energy pathways

owing to its low fractionation (circa 0.4-1‰) with each in trophic transfer (Post, 2002). In contrast, $\delta^{15}N$ has a higher fractionation (3-4‰) per trophic transfer but can be used to determine the trophic position of a species relative to others within the same food web or, with the appropriate baselines, to compared trophic functionality among food webs (Post, 2002).

Given the importance of size and condition for influencing marine survival, the objective of this study was to determine the significance of differences in smolt freshwater feeding ecology on fork length and condition factor in three genetically distinct Eastern Canadian rivers (Lehnert *et al.,* 2023; Bradbury *et al.,* 2021). Size often correlates with age (Jonsson and Jonsson, 2011) and the effects of sex were also considered for this study. Stable isotope data obtained from out-migrating smolts were combined with age and sex data to test the hypotheses: [1] length and condition factor remained stable over time (i.e. from 2000 – 2016) but were positively correlated with stable isotope values, increasing as smolts fed higher in the food web, i.e. positively correlated with $\delta^{15}N$, and relied more on benthic prey, i.e. positively correlated with $\delta^{13}C$; [2] that length and condition factor were similarly correlated to age, but not sex; and [3] that correlations among the variables were strongest in rivers in boreal-dominated ecoregions.

2.2 METHODS

Sample collection

Atlantic salmon smolt biological data consisting of fork length, weight, age, sex, and carbon and nitrogen stable isotope values were obtained from three rivers located in Eastern Canada: Conne, Trinité, and St. Jean from 2000-2016 (Figure 1). These

represent three of the 19 unique Atlantic salmon regional groups (designated units (DU)) identified using genetic and genomic data for North America by Lehnert *et al.* (2023): South Newfoundland - West (Conne River), Quebec Western North Shore (Trinité River), and Gaspé (St. Jean River). Conne River and Trinité River share similar watershed characteristics as both are located in the Boreal Shield ecoregion, have boreal forest types, and lacustrine dominated watersheds. St. Jean River is situated in the Atlantic maritime ecoregion, has a Great Lake – St. Lawrence/boreal forest, and has minimal lacustrine habitats within its watershed (Hedger *et al.,* 2004).

Samples were collected by the Department of Fisheries and Oceans Canada Newfoundland and Labrador region (DFO) and the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (MELCCFP) Québec as a part of on-going smolt monitoring programs. Smolt (n=19-30 per river/year, see appendix A) were selected haphazardly based on visual inspection for midsize smolts from the fish counting fence traps (Conne) and rotary traps (Trinité and St. Jean) and were measured for fresh fork length (mm) and fresh weight (g) in the field during the spring (May-June) smolt run from 2000-2016. Length and weight data were used to compute condition factor as Fulton's K (Fulton, 1904; Froese, 2006). Data from 2015 were excluded from this study for St. Jean River as no samples were collected in that year for St. Jean. Smolts were lethally sampled for sex, age, and dorsal muscle tissue for stable isotope analysis. Age data were determined and provided by DFO Newfoundland and Labrador region for the Conne River and by MELCCFP for Trinité and St. Jean rivers. Data were obtained through joint collaboration with researchers at DFO and the University of Waterloo.

Smolt dorsal muscle tissue samples were taken posterior to the dorsal fin above the lateral line. Samples were analyzed at the University of Waterloo Environmental Isotope Laboratory using a Micromass VG Isochrom continuous-flow isotope ratio mass spectrometer connected to a Carlo Erba elemental analyzer (Sinnataby *et al.,* 2009). For each sample the stable isotopes were calculated relative to international standards; Vienna Pee Dee Belemnite (VPDB) standard for $\delta^{13}C$ (Craig, 1957) and atmospheric nitrogen for $\delta^{15}N$ (Mariotti, 1983). All results are expressed in standard delta notation as parts per thousand. R represents the ratio of heavy to light isotopes in the sample.

$$(\delta, ‰) = [(R_{sample} / R_{standard}) / R_{standard}] \times 1000‰$$

Internal laboratory standards cross-calibrated against International Atomic Agency CH3, CH6 standards for carbon and the N1, N2 standards for nitrogen were inserted at the beginning, middle and end of each sample run to measure accuracy, determined for reporting purposes as ± 0.2‰ and ± 0.3‰, respectively, for $\delta^{13}C$ and $\delta^{15}N$. The percent elemental carbon to nitrogen ratios for smolt muscle tissues ranged from 2.96 to 3.74 for Conne River, 2.81 to 4.06 for Trinité River, and 2.88 to 3.71 for St. Jean River. No mathematical lipid correction was applied to the muscle tissue as the mean C:N ratio for all smolt muscle tissues was less than the 3.5 C:N ratio threshold (Kiljunen *et al.,* 2006).

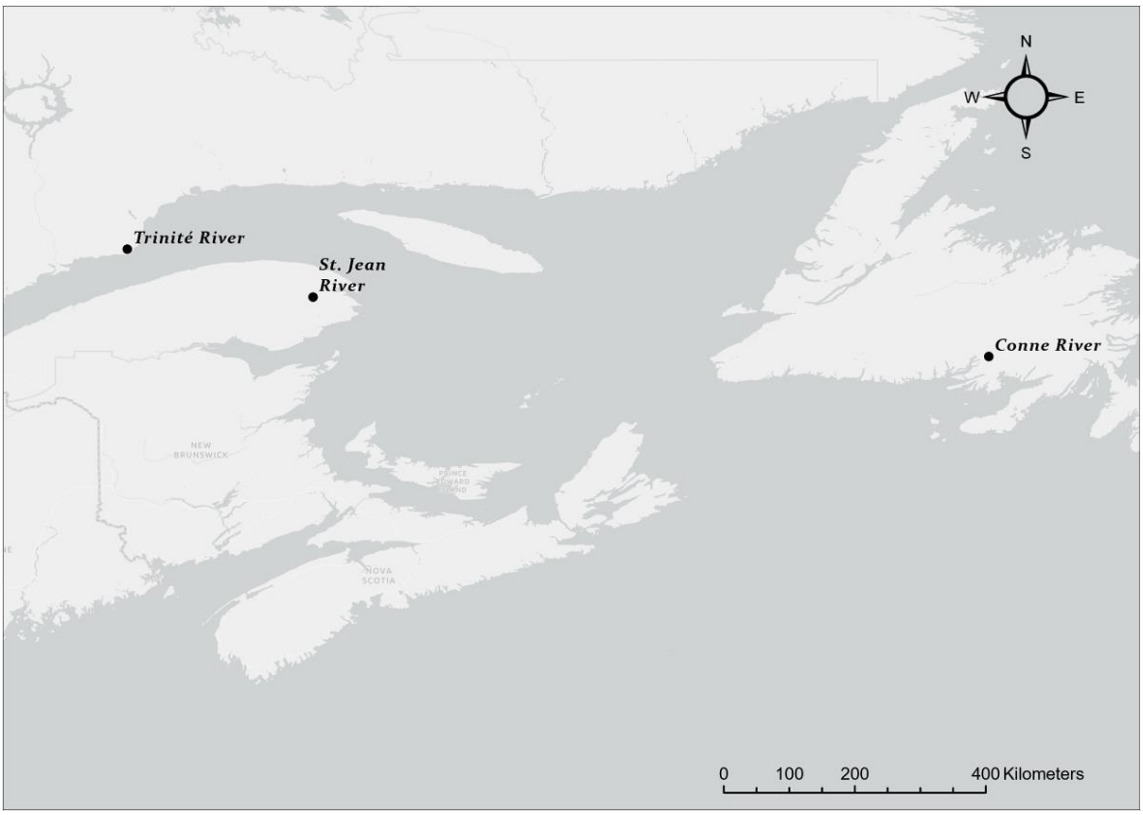


Figure A. Sample locations for Atlantic salmon smolt counting fences in the Conne River (South Newfoundland – West (DU)), Trinité River (Quebec Western North Shore (DU)), and St. Jean River (Gaspé (DU)). Locations indicate sites where smolt were collected in the spring smolt run from 2000-2016.

Statistical analysis

Mean fork length, condition factor, age, $\delta^{13}C$, and $\delta^{15}N$ were compared among sites using a one-way analysis of variance (ANOVA) followed by a Tukey's Post-hoc when significant differences were found. Smolt body size was examined using fork length (mm) and condition factor as dependent variables in separate multiple linear regressions (MLR) models. Pearson's correlation coefficient was used to examine the correlation of fork length and condition factor over time.

The predictor variables examined as having a possible influence on smolt body size included: Year (2000-2016) as a random factor, River (Conne, Trinité, and St. Jean) as a fixed factor, Sex as a fixed factor, Age as a fixed factor (year 2-5 smolt), $\delta^{15}N$ as a

continuous variable, and $\delta^{13}C$ as a continuous variable. The continuous variables were centered for use in the mixed-effects MLRs. An interaction between sex and age was used to account for variation in body size at age and an interaction between $\delta^{15}N$ and $\delta^{13}C$ allowed for links between trophic position and resource use. The models used for the model selection purposes are presented below and were estimated in R v.4.3.2. using the lmer() function, and the MuMIn and car packages (R core team 2023; Bartoń 2023; Fox and Weisberg 2019; Pena and Slate 2019).

(A) Fork length (mm) ~ River + Sex + Age + (Sex*Age) + $\delta^{15}N$ + $\delta^{13}C$ + ($\delta^{15}N$*$\delta^{13}C$) + (1| Year)

(B) Condition factor ~ River + Sex + Age + (Sex*Age) + $\delta^{15}N$ + $\delta^{13}C$ + ($\delta^{15}N$*$\delta^{13}C$) + (1|Year)

Model selection was performed using the corrected Akaike information criterion (AICc). The dredge () function in the MuMIn R package was used to determine the best-fit model and list all models with ΔAICc scores <11 (Burnham and Anderson, 2004; Bartoń, 2023). The 95% confidence set of models was presented for each model selection and the best-fit models coefficient confidence intervals. The models' performance was evaluated using marginal (R^2m), which indicates the proportion of variance explained by the fixed effects, and conditional (R^2c) coefficients which explains the variance by the full model. For the ANOVAs, Pearson's correlation test and MLRs, the test assumptions for normality, linearity, lack of influential outliers, homoscedasticity, autocorrelation, and multicollinearity, were assessed using residual plots (Fernandez, 1992). A significance level of $\alpha = 0.05$ was used for all tests.

2.3 RESULTS

Test of the residuals indicated none of the reported residuals violated the ANOVAs, Pearson's correlation tests, or multiple linear regression assumptions (i.e. normality, linearity, lack of influential outliers, autocorrelation and homoscedasticity).

Descriptive statistics

Smolt fork length and weight were significantly different among the three study rivers (highest in the Conne and lowest in the St. Jean), and condition factor was significantly higher in Trinité River compared to the other two sites (Table 1). For all three rivers, female smolts dominated the samples: Conne (F=264, M=75), Trinité (F=218, M=131), and St. Jean (F=220, M=115). The mean smolt age differed among the rivers, with Conne River having the highest and Trinité the lowest smolt age (Table 1). For resource use, $\delta^{13}C$ and $\delta^{15}N$ were significantly different among the three rivers, with the lowest isotopic values found in the St. Jean River and the highest in the Conne River (Table 1).

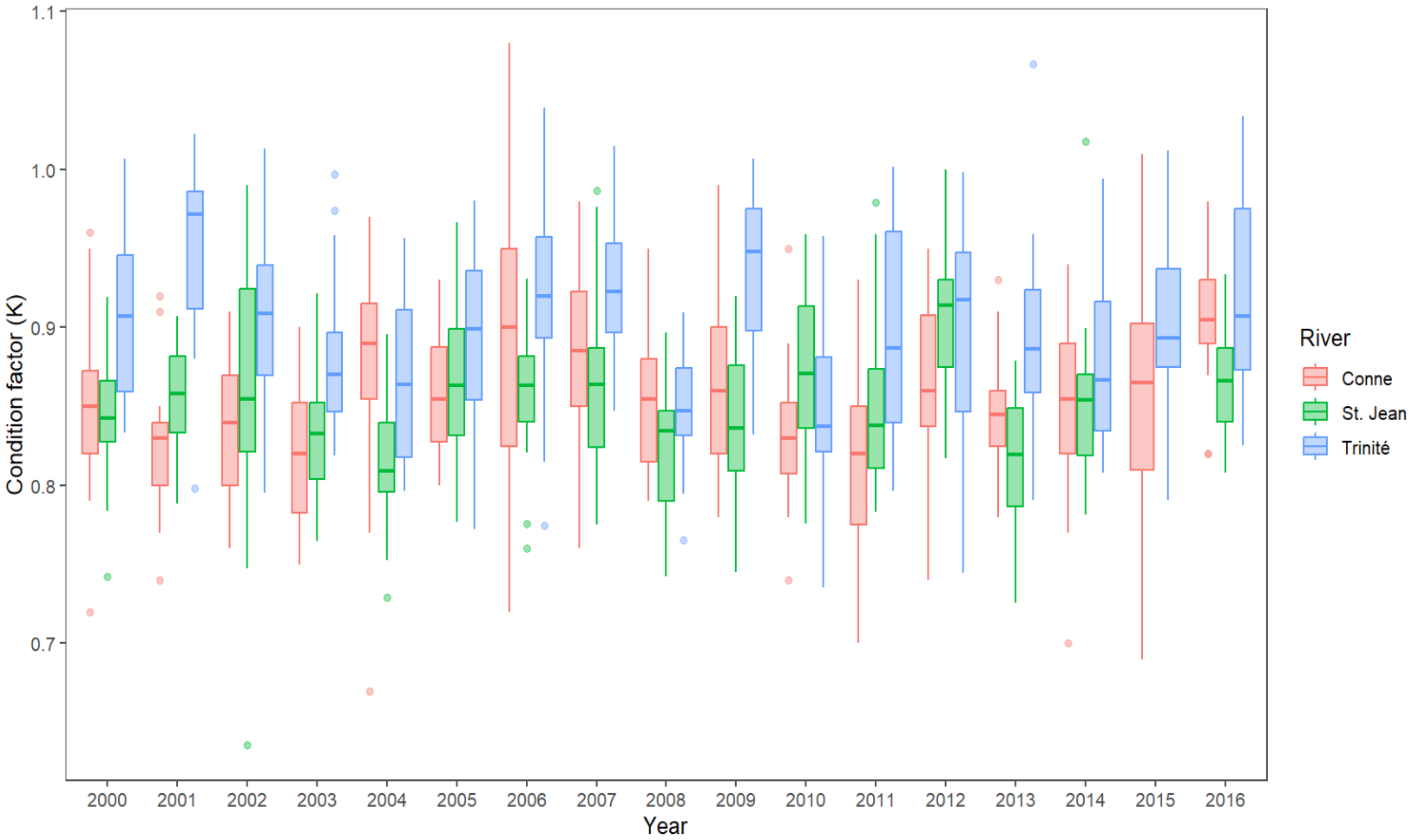
Years showed varying increases and reductions in both smolt fork length and condition factor among all three rivers (Figure 2). There were no significant trends in length for Conne and Trinité rivers (Pearson's correlations: Conne $r = -0.0956$, $p = 0.08$; Trinité $r = 0.00416$, $p = 0.938$) and only a weak negative correlation was significant for St. Jean ($r = -0.122$, $p = 0.03$), and no significant trends in condition factor (Condition factor; Conne $r = 0.105$, $p = 0.05$; Trinité $r = -0.0464$, $p = 0.388$; St. Jean $r = 0.0620$, $p = 0.257$) over the sixteen-year time series.

Table 1. Descriptive statistics showing the mean, standard error of the mean (SEM), minimum observation, and maximum observation for smolt fork length, weight, age, condition factor, $\delta^{13}C$, and $\delta^{15}N$ for Atlantic salmon smolt from Conne (n=339), Trinité (n=349) and St. Jean River (n=335). Data were collected during May-June from 2000-2016. River names that do not share a letter are significantly different from each other for each variable mean.

Variable	River	Mean	SEM	Minimum	Maximum
Fork length (mm)	Conne[a]	154.59	0.85	121.00	197.00
	St. Jean[c]	117.61	0.53	94.00	149.00
	Trinité[b]	122.85	0.64	95.00	163.00
Weight (g)	Conne[a]	32.42	0.53	15.80	64.40
	St. Jean[c]	14.11	0.20	7.31	27.10
	Trinité[b]	16.83	0.27	7.60	38.60
Age	Conne[a]	3.42	0.028	2	5
	St. Jean[b]	3.32	0.033	2	5
	Trinité[c]	2.96	0.027	2	5
Condition Factor	Conne[b]	0.855	0.00328	0.670	1.080
	St. Jean[b]	0.852	0.00287	0.635	1.018
	Trinité[a]	0.898	0.00327	0.735	1.067
$\delta^{13}C$	Conne[a]	-25.96	0.070	-30.19	-22.66
	St. Jean[c]	-30.34	0.106	-36.42	-25.02
	Trinité[b]	-26.79	0.056	-30.43	-22.98
$\delta^{15}N$	Conne[a]	8.34	0.032	6.75	10.29
	St. Jean[c]	6.90	0.026	4.73	8.69
	Trinité[b]	7.36	0.027	5.40	9.50

Figure B. Boxplot of (A) fork length and (B) condition factor for Atlantic salmon smolts for each sample river; Conne (n = 339), Trinité (n=349) and St. Jean (n=335) collected in the spring from rotary traps from 2000 - 2016. Boxplot is showing the median, interquartile range, whiskers and outliers. n = 19-30 smolt per year in each river (Appendix A)

Figure C. Boxplot of condition factor for Atlantic salmon smolts for each sample river; Conne (n = 339), Trinité (n=349) and St. Jean (n=335) collected in the spring from rotary traps from 2000 - 2016. Boxplot is showing the median, interquartile range, whiskers and outliers. n = 19-30 smolt per year in each river (Appendix A)



MLR and model selection

Table 2. Model selection table for the best-fit multiple linear regression model using the corrected Akaike information criterion (AICc) showing the top five results for ΔAICc < 11 for both fork length (mm) and condition factor as dependant variables for Atlantic salmon smolt. Empty sections indicate variables left out of the models.

Dependant variable	**Inter-cept**	**Age**	**$\delta^{13}C$**	**$\delta^{15}N$**	**River**	**Sex**	**Age: Sex**	**$\delta^{15}N$: $\delta^{13}C$**	**df**	**AICc**	**delta**	**Weight**
Fork length (mm)	144.3	+	-0.046	0.094	+	+		1.07	12	8023.6	0.00	0.665
	145.0	+	-0.036	0.087	+	+	+	1.09	15	8025.5	1.93	0.253
	144.6	+	0.030	0.172	+			1.036	11	8027.8	4.20	0.081
Condition Factor	0.852		-0.00124	0.00224	+			0.00234	8	-2958.2	0.00	0.155
	0.858		-0.00179		+				6	-2957.4	0.78	0.105
	0.855				+				5	-2957.3	0.87	0.100
	0.864	+	-0.00121	0.00258	+			0.00233	11	-2957.1	1.03	0.093
	0.867	+			+				8	-2956.3	1.86	0.061

The best-fit model for fork length used River, Age, Sex, $\delta^{15}N$, $\delta^{13}C$, and the interaction of $\delta^{15}N$*$\delta^{13}C$ as predictor variables and explains 66.5% of the variance in smolt fork length ($cR^2 = 0.665$, $mR^2 = 0.649$). Models within the confidence set of 10% of the top model included the top three models for fork length with the top model being 2.6x more likely to explain the variation in fork length compared to the model where the interaction of sex and age is added (Table 2). The top model suggests that smolts have a larger fork length if they are males, as age increases, and with higher $\delta^{15}N$: $\delta^{13}C$ ratios (Table 3). After river site, Age 4 and 5 smolts had the highest coefficients for predicting the variation in fork length (Table 3).

For condition factor the best-fit model was found to have River, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{15}N$* $\delta^{13}C$ and explains 19.1% of the variance in condition factor ($cR^2 = 0.191$, $mR^2 = 0.126$). Models within the confidence set of 10% of the top model for condition factor include the top eighteen models (Table 2), with the top model being 1.5x more likely to explain the variation in condition factor compared to the second model where $\delta^{15}N$ and the interaction of $\delta^{15}N$* $\delta^{13}C$ are removed (Table 2). The results suggest there is insufficient evidence to consider the interaction between sex and age in the model to explain the variation in condition factor. River was found to have the greatest coefficients in the best fit model for condition factor (Table 3).

Table 3. Coefficient table of the fixed predictors, estimates, standard error, t-values, and the upper and lower 95% confidence interval (CI) for each fixed predictor for the best fit models for fork length ~ River + Age + Sex + $\delta^{15}N$ + $\delta^{13}C$ + $\delta^{15}N$*$\delta^{13}C$ + (1|Year) and Condition factor ~ River + $\delta^{13}C$ + $\delta^{15}N$ + $\delta^{15}N$*$\delta^{13}C$ + (1|Year) for Atlantic salmon smolt.

Dependant variable	Predictor	Estimate	Std. Error	t value	95% CI lower	95% CI upper
Fork length	Intercept	144.26	1.998	72.192	140.375	148.169
	Trinité	-28.836	1.27	-22.706	-31.323	-26.361
	St. Jean	-37.17	1.767	-21.035	-40.634	-33.729
	Male sex	2.096	0.837	2.506	0.453	3.725
	Age 3	6.735	1.61	4.185	3.577	9.869
	Age 4	10.819	1.775	6.094	7.341	14.277
	Age 5	7.207	3.868	1.863	-0.366	14.745
	$\delta^{13}C$	-0.046	0.277	-0.165	-0.586	0.494
	$\delta^{15}N$	0.1	0.747	0.134	-1.367	1.555
	$\delta^{13}C$: $\delta^{15}N$	1.07	0.243	4.404	0.595	1.544
Condition Factor	Intercept	0.852	0.00623	136.611	0.840	0.864
	Trinité	0.0483	0.00574	8.421	0.037	0.060
	St. Jean	-0.00671	0.00814	-0.825	-0.023	0.009
	$\delta^{13}C$	-0.00124	0.00128	-0.966	-0.004	0.001
	$\delta^{15}N$	0.00228	0.00345	0.659	-0.005	0.009
	$\delta^{13}C$:$\delta^{15}N$	0.00234	0.00113	2.067	0.0001	0.005

2.4: DISCUSSION

The goal of this study was to examine Atlantic salmon smolt length and condition from three Eastern Canadian rivers and examine factors that contribute to the variation in smolt size. Within each river, while there were some fluctuations between consecutive years, there were no notable trends observed over the 16-year time-series, suggesting that smolt length and condition stayed relatively stable within Conne, Trinité, and St. Jean rivers over the sampled years.

Smolts from all three rivers were significantly different for fork length, and Trinité River had significantly higher condition factors compared to the Conne and St. Jean rivers. Additionally, the smolt river of origin had the highest coefficients in the MLRs, suggesting that smolt origin group (i.e. DUs; Lehnert *et al.,* 2023; Bradbury *et al.,* 2021) may have a large influence on body size. Conne and Trinité rivers are both located in the Boreal Shield ecoregion and were found to have greater overall smolt lengths and condition factors compared to St. Jean; this could also suggest that site environment is a factor driving body size. The Atlantic maritime ecozone is the warmest ecoregion in Eastern Canada with mean summer temperatures 3-4ºC warmer than those of the Boreal Shield ecozone which are reported between 8.5 – 12.5ºC (Vasseur *et al.,* 2007). In Atlantic salmon increased air and water temperatures have been shown to produce younger out-migrating smolts (Swansburg *et al.,* 2002). With increasing temperatures associated with climate change affecting smolt growth and size, understanding additional factors influencing their development is critical for managing juveniles and increasing marine return rates (Russel *et al.,* 2012). Additional river characteristics such as water velocity, turbidity, in-river temperature, lacustrine/lentic

habitats, contaminants, and resource abundance should be observed as they have been known to impact the size of smolt (Russel *et al.,* 2012; Pakkasmaa and Piironen, 2000; Dempson *et al.,* 2004; McCormick *et al.,* 2009).

Carbon and nitrogen stable isotopes were found to be explanatory variables in the best-fit models for both fork length and condition factor. However, for both models $\delta^{13}C$, $\delta^{15}N$, and $\delta^{15}N*\delta^{13}C$ had the lowest coefficients of all the predictors. Both models showed a negative coefficient for $\delta^{13}C$ and a positive coefficient for $\delta^{15}N$ which indicates that as length and condition factor increase, $\delta^{13}C$ are predicted to be more depleted and $\delta^{15}N$ more enriched. However, the low coefficient results suggest resource ($\delta^{13}C$) and trophic ($\delta^{15}N$) diversity of out-migrating smolt does not have a major impact on length and condition factor, as opposed to other predictors such as origin river, age, and sex.

Food availability and feeding conditions are known to affect length negatively if freshwater conditions are poor (Armstrong *et al.,* 2018). However, $\delta^{13}C$ is not an indicator of food abundance and availability; rather, it is more indicative of the energy source (Post, 2002). Juvenile salmon are opportunistic drift feeders and eat prey based on the availability of prey (Thonney and Gibson, 1989; Gibson and Cunjak, 1986). Prey selectivity may also differ more between populations rather than within populations due to energy requirements and resources present in rivers. This may explain why the river predictor had a larger coefficient for length and condition compared to $\delta^{13}C$ or the smolts are all targeting similar resources (Reiriz *et al.,* 1998).

$\delta^{15}N$ had larger coefficients compared to $\delta^{13}C$ in the best-fit models. $\delta^{15}N$ has been observed to increase with increasing fish body size including juvenile Atlantic salmon (Jennings *at al.,* 2002), as individuals shift to a higher trophic level and consume larger

prey (DiNero and Epstein, 1981; Power *et al.,* 2002; Jennings *et al.,* 2008). However, a specific trend between body size and $\delta^{15}N$ has not been concluded as it is strongly dependant on sample size (Galvan *et al.,* 2010). In this study we only examined one life stage of salmon and all smolt collected were most likely feeding on similar prey due to gape size limitations, this suggests $\delta^{15}N$ may be independent of body size (Galvan *et al.,* 2010). Increasing size with increasing $\delta^{15}N$ may apply more prey switching for example when post-smolt begin feeding in a new environment and have access to larger prey (Sinnatamby *et al.,* 2008).

Age and sex were significant predictors only for the model predicting fork length. Conne River had the largest smolt lengths and the oldest smolt population. The older age and slower growth rates of smolts in the Conne River may contribute to their increased body size. However, the relationship between freshwater growth and marine growth is not well understood (Simmons *et al.,* 2022). Smolts that spend more time in freshwater environments and enter the ocean at a larger size and older age may have a better chance of escaping predation (Friedland *et al.,* 2006). Therefore, larger smolt entering the ocean may still have an advantage for higher marine return rates.

The results indicated that male smolts were associated with greater fork lengths, suggesting that male smolts are predicted to have longer body size compared to females. A higher ratio of females to males was collected throughout this study with female smolt dominating in every river, a trend consistent with previous studies on Atlantic salmon (Dahl, 1910; Osterdahl, 1969; O'Connell and Ash, 1993). A high abundance of female smolt outmigration may be linked to males having a higher propensity to mature in freshwater without having to migrate; this is especially seen in lacustrine habitats

(Jonsson, 1989; O'Connell and Ash, 1993). While female and male smolt typically do not exhibit sex-based differences in length at migration (Fängstam *et al.,* 1983), the high abundance of female smolt collected in this study may have influenced the coefficient results. Although previous studies have found that males are larger in older smolts (i.e., Jonsson *et al.,* 1998), there was no evidence to support the interaction of sex and age in the predictive model for smolt fork length (Jonsson *et al.,* 1998).

The low explained variation by the best-fit model for condition factor could be observed since condition factor is calculated using a ratio for smolt length and weight so the model must predict the relationship of two dependent variables (Bolger and Connolly, 1989). In addition, the weight of an individual at any given time is more variable compared to their length as it can fluctuate depending on the species recent feeding habits, which could result in the model having a lower multiple R^2 value (Malenfant *et al.,* 2018). The low multiple R^2 suggest the model would not be very useful in practical settings and smolts would still have to be weighed to obtain an accurate value for condition factor.

Condition factor was significantly higher in Trinité River compared to both St. Jean and Conne; all populations were found to have a condition factor 0.8-0.9. For most species a condition factor under one would be indicative of a poor fish condition; however, a decrease in condition factor is typically examined in smolt as they experience a rapid growth in length (McCormick, 2012; Ravikumar *et al.,* 2023). Overall, the condition factors for all three rivers fell within the range for typical out-migrating smolt and did not give a strong indication of populations with poor conditions (Persson *et al.,* 2019; Whalen *et al.,* 2019). However, St. Jean River had both the lowest mean fork

length and condition factors which could indicate some environmental influence on body condition.

In conclusion, fork length and condition factor stayed relatively stable within each river throughout the 16-year time-series. Smolt from all three rivers were found to differ significantly in body size, with Conne River (South Newfoundland - West group) having the highest fork lengths and Trinité River (Quebec Western North shore group) having the highest condition factor; both rivers located in the Boreal Shield ecoregion. Resource use, as inferred by $\delta^{13}C$ and $\delta^{15}N$, was a useful predictive variable for explaining both smolt fork length and condition factor for the three rivers. However, $\delta^{13}C$ and $\delta^{15}N$ explained the least amount of variation on body size compared to all other predictor variables in the models. Age and sex were found to influence length but not condition factor, with length increasing at older ages and males having predicted longer fork lengths compared to females. This study provides information on what smolt, or habitat characteristics are predictive for greater body sizes which can aid in determining smolt size drivers to maintain larger smolt populations for higher marine survival.

Future work

Future studies should explore additional rivers within the South Newfoundland – West, Quebec Western North Shore, and Gaspé Atlantic salmon groups to see if similar results are found and assess whether rivers within the same population display consistent body sizes. By including more rivers, we can also determine whether body size is more influenced by smolt population-level group assignment or by river characteristics. For instance, Conne River consistently yielded the longest smolt over a 16-year period, determining if this pattern extends across other rivers in the South Newfoundland – West

group could help determine why larger smolt are found in this group. Gathering data from more rivers in each ecoregion, with additional information on river characteristics, may also provide insight into the influence of surrounding wetland landscapes on smolt size.

Moreover, increasing sample sizes for Age 2, Age 5 and male smolt may also help even out predictor sample sizes to better examine age and sex-specific trends. In addition to increasing the study size, correcting the isotope data with baselines may help eliminate the bias of environmental influence for each river site and allow for better comparison of $\delta^{13}C$ and $\delta^{15}N$ between sites (Olsson *et al.,* 2009; Anderson and Cabana, 2007). Despite the low coefficient produced by $\delta^{13}C$, comparing isotopic data with baselines would enable us to infer prey diversity among rivers, potentially changing the effects of $\delta^{13}C$ as a predictor variable.

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APPENDIX A

Table A. Smolt sample sizes (n) for Conne, Trinité and St. Jean rivers for each year from 2000-2016, with no samples collected from St. Jean in 2015. Smolt were collected during the spring smolt run from counting fence traps and selected haphazardly.

Year	Conne	Trinité	St. Jean
2000	20	20	20
2001	20	20	20
2002	20	21	25
2003	20	25	20
2004	20	21	20
2005	20	22	21
2006	19	20	20
2007	20	20	30
2008	20	20	20
2009	20	20	20
2010	20	20	20
2011	20	20	20
2012	20	20	20
2013	20	20	20
2014	20	20	20
2015	20	20	0
2016	20	20	19

CHAPTER 3: STABLE ISOTOPE ASSESSMENT OF ATLANTIC SALMON (SALMO SALAR) SMOLTS ACROSS REGIONAL POPULATIONS IN EASTERN CANADA

3.0 ABSTRACT

Atlantic salmon (*Salmo salar*) are widely distributed across Eastern Canada; however, many populations are threatened or at risk. At-sea mortality poses a significant challenge for Atlantic salmon. To support management efforts, we focused on the life stage prior to sea entry; smolts, aiming to gather ecological information that could inform conservation strategies. We sampled smolt collaboratively through accidental mortalities collected in seven smolt wheels and two fish counting fences to investigate dietary patterns at the beginning of their out-migration and assess changes in their resource use within and between rivers in Eastern Canada using stable isotope analysis. Specifically, we analysed carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes from smolt fin, muscle and liver tissues as well as body size as an indicator of condition. Our findings revealed high individual dietary specialization for smolt across tissues. Within rivers, we observed a consistent decreasing trend in $\delta^{15}N$ from long-term (fin and muscle) to short-term (liver) tissues, suggesting active feeding behaviour. However, while most rivers within the same genetically assigned population exhibited consistent patterns in resource use, a few rivers showed notable deviations. This suggests that environmental factors associated with river systems may exert a stronger influence on resource utilization than genetic similarities.

3.1 INTRODUCTION

In Canada, Atlantic salmon are native to Nova Scotia, New Brunswick, Newfoundland and Labrador, Prince Edward Island, Quebec and Ontario (extirpated). There are currently 16 populations of Atlantic salmon designated by the Committee on

the Status of Endangered Wildlife in Canada (COSEWIC), with five listed as endangered and another five as threatened or of special concern, many of which are found in the southern region of Eastern Canada (COSEWIC, 2010; Aas *et al.,* 2010). Recently, 19 Atlantic salmon groups have been distinguished in Eastern Canada using genetic and genomic data (Lehnert *et al.,* 2023; Bradbury *et al.,* 2021). The distribution of Atlantic salmon spans four ecoregions in Canada: the Atlantic Maritime, the Boreal Shield, the Taiga Shield, and the Arctic Cordillera. The wide distribution of habitat and multiple life stages of Atlantic salmon cause variation in their freshwater rearing habitats in regard to river chemistry, riparian ecosystems, and anthropogenic effects (Finstad *et al.,* 2011; Bradbury *et al.,* 2021; Armstrong *et al.,* 1998).

One of the major drivers of population declines in salmon is at-sea mortalities (Dadswell *et al.,* 2022). This issue is challenging because the root cause of the mortalities is unclear and multiple factors including climate change could be contributing. However, increasing smolt survivability during seaward migration may help give post-smolts a better chance of surviving the ocean environment (Russell *et al.,* 2012). Multiple studies have examined connections between out-migrating smolt and adult return rates (Armstrong *et al.,* 2018; Otero *et al.,* 2014; Russell *et al.,* 2012) and have determined direct relationships with increased body size of smolt (Gregory *et al.,* 2019; Armstrong *et al.,* 2018), increased freshwater food abundance and availability, and migration timing (Otero *et al.,* 2014).

Increasing the number of out-migrating smolts as well as their size could help increase the amount of marine survivals. Smolt size is influenced by the growth of the parr (Thorstad *et al.,* 2012) which is driven by the condition of the freshwater

environment (Finstad *et al.,* 2011). In productive freshwater systems, parr feed opportunistically on benthic macroinvertebrates with a selection towards larger prey such as caddisfly, mayfly and dragonfly larvae (Gibson, 1993). However, the effects of feeding during the out-migration on the survival of smolt have not been well studied. Larsson *et al.,* (2011) examined the number of feeding smolts and their stomach contents in wild and hatchery Atlantic salmon in River Tornionjoki; bordering Finland and Sweden, and found that wild smolt demonstrated selective feeding during their migration. It was concluded that feeding during migration is important for smolts in the Tornionjoki system (Larsson *et al.,* 2011), in contrast to previous studies that suggest smolts exhibit minimal feeding throughout their migration (McCormick and Saunders, 1987; Stefansson *et al*., 2003).

Stable isotope analysis

Stomach content analysis is a useful method for examining the short-term diet of a species; however, it may be biased to hard bodied prey (Post, 2002; Ibáñez *et al.,* 2021). Other dietary assessment tools such as stable isotope analysis (SIA) can offer a more long-term perspective into a species' diet. Assimilated stable isotopes in tissues such as $\delta^{13}C$ and $\delta^{15}N$ can be used to examine energy sources of consumers and determine their trophic position (Post, 2002). The relationship between resource use ($\delta^{13}C$) and body size is not well understood; however, $\delta^{13}C$ can indicate if fish are gaining their energy from benthic or pelagic sources in aquatic environments. Moreover, an increase in body size has been observed with an increase in $\delta^{15}N$ as species move up in trophic levels, however, this relationship has yet to be established (Galvan *et al.*, 2010). In addition, isotopic values vary geographically and temporally based the organic

and inorganic nutrients present at a given location (Glibert *et al.,* 2019; Hobson, 1999). Therefore, tracing the stable isotope composition of consumers can provide information about their resource use, condition, and foraging location.

Most stable isotope studies are invasive and typically lethal since tissues must be removed and processed for analysis. There are a few alternatives to lethal sampling in stable isotope ecology such as using fish fin clips (Jardine *et al.,* 2005), fish scales (Dempson and Power, 2004), or hair in mammals (Bentsen *et al.,* 2014). In fish biology, the use of fin tissue as a non-lethal alternative to white muscle has been commonly used in stable isotope studies (Hanish *et al.,* 2010; Jardine *et al.,* 2005). However, there has been ongoing research about the specific metabolic turnover rate of fin tissue and if it is a good surrogate for muscle given its heterogenous structure (Hayden *et al.,* 2015; Jardine *et al.,* 2005).

Not all tissues coming from the same species will carry the same metabolic turnover rates for SIA which allows us to examine a species' diet at multiple times (Boecklen *et al.,* 2011). Tissues with slower metabolic turnovers such as the muscle and fin will give a longer snapshot into a species' diet whereas tissues with faster turnover rates such as the liver will give us a shorter timeframe (Vander Zanden *et al.,* 2015; Dvergedal *et al*., 2019). Fin and muscle have been found to carry similar turnover rates of about 3-5 months (Church *et al.,* 2009; Willis *et al.,* 2013) while the liver in fish has a faster turnover rate of about 2 weeks because it is more metabolically active (Thomas and Crowther, 2015). Therefore, we can determine ecological information such as movement patterns, foraging, and prey selection over time based on varying metabolic turnover rates from different tissues (Cunjak *et al.,* 2005; Peterson and Fry, 1987).

In addition to energy source and trophic position, we can calculate the isotopic niche width and proportional prey usage using SIA. The isotopic niche width refers to the total area of the $\delta^{13}C$ and $\delta^{15}N$ data expressed as ‰², which can be applied to single organisms within communities to determine their dietary niche width (Jackson *et al.,* 2011; Layman *et al.,* 2007). Isotopic niche width is useful in comparing populations to examine differences in the nutritional spread of resources used, to examine specialist vs generalist populations, as well as changes in resource use over time at a population level (Layman *et al.,* 2007). Comparing isotopic niche widths of multiple tissues between and within rivers will allow us to examine changes in dietary breadth during the migration and examine if smolt remain generalist feeders throughout migration or specialize in their dietary preferences (Dixon *et al*., 2012; Thonney and Gibson, 1989; Aas *et al.,* 2010).

The objective of this study is to gain ecological information on Atlantic salmon smolts through passive sampling of animals that were euthanized for other ecological studies. The focus of this study is on the resource use of smolt prior to sea entry. Data on smolt stable isotopes and body size were obtained from multiple rivers to test the hypotheses that: [1] resource use of smolts, quantified by $\delta^{13}C$ and $\delta^{15}N$ will not change from their parr life stage as they continue to inhabit freshwater environments, [2] resource use will vary regionally among rivers in Eastern Canada with smolt coming from the same genetic origin group showing similar $\delta^{13}C$ and $\delta^{15}N$ values, and [3] rivers with enriched $\delta^{13}C$ and $\delta^{15}N$ values may smolts increased smolt body size, i.e., fork length.

3.2 METHODS

Sample collection

Atlantic salmon smolt were collected opportunistically through accidental mortalities in smolt wheels and fish counting fences from monitoring projects that conducted studies on smolts in Eastern Canada from 2022 – 2023 by collaborative parties: Department of Fisheries and Oceans Canada Newfoundland and Labrador, and Gulf regions (DFO), the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (MELCCFP) Québec, Nova Scotia Salmon Association (NSSA), Gespe'gewa'gi Institute of Natural Understanding (GINU), and Miramichi Salmon Association (MSA). Samples were sent to the University of New Brunswick Fredericton campus and stored in a freezer (-20°C) until they were thawed, measured and samples collected for condition and stable isotope analysis.

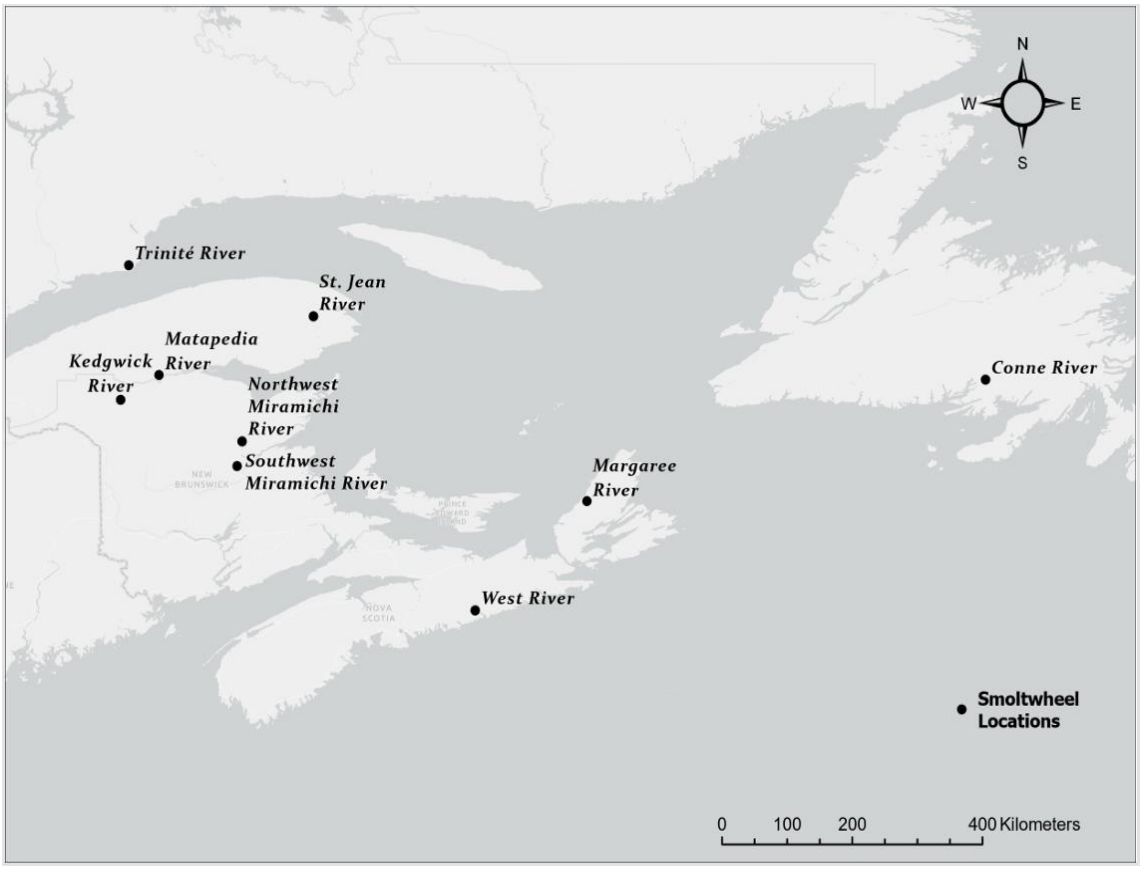

Figure D. Smolt wheel locations of the nine rivers sampled for smolt during the spring 2022 and 2023 smolt run. Rivers include Conne (Newfoundland), Trinité, St. Jean, and Matapedia Rivers (Québec), Kedgwick, Northwest Miramichi, and Southwest Miramichi (New Brunswick), Margaree and West River (Nova Scotia).

Smolt condition measurements including fork length, weight, hepatosomatic index (HSI), lipid content, and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were obtained for all rivers with smolt sample size greater than five: Conne River, Trinité River, Kedgwick River, Margaree River, Matapedia River, Northwest Miramichi River, St. Jean River, Southwest Miramichi River, and West River (Figure 3). Nineteen designated units (DUs) of Atlantic salmon have been designated using genetic and genomic data in Canada by Lehnert *et al.* (2023) and the rivers sampled for this study cover five of these DUs (Table 4).

Table 4. Rivers where Atlantic salmon smolt were collected from smolt wheels and fish counting fences, their groups designated by Lehnert *et al.,* 2023, collection year and sample size.

River	Group	Year	Sample size
Conne	South Newfoundland – West group	2023	30
Trinité	Quebec Western North shore group	2022	20
		2023	20
Kedgwick	Gaspé group	2023	6
Matapedia	Gaspé group	2023	35
St. Jean	Gaspé group	2022	20
		2023	20
Margaree	Southern Gulf of St. Lawrence and Cape Breton group	2022	30
		2023	35
Northwest Miramichi	Southern Gulf of St. Lawrence and Cape Breton group	2022	17
		2023	10
Southwest Miramichi	Southern Gulf of St. Lawrence and Cape Breton group	2023	5
West River	Nova Scotia Southern Upland -East group	2022	15

Baseline samples were collected from five of the rivers during June – September 2023 from the locations where smolts were collected. Rivers that were sampled for benthic macroinvertebrates (BMIs) using a kick net and methods from the Canadian Aquatic Biomonitoring Network (CABIN) protocol include Matapedia River (n=19), Kedgwick River (n=17), Margaree River (n=18), West River (n=20), and Northwest Miramichi (n=15). BMIs were identified to order level and sent to the Stable Isotope in Nature Laboratory (SINLAB; Fredericton, NB) for stable isotope analysis (Appendix B). BMIs were not collected for the other rivers due to sampling time and accessibility constraints. BMIs identified in the same order level were grouped together to fill 1.5 ml microcentrifuge tubes and sent for SIA (see Appendix C and D).

Lipid measurements

Lipid measurements were taken using two methods: bioelectrical impedance meter (detailed below) and the Distell Fish Fat Meter (Models FFm-992 and 692, Distell Fauldhouse, UK). Smolt samples were taken out of the freezer to thaw at room temperature (22.5ºC). Smolt samples were left to thaw until the muscle temperature reached 4ºC which was measured on their lateral muscle just below the dorsal fin using a thermometer. Smolts were wiped to remove excess water according to the whole – fish carcass ("Salmon-2") protocol for the Distell Fish Fat Meter Calibration Table. Two different models of meters were used throughout sampling as one had to be sent off for maintenance. Model FFM-992 was a smaller model and took the mean of 8 lipid readings (4 per side), while Model FFM- 692 had a larger sensor head and took the mean of 6 lipid readings (3 per side). Only smolts with a fork length >125mm were sampled, as the sensor heads were larger than the lateral surface area of smaller smolts. Values are expressed as % total lipid, and since measurements were taken from 2-15% we can expect an accuracy range of +/- 0.5-1.0% (Distell fish fat analyser, 2020).

The bioelectrical impedance measurements (BIM) were taken using a Quantum III Bioelectrical Body Composition Analyzer (RJL Systems, Michigan, US) with four stainless, 28-gauge (0.32 mm) needle electrodes (Grass Telfactor, Rhode Island) which were inserted in two locations above the lateral line (Vue *et al.,* 2015; Hartman *et al.,* 2015). Internal temperature was measured using a thermometer, and the distance between electrodes (mm) was measured using a ruler, and reactance and resistance were measured for each smolt. Temperature of the smolt was measured between 7-22ºC to mimic optimum smolt temperature from a field setting (Vue *et al.,* 2015). Total lipid content (g) was calculated using methods and equations provided by Vue *et al.* (2015).

Dissection

Smolt dissections were completed after thawing and lipid measurement were taken. Smolt were dissected for a sample of their muscle, caudal fin, and liver. Muscle samples were taken from the lateral side of the smolt opposite to where the bioelectrical probes were inserted, above the lateral line and below the dorsal fin. A clip of the caudal fin was taken posterior to the fork in the caudal fin, and the whole liver was removed and weighed for HSI calculation. A few smolts (n=6) provided from Trinité River were missing internal organs and therefore only the fin and muscle tissue were sampled. Dissection tools were wiped with 95% ethanol between each smolt sample to reduce the chances of contamination between individuals. All three tissues were sent to SINLAB for stable isotope analysis.

Stable isotope analysis (SIA)

Smolt tissues and BMI samples were placed into separate 1ml test tubes and oven dried at 60°C for 48 hours. Dried samples were homogenized individually by using a mortar and pestle and grinding into a fine powder and then placed in a tin capsule for SIA. Between grinding each tissue sample, the tools and workspace were wiped with ethanol. Samples weighing 1.00-1.20mg were sent to SINLAB. Samples weighing between 0.371-1.310mg were processed at the SINLAB and calculated relative to international standards; Vienna Pee Dee Belemnite (VPDB) standard for carbon (Craig, 1957) and atmospheric air for nitrogen (Mariotti, 1983). The results for the stable isotope values were expressed in a delta notation (δ) as parts per thousand (‰). The same process was then repeated for the BMI samples from each river. R represents the ratio of heavy to light isotopes in the sample.

$$(\delta, ‰) = [(R_{sample} / R_{standard}) / R_{standard}] \times 1000‰$$

Stable isotope analytical precision was found to be ±0.19‰ standard deviations for $\delta^{13}C$ and ±0.14‰ standard deviations for $\delta^{15}N$ for USGS61, BLS and MLS secondary standards. Carbon to nitrogen ratios ranged from 3.1 – 3.5 in the smolt muscle tissue samples, 3.5 – 7.2 for the liver samples, 2.9 – 4.6 for the fin samples, and the BMI samples ranged from 3.66 – 10.33. SINLAB produced replicates for every 20th sample and these replicates were removed from the analysis as very little variation was observed between replicates. A mathematical lipid normalization calculation was applied to the liver samples since the C:N ratios were found to be high (mean C:N > 3.5) (Kiljunen *et al.,* 2006, Logan *et al.,* 2008):

$$\delta^{13}C' = \delta^{13}C + D \times (\theta + 3.91 + 287/L)$$

D = the isotopic difference between protein and lipid

θ = constant

L = the proportional lipid content of the sample:

$$L = \frac{93}{1 + (0.246\ X\ (C{:}N_{Liver}) - 0.775^{-1}}$$

A correction using specific fish liver tissue model by Logan *et al.* (2008) was applied to the liver samples where D = 7.209 ± 0.3285 and θ = -0.054 ± 0.0264.

For rivers where BMIs were collected a baseline correction was applied to both the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values. Baselines used for both the carbon and nitrogen corrections were of the common groups, mayflies, caddisflies, dragonflies, craneflies,

stoneflies, gastropods, and water penny beetles (Appendix B.1). Carbon was corrected using the equation provided by Olsson *et al.,* (2009) where δ^{13}C smolt is the δ^{13}C of the smolt sample, δ^{13}C (mean baseline) is the mean δ^{13}C invertebrate value, and C (baseline range) is the carbon range for the same baselines used to calculate the mean (δ^{13}Cmax–δ^{13}Cmin):

$$\delta^{13}\text{Ccorr} = \frac{\delta^{13}\text{C smolt} - \delta^{13}\text{C (mean baseline)}}{C(baseline\ range)}$$

Nitrogen was corrected by calculating the trophic position (TP) for each smolt where δ^{15}Ncn is the nitrogen value of the consumer, δ^{15}Nprey is the mean nitrogen value of the prey collected at that site, 2 is the expected trophic position of the baselines, and f is the fractionation value where a value of 3.4‰ was used (Anderson and Cabana, 2007; Post, 2002).

$$\text{Trophic position: TP (consumer)} = ([\delta^{15}\text{Ncn} - \delta^{15}\text{Nprey}]/f) + 2$$

Statistical analysis

All Statistical analyses and calculations were performed using Minitab v. 20.2 and Rstudio v. R4.1.1. (Minitab 20.2, 2021; R Core Team, 2021). Permutational analysis of variance (PERMANOVA) were used to pool 2022 and 2023 data into one dataset for the fork length and stable isotope data. PERMANOVAs were performed using R statistical software with the "Vegan" package, and 99999 permutations were run for both tests (McArdle and Anderson, 2001). Descriptive statistics on the mean, standard deviation, min and max were calculated for the fork length and condition data. Correlation coefficients for the ratio of carbon to nitrogen for fork length were computed to examine a relationship between the isotope results and size of the smolt.

To check for variance within individual smolt diet, correlation tests using Pearsons's correlation coefficient were conducted to examine the relationship of $\delta^{13}C$ and $\delta^{15}N$ values between fin, muscle and liver tissues. A high correlation between tissues indicates strong diet consistency. Tissue stable isotope results were compared within sites using one-way analysis of variance (ANOVA). If significant results were found, a Tukey's post-hoc test was used to identify the significant difference between specific tissues using the "TukeyC" package in R. A boxplot was produced to show the trend for carbon and nitrogen between tissues within each river. Niche width was calculated for each tissue using the SIBER package to determine changes in niche at different periods prior to the smolts migration (Jackson *et al.,* 2011). The corrected standard area ellipses (SEAc) were used to examine niche width size and were expressed as ($‰^2$) following the protocol from Jackson *et al.* 2011.

Comparisons between populations were performed using the uncorrected and corrected stable isotope data from the muscle tissue. Muscle tissue was selected because it is widely utilized and a reliable indicator in trophic ecology. Separate one-way ANOVAs were run to compare the $\delta^{13}C$ and $\delta^{15}N$ muscle tissues of all 9 uncorrected rivers and the 5 baseline corrected rivers. Assumptions for all ANOVAs and Pearson's correlation coefficients (normality, homogeneity of variance, and linearity) were assessed using residual plots (Appendix B.2, B.3) (Fernandez, 1992). If assumptions were not met for ANOVAs, a non-parametric Kruskall-Wallis test was conducted and if significant a Dunn's post-hoc test was performed using the Bonferroni method. A significance level of $\alpha = 0.05$ was used for all tests.

3.3 RESULTS

Effects of year, river and tissue

Year did not have a significant effect on the stable isotope values, but river and tissue did (Table 5). Although there was a significant interaction between year and river, river had a much larger effect on the stable isotope ratios (based on the R^2 values) and therefore river $\delta^{13}C$ and $\delta^{15}N$ data for 2022 and 2023 could be pooled.

Table 5. Fixed effect permutational multivariate analysis of variance (PERMANOVA) testing the stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) for river, year, and tissues of rivers that were collected in 2023 and 2022. After 99999 permutations produced on R using adronis2 function and vegan package.

	DF	SS	R^2	F	P
Year	1	9.9	0.00161	1.7846	0.169
River	3	3171.9	0.51445	190.6141	<0.001
Tissue	2	210.2	0.03410	18.9524	<0.001
Year:River	3	51.3	0.00832	3.0844	0.019
Year:Tissue	2	2.2	0.00035	0.1944	0.897
River: Tissue	6	24.2	0.00393	0.7284	0.656
Year:River:Tissue	6	5.7	0.00092	0.1701	0.997
Residual	485	2690.2	0.43632		
Total	508	6165.6	1.00000		

Individual tissue variation

Tissue correlation coefficients for $\delta^{13}C$ were >0.800 in all river sites except for the correlation of fin-liver in Northwest Miramichi (0.691; Table 6; Figures 3.2, 3.3, 3.4). The $\delta^{15}N$ correlation coefficients were more variable with a range of 0.365-0.988 for fin-muscle, 0.315-0.936 for muscle-liver, and 0.647-0.955 for fin-liver (Table 6;

Figures 4, 5, 6). Trinité river was the only site that had correlation coefficient estimates below 0.500.

Table 6. Pearsons's correlation coefficients for the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of smolt tissues between fin – muscle, muscle – liver, and fin – liver for nine rivers in Eastern Canada.

	$\delta^{13}C$			$\delta^{15}N$		
River	Fin – Muscle	Muscle – Liver	Fin – Liver	Fin – Muscle	Muscle – Liver	Fin – Liver
Conne	0.963	0.945	0.920	0.697	0.602	0.809
Trinité	0.915	0.949	0.951	0.365	0.315	0.725
Kedgwick	0.905	0.936	0.869	0.988	0.582	0.647
Matapedia	0.810	0.834	0.846	0.898	0.855	0.894
Saint- Jean	0.940	0.930	0.928	0.677	0.787	0.773
Margaree	0.945	0.958	0.971	0.931	0.936	0.955
Northwest Miramichi	0.881	0.899	0.691	0.548	0.802	0.659
Southwest Miramichi	0.952	0.985	0.944	0.925	0.679	0.851
West River	0.902	0.937	0.900	0.897	0.908	0.899

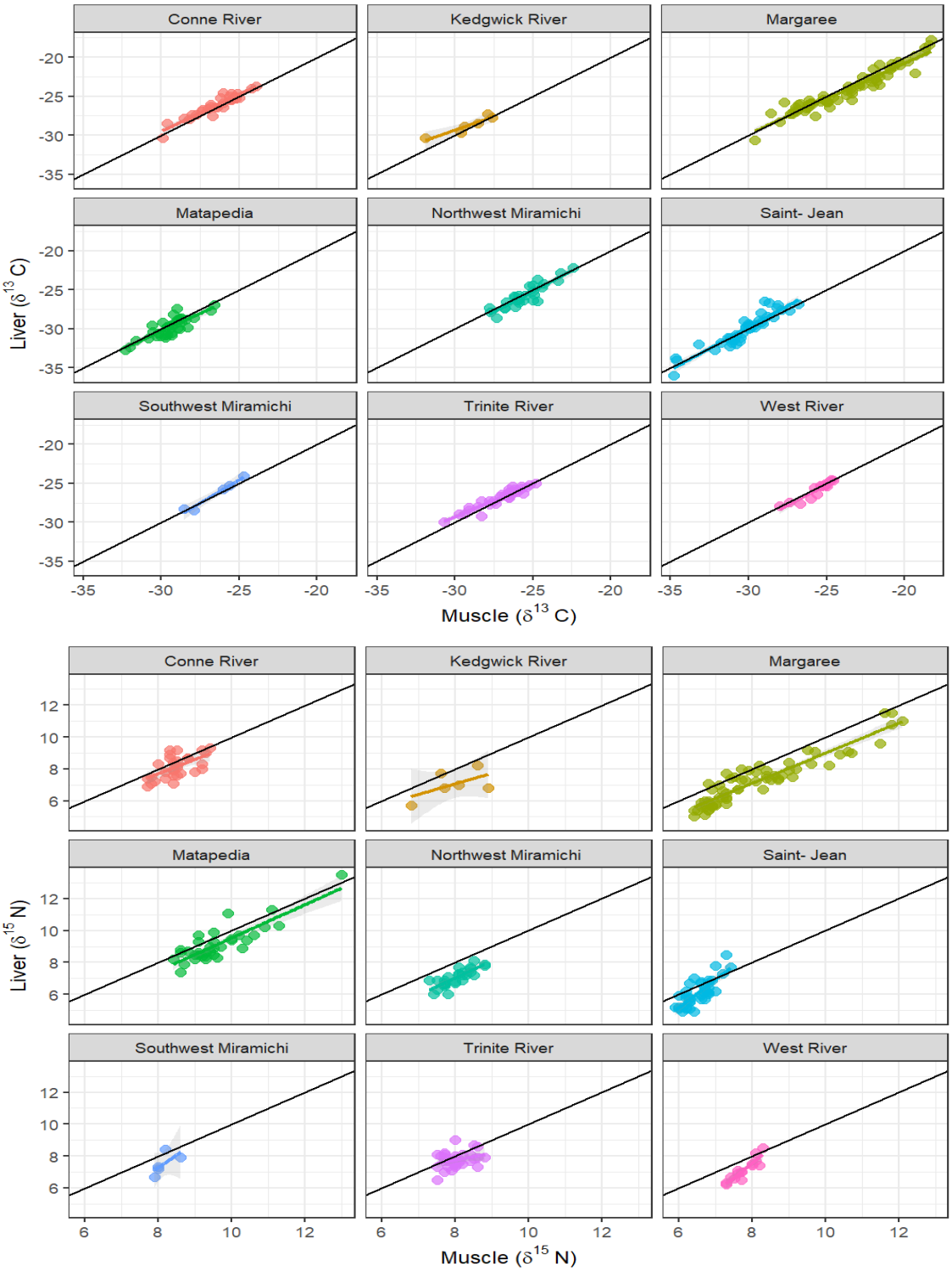

Figure E. Stable isotope ratios of liver $\delta^{13}C$ to muscle $\delta^{13}C$ (top), liver $\delta^{15}N$ to muscle ($\delta^{15}N$) (bottom), from the smolt sampled from nine rivers in Eastern Canada in 2022 and 2023. Each circle represents an individual smolt, and the fitted line represents a 1:1 ratio.

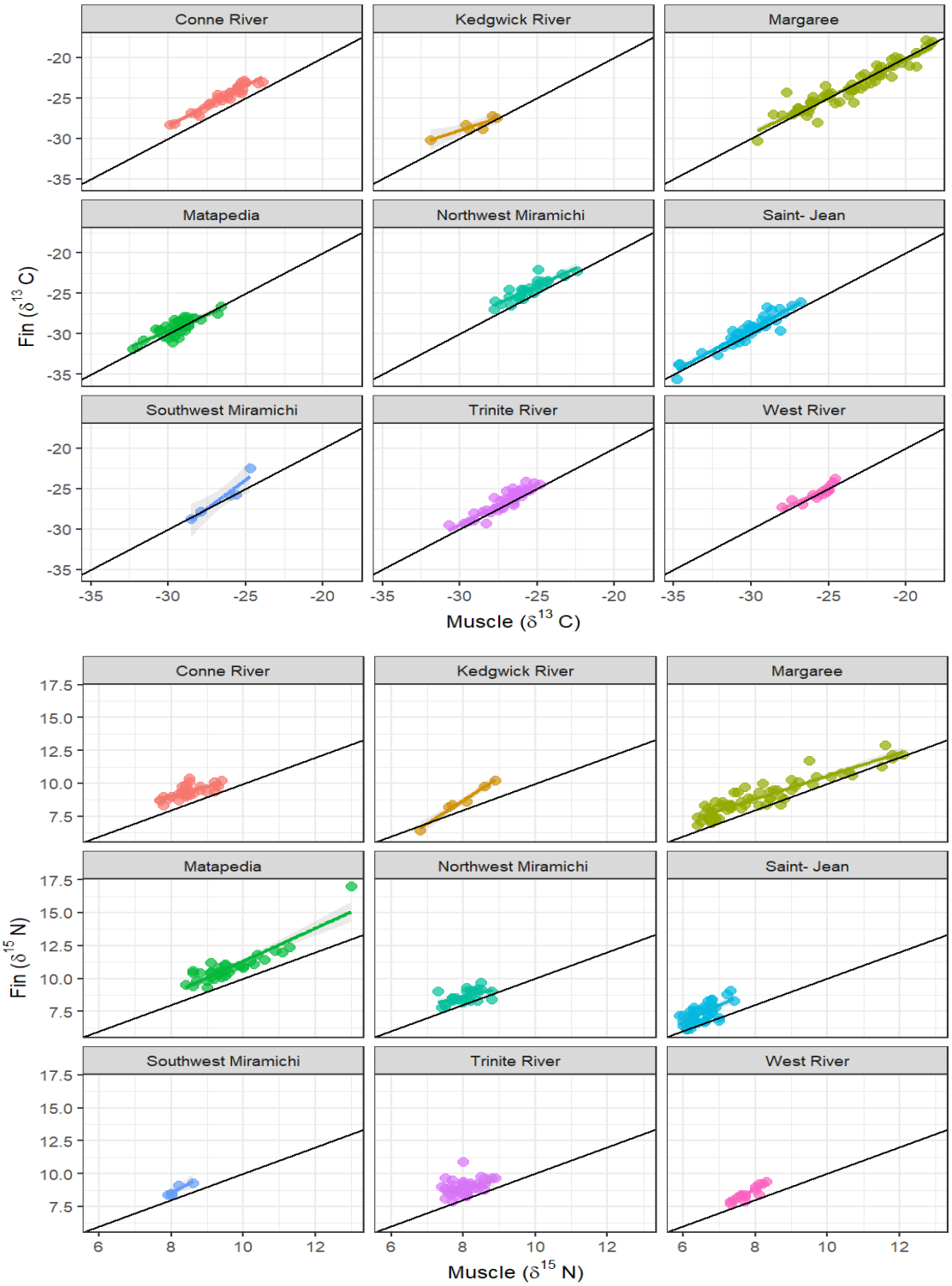

Figure F. Stable isotope ratios of fin $\delta^{13}C$ to muscle $\delta^{13}C$ (top), fin $\delta^{15}N$ to muscle ($\delta^{15}N$) (bottom), from the smolt sampled from nine rivers in Eastern Canada in 2022 and 2023. Each circle represents an individual smolt, and the fitted line represents a 1:1 ratio.

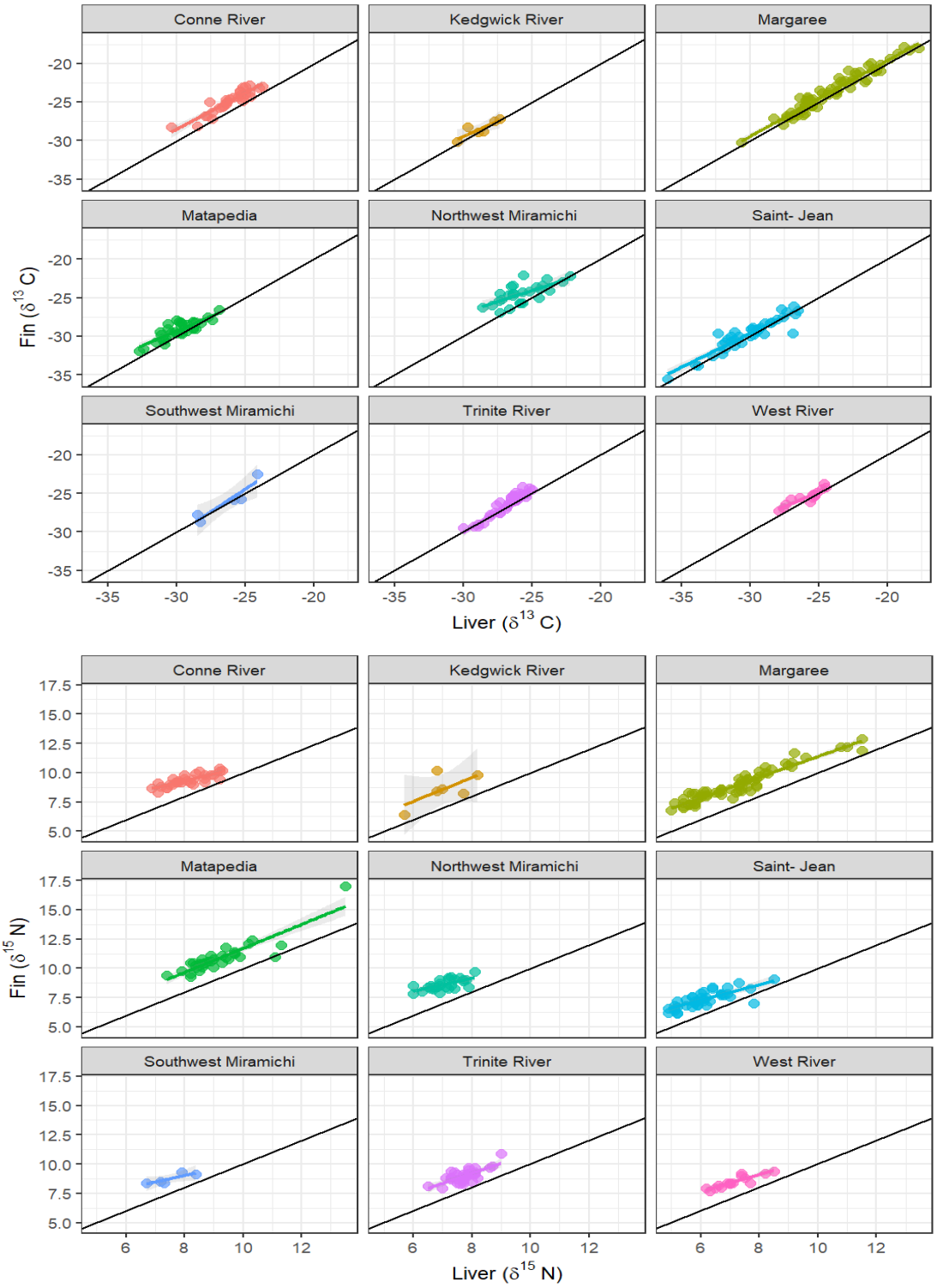

Figure G. Stable isotope ratios of fin $\delta^{13}C$ to liver $\delta^{13}C$ (top), fin $\delta^{15}N$ to liver ($\delta^{15}N$) (bottom), from the smolt sampled from nine rivers in Eastern Canada in 2022 and 2023. Each circle represents an individual smolt, and the fitted line represents a 1:1 ratio.

Migration resource use

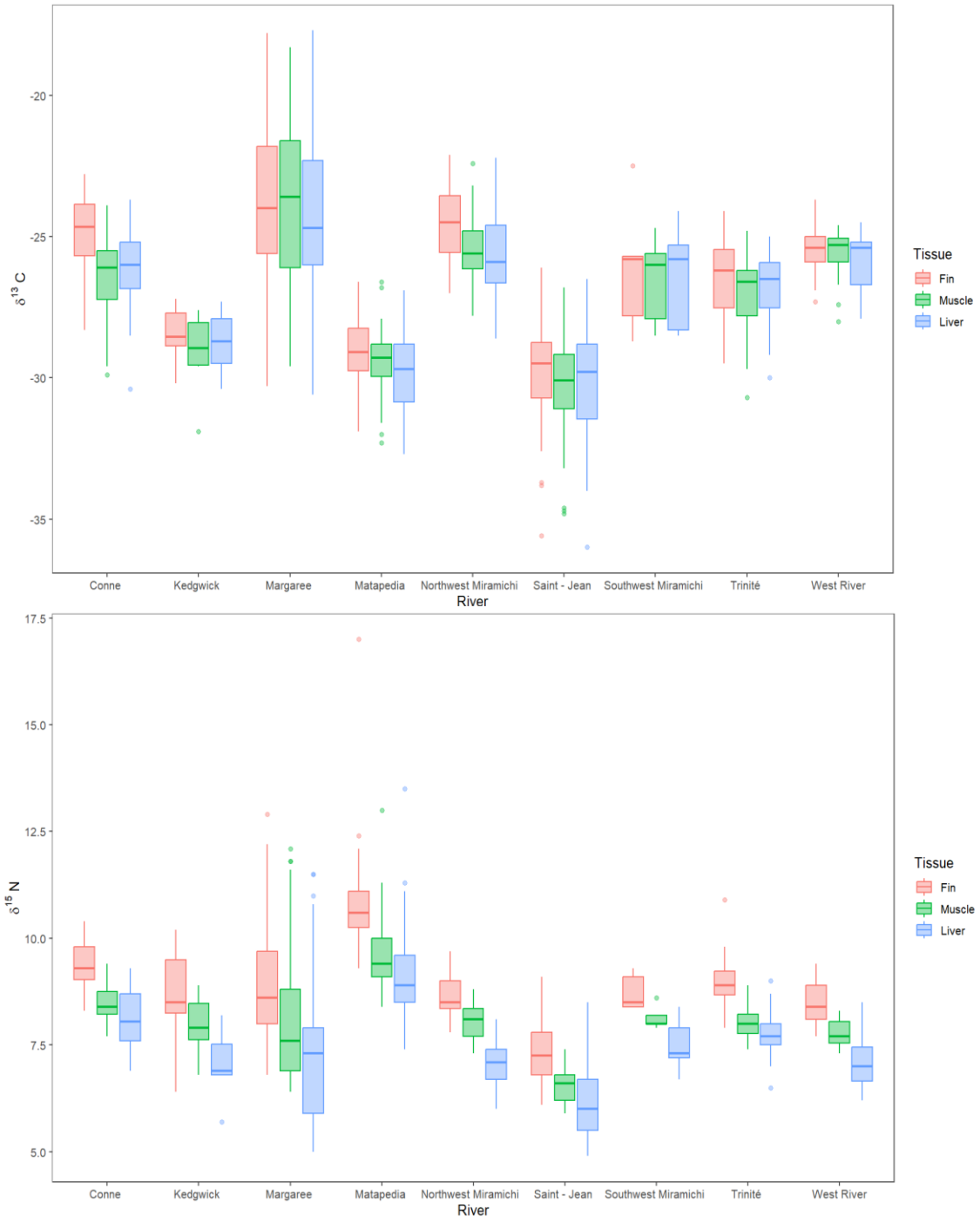

Figure H. Carbon ($\delta^{13}C$) (top) and Nitrogen ($\delta^{15}N$) (bottom) boxplots showing the median, interquartile range, whiskers and outliers for fin, muscle and liver tissues of Atlantic salmon smolt collected from rivers in Eastern Canada in 2022 and 2023, with data for the two years pooled.

A significant difference in $\delta^{13}C$ was found for Conne River ($p < 0.001$) with fin being significantly enriched compared to muscle ($p < 0.001$) and liver ($p = 0.00463$) and Northwest Miramichi ($p = 0.00361$) with fin being significantly enriched compared to muscle ($p=0.028$) and liver (0.00426) (Figure 7). All other rivers were found not to differ significantly from each other between all tissues ($p > 0.05$).

All rivers except for Kedgwick ($p=0.054$) were found to be significantly different in $\delta^{15}N$ for all tissues (Table 7). All rivers excluding Kedgwick were found to be significantly different when comparing the $\delta^{15}N$ values of fin and muscle except Southwest Miramichi (Table 7). A significant difference between muscle and liver was found in Trinité, Margaree, Northwest Miramichi, St. Jean and West River. All significant rivers were found to be significantly different in their liver to fin comparisons (Table 7).

Table 7. One-way analysis of variance (ANOVA) comparing tissue $\delta^{15}N$ values for each river indicating the degree of freedom, F-value, P-value and results from a Tukeys post-hoc test if significant for the pair-wise comparisons of fin (F), muscle (M), and liver (L) . * Denotes significant results in the Tukey's test.

River	ANOVA	p value	Tukey (F-M)	Tukey(M-L)	Tuckey (L-F)
Conne	F(2,87) = 37.83	<0.001	<0.001*	0.0512	0.00463*
Trinité	F(2,111) = 68.98	<0.001	<0.001*	0.0231*	<0.001*
Kedgwick	F(2,15) = 3.577	0.0537			
Margaree	F(2,192) = 21.13	<0.001	0.00641*	0.00232*	<0.001*
Matapedia	F(2,102) = 20.77	<0.001	<0.001*	0.197	<0.001*
Northwest Miramichi	F(2,78) =73.78	<0.001	<0.001*	<0.001*	<0.001*
St. Jean	F(2,117) = 36.02	<0.001	<0.001*	0.0147*	0.00409*
Southwest Miramichi	F(2,12) = 8.287	0.00549	0.162	0.131	<0.001*
West River	F(2,42) = 24.76	<0.001	0.00164*	0.00539*	<0.001*

Margaree River had the largest niche width size (based on SEAc calculations) across all tissue types, with the smallest being West River (based on fin and muscle), and Trinité River (based on liver) (Table 8). No consistent increasing or decreasing patterns were seen between tissues across all rivers.

Table 8. Niche width size estimated from Standard Area Ellipses (corrected for small sample sizes; SEAc) of fin, muscle, and liver for each river calculated using the SIBER package in Rstudio.

River	Fin (‰²)	Muscle (‰²)	Liver (‰²)
Conne	2.41	3.32	2.22
Trinité	2.62	1.54	1.96
Kedgwick	3.94	3.84	3.58
Margaree	11.94	13.28	12.7
Matapedia	4.92	3.61	4.72
Northwest Miramichi	1.89	1.67	2.78
St. Jean	4.44	2.11	4.66
Southwest Miramichi	4.21	1.78	5.26
West River	1.65	1.08	2.39

Comparison between populations and groups – Muscle tissue

The uncorrected muscle $\delta^{13}C$ values were significantly different across rivers ($p<0.001$) with Margaree having significantly enriched $\delta^{13}C$ values compared to all other rivers (Table 9; Figure 8). Moreover, the corrected $\delta^{13}C$ data was also different between rivers (Chi-squared = 105.63, df = 4, $P<0.001$) (Table 9; Figure 8). The Dunn's post-hoc test revealed that four River interactions did not differ significantly for the corrected $\delta^{13}C$: Kedgwick and Matapedia, Margaree and Northwest Miramichi, Matapedia and West River, and Kedgwick and West River.

Uncorrected muscle $\delta^{15}N$ values for all nine rivers were significantly different from each other (Chi-squared = 148.67, df = 8 $p < 0.001$). Dunn's post-hoc test results are presented in Table 10 with nineteen interactions showing a significant difference between rivers. The corrected $\delta^{15}N$ values were also found to be significantly different from each other (Chi-squared=61.266, df = 4, $P>0.001$) with eight river interactions showing a significant difference between corrected $\delta^{15}N$ (Table 10; Figure 9).

Table 9. Tukey results from one-way ANOVA for uncorrected $\delta^{13}C$ compared across rivers and the Dunns post-hoc test from the Kruskall-Wallis non-parametric test for the baseline corrected $\delta^{13}C$. G indicates the group association from the Tukey's post-hoc test, rivers that do not share letters are significantly different from each other. Baselines were not sampled for Conne, Southwest Miramichi, Trinité, and St. Jean rivers.

River	Group	Mean $\delta^{13}C$	G1	G2	G3	G4	G5	Mean $\delta^{13}Cc$	G1	G2
Margaree	Southern Gulf of St. Lawrence and Cape Breton	-23.71	a					2.18	a	
Northwest Miramichi	Southern Gulf of St. Lawrence and Cape Breton	-25.5		b				2.33	a	
West River	Nova Scotia Southern Upland - East	-25.55		b	c			0.26		b
Conne	South Newfoundland - West	-26.42		b	c					
Southwest Miramichi	Southern Gulf of St. Lawrence and Cape Breton	-26.54		b	c	d				
Trinité	Quebec Western North Shore	-27.06			c	d				
Kedgwick	Gaspé	-29.15				d	e	0.21		b
Matapedia	Gaspé	-29.43					e	0.17		b
St. Jean	Gaspé	-30.27					e			

Table 10. Dunns post-hoc test from the Kruskall-Wallis non-parametric test for mean uncorrected $\delta^{15}N$ (Mean $\delta^{15}N$) and the for the mean baseline corrected $\delta^{15}N$ (Mean trophic position (TP)) compared across rivers with their respective group assignment. G indicates the group association from the Tukey's post-hoc test, rivers that do not share letters are significantly different from each other. Baselines were not sampled for Conne, Southwest Miramichi, Trinité, and St. Jean rivers.

River	Group	Mean $\delta^{15}N$	G1	G2	G3	G4	Mean TP	G1	G2	G3
Matapedia	Gaspé	9.63	a				2.25	a		
Conne	South Newfoundland - West	8.47		b						
Southwest Miramichi	Southern Gulf of St. Lawrence and Cape Breton	8.14		b	c					
Margaree	Southern Gulf of St. Lawrence and Cape Breton	8.12		b	c		1.9		b	
Trinité	Quebec Western North Shore	8.05		b	c					
Northwest Miramichi	Southern Gulf of St. Lawrence and Cape Breton	8.03		b	c		1.59			c
Kedgwick	Gaspé	7.95		b	c		2.46	a		
West river	Nova Scotia Southern Upland - East	7.62			c		2.06	a	b	
St. Jean	Gaspé	6.53				d				

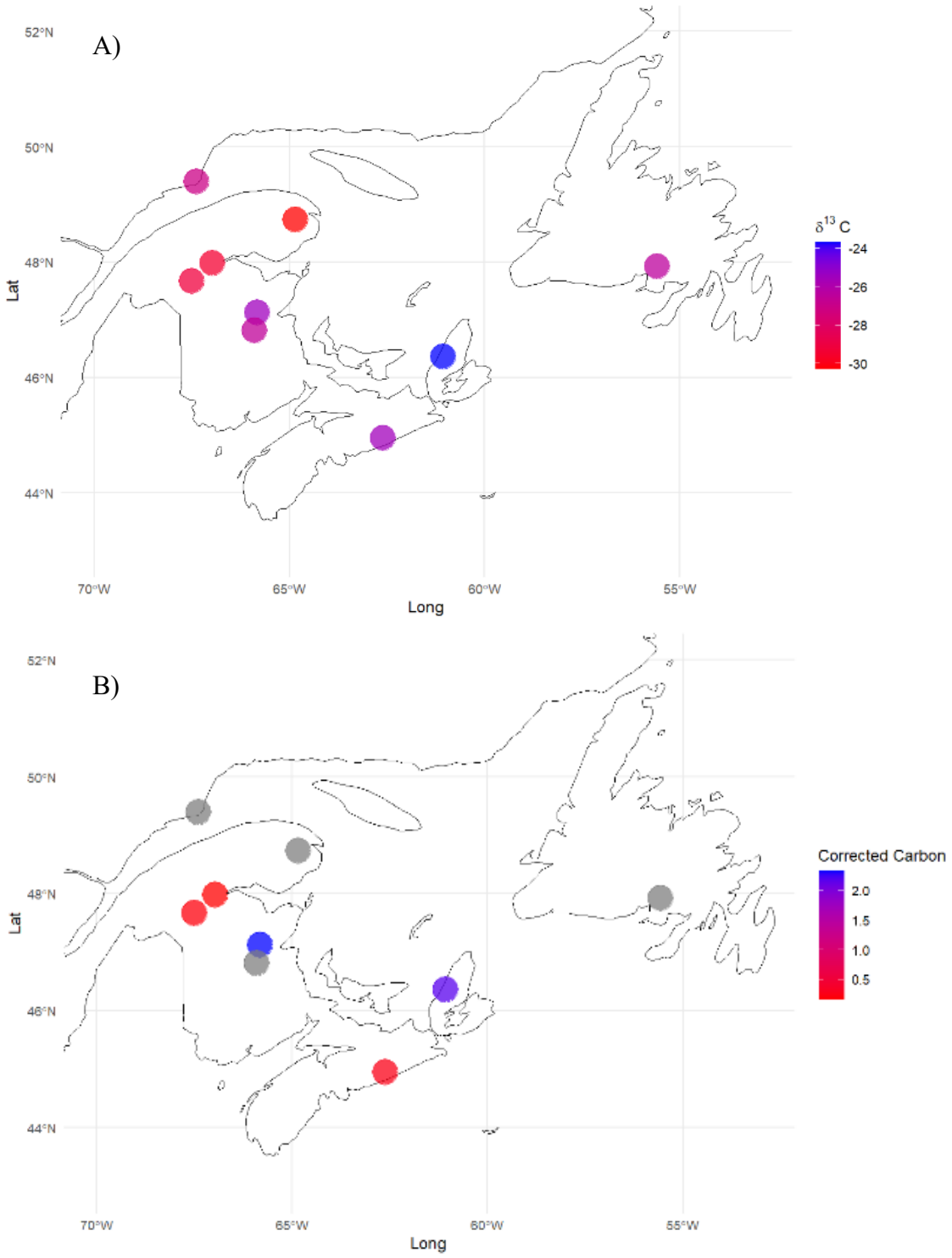

Figure I. Heat map of mean uncorrected $\delta^{13}C$ of Atlantic salmon smolt muscle tissues from all nine rivers (A) and the mean baseline corrected carbon ($\delta^{13}Cc$) of smolt muscle tissue from the five rivers where baselines were collected (B). Blue colouring indicates enriched $\delta^{13}C$, red indicates more depleted $\delta^{13}C$, and grey indicates sites with no baseline collection.

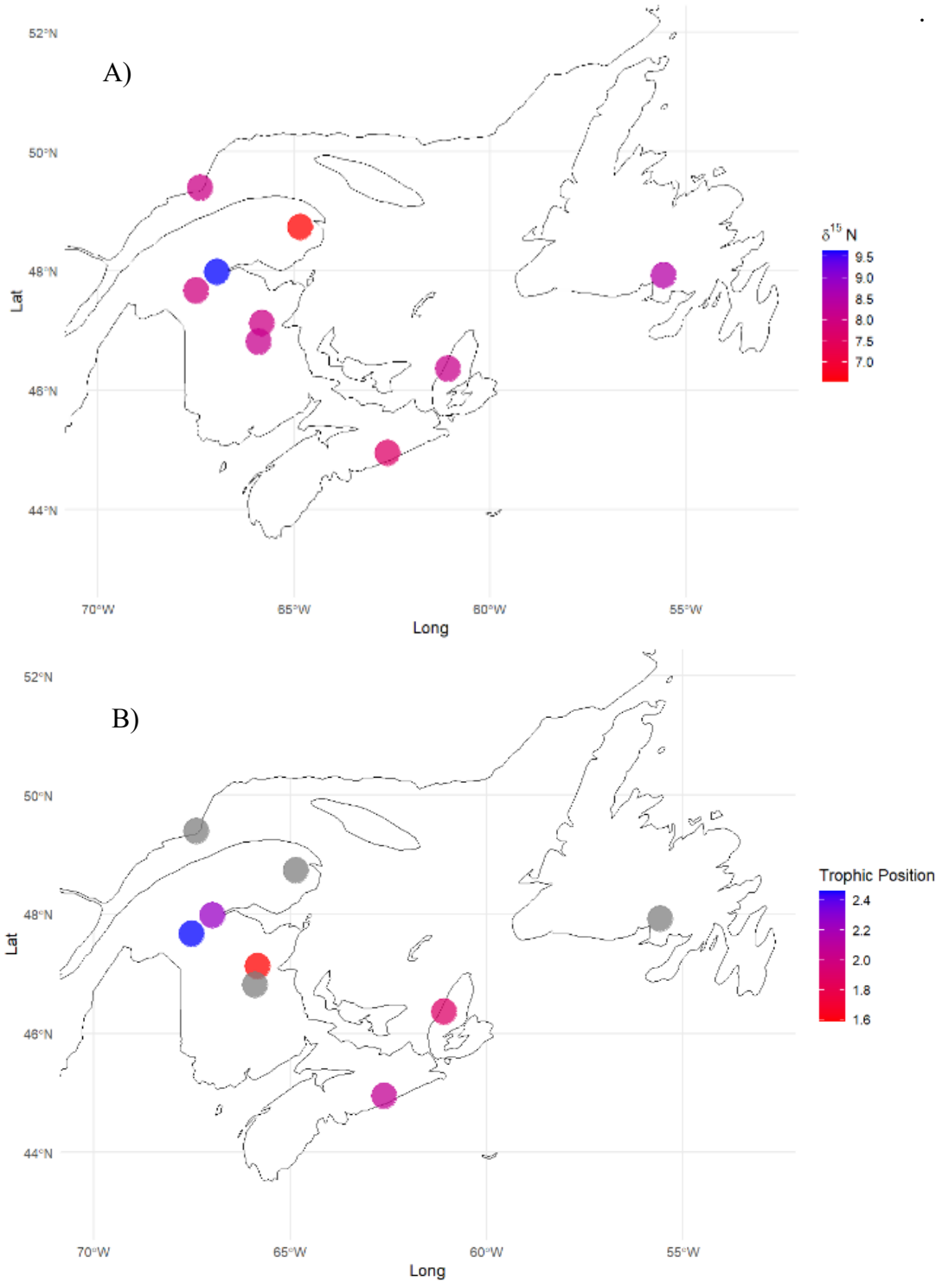

Figure J. Heat map of mean uncorrected $\delta^{15}N$ of Atlantic salmon smolt muscle tissues from all nine rivers (A) and the mean baseline corrected $\delta^{15}N$ (Trophic position) of smolt muscle tissue from the five rivers where baselines were collected (B). Blue colouring indicates enriched $\delta^{15}N$, red indicates more depleted $\delta^{15}N$, and grey indicates sites with no baseline collection.

In the baseline corrected stable isotope values, Kedgwick, Matapedia and West rivers were found to have a very similar distribution on the corrected carbon (δ^{13}Cc) axis (Table 9; Figure 10). Margaree ($\bar{x}$ = 2.18) and Northwest Miramichi ($\bar{x}$ = 2.33) were found to have larger δ^{13}Cc values (Figure 10). The trophic position (TP) of smolt from Margaree had the largest range from 1.39 to 3.06, followed by Matapedia (TP: 1.89 – 3.24), Kedgwick (TP: 2.12 – 2.74), Northwest Miramichi (TP: 1.37 – 1.81) and West River (TP: 1.93-2.22) (Figure 10).

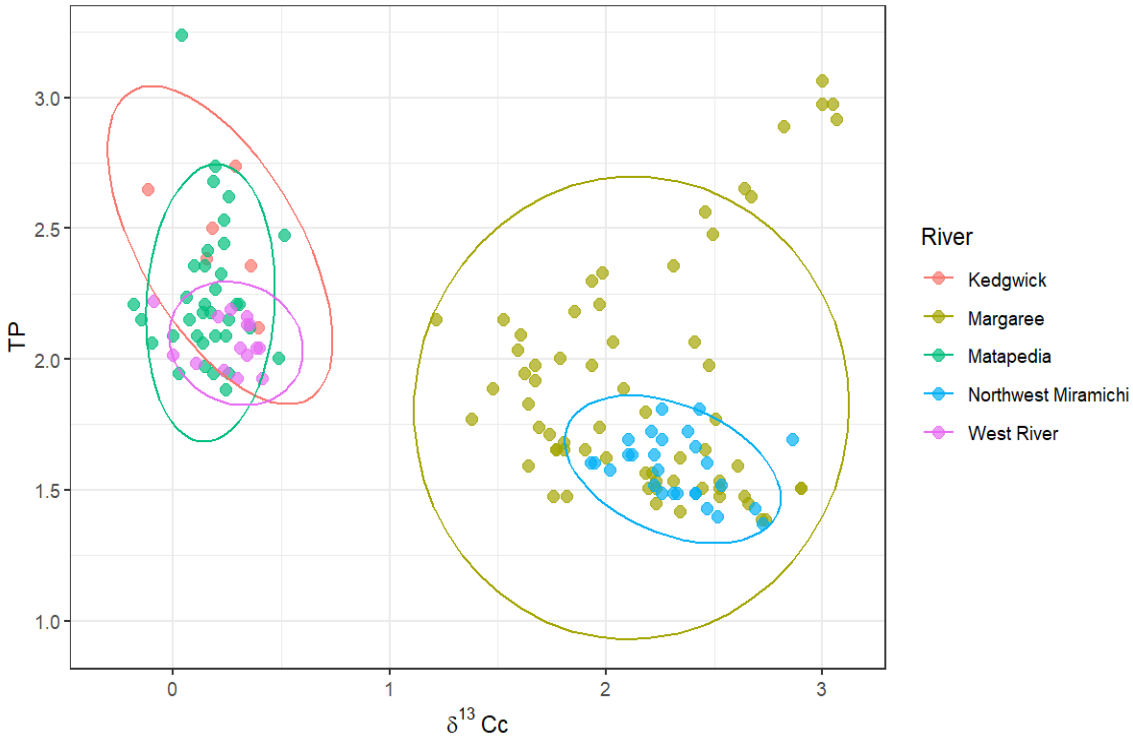

Figure K. Stable isotope biplot of the baseline corrected δ^{13}C (δ^{13}Cc) and corrected δ^{15}N (trophic position) of Atlantic salmon smolt muscle tissue from Kedgwick River, Margaree River, Matapedia River, Northwest Miramichi River and West River. A standard ellipsis representing 95% distribution of the data was added to the biplot.

Effect of year on smolt fork length

Year did not have a significant effect on fork length (p=0.621) while river did (p < 0.001) for smolts collected in 2022 and 2023. Although there was a significant

interaction of between year and river (F=3.30, R^2 = 0.016, p = 0.020), river had a much larger effect on the stable isotope ratios (F= 34.57, R^2=0.169) compared to year (F= 0.250, R^2 = 0.00041) and therefore river fork length data for 2022 and 2023 could be pooled (Table 11).

Table 11. Sample size, mean, standard deviation, minimum and maximum for fork length (mm) for Atlantic salmon smolt collected from nine rivers in Eastern Canada in 2022 and 2023.

River	N	Mean	SD	Min	Max
Conne	30	146.57	16.15	116	183
Trinité	40	128.85	9.77	110	152
Kedgwick	6	125.33	14.75	108	143
Matapedia	35	140.34	12.67	109	161
St. Jean	40	118.42	8.68	103	151
Margaree	65	137.14	21.96	102	259
Northwest Miramichi	27	128.11	10.27	105	153
Southwest Miramichi	5	125.8	16.81	105	150
West River	15	137.13	5.07	126	145

Conne River had the largest mean fork length while St. Jean River had the smallest mean fork length (Table 11; Figure 11). Margaree had the largest size range (157mm) in smolt with it having the largest and smallest individual smolt based on fork length, with two large outlier smolt found at 178mm and 259mm (Figure 11). Trinité had an outlier at 152mm and St. – Jean had an outlier at 151mm. No strong correlations were observed for Carbon:Nitrogen to fork length for each river with all correlation coefficients less than less than 0.500 and greater than -0.500, except Southwest Miramichi (0.515) and Kedgwick (0.813); however, the sample size for these two rivers were much lower than the other rivers sampled (Figure 12).

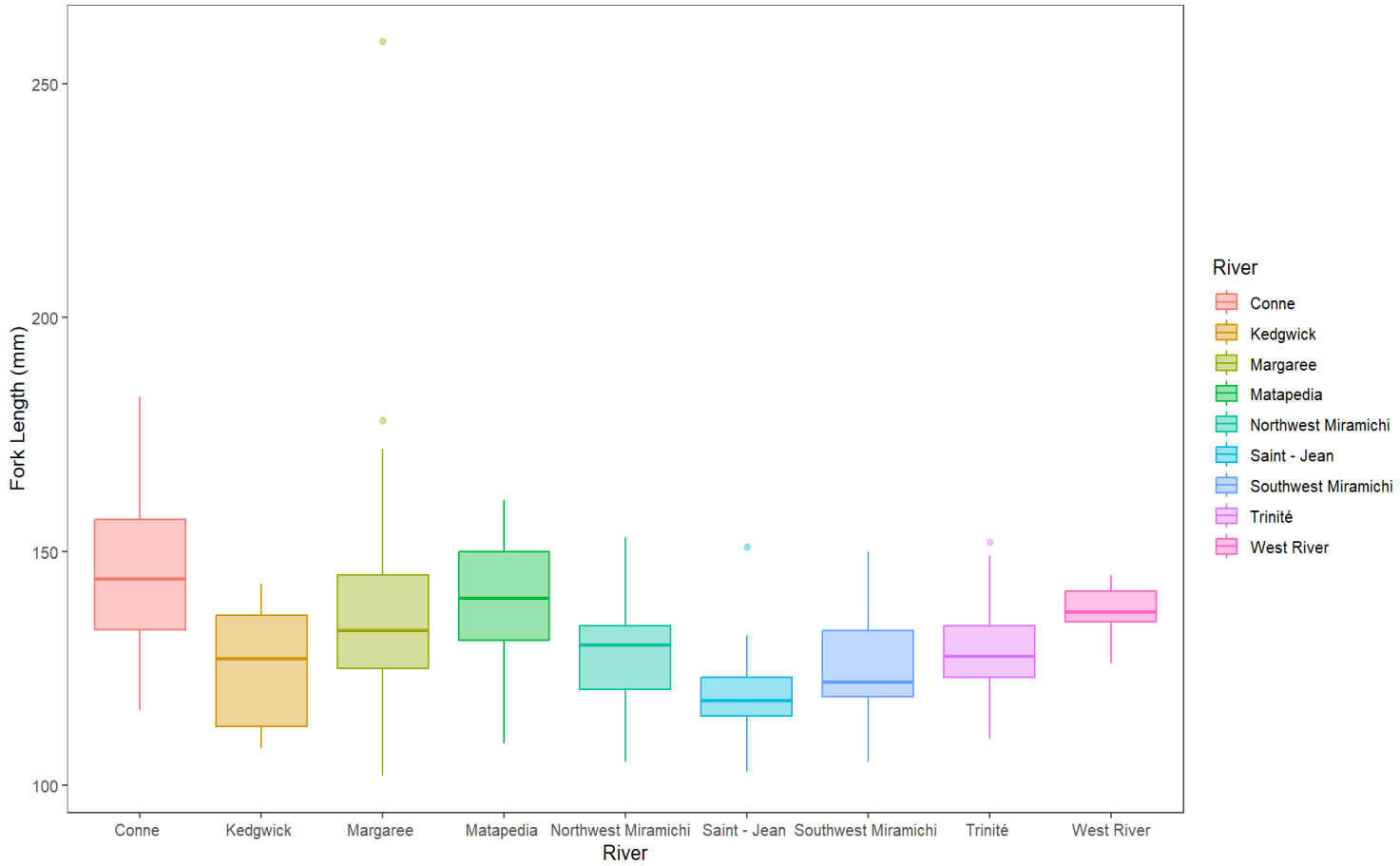

Figure L. Boxplot displaying the median, interquartile range, tails and outliers for fork length (mm) of Atlantic salmon smolt collected in nine rivers. Trinité, Margaree, and St. Jean showing outliers of larger smolt.

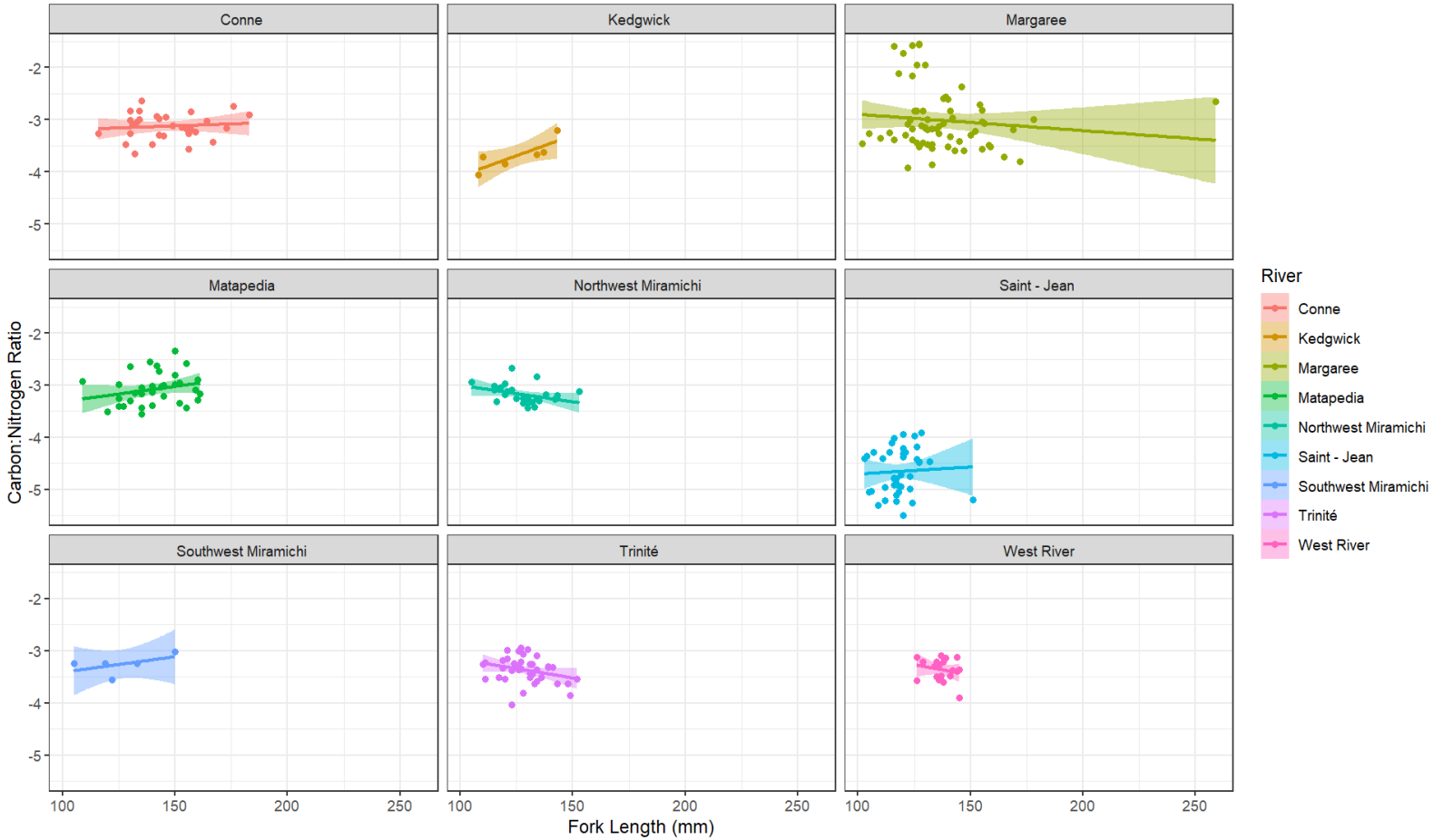

Figure M. $\delta^{13}C$:$\delta^{15}N$ ratio to fork length (mm) correlation for rivers where Atlantic salmon smolt were collected. Shaded area represents the 95% confidence range for the linear relationship.

Table 12. Biological condition measurements including the sample size (n), the mean and standard deviation (SD) of weight (g), condition factor (K), heptosomatic index (HSI), bioelectrical impedance meter (%) total lipid, carbon: nitrogen (C:N) ratios in muscle tissue, Distell Fish Fat Meter (%) total lipid for all smolt collected in 2022 and 2023 from the nine sampled rivers in Eastern Canada. * Indicates no standard deviation value due to sample size of 1 for that measurement.

Year	River	n	Weight	SD	Condition Factor	SD	HSI	SD	BIM mean	BIM SD	C:N mean	C:N SD	n	Distell mean	Distell SD
2022	Trinité	20	19.55	5.85	0.85	0.06	0.72	0.15	0.29	0.30	3.25	0.051	9	3.68	0.48
	St. Jean	20	13.67	4.34	0.79	0.6	0.70	0.15	0.37	0.36	3.37	0.098	1	6.3	*
	Margaree	30	23.15	19.68	0.87	0.12	1.10	0.24	0.13	0.41	3.25	0.068	20	4.39	1.68
	Northwest Miramichi	17	22.8	5.96	1.01	0.08	0.93	0.07	-0.039	0.20	3.35	0.062	11	4.32	1.00
	West River	15	26.1	3.17	1.01	0.05	1.10	0.26	-0.58	0.35	3.25	0.052	15	6.85	2.80
2023	Conne	30	32.02	1.83	0.99	0.016	0.72	0.03	0.31	0.21	3.27	0.071	29	2.62	0.84
	Trinité	20	17.50	0.68	0.85	0.017	0.77	0.12	0.44	0.22	3.24	0.049	14	3.44	2.48
	Kedgwick	6	18.73	2.37	0.94	0.056	1.23	0.12	0.29	0.19	3.27	0.12	3	3.7	0.2
	Matapedia	35	24.74	1.1	0.87	0.011	1.23	0.04	0.17	0.33	3.22	0.038	31	4.75	1.23
	St. Jean	20	13.27	0.64	0.80	0.012	1.10	0.05	0.70	0.17	3.22	0.037	4	2.3	0.58
	Margaree	35	26.31	1.6	0.93	0.013	1.09	0.04	0.076	0.27	3.28	0.10	29	4.89	1.23
	Northwest Miramichi	10	18.31	1.93	0.92	0.031	0.99	0.09	0.25	0.39	3.26	0.070	5	4.72	0.96
	Southwest Miramichi	5	19.65	3.6	0.96	0.058	1.00	0.11	0.23	0.28	3.20	0	2	4.2	1.56

Condition measurements

In addition to having the longest average smolts, Conne River was also found to have the heaviest average smolts and St. Jean had the lowest mean weight and fork length smolts (Table 12). For all rivers condition factors were found to be between 0.79 – 1.01. HSI was found to range between 0.70 – 1.23 with St. Jean, Trinité, and Conne having the lowest HSI values. Lipid results varied between the two methods used to measure total % lipid. The bioelectrical impedance meter produced mean % lipid for smolt between -0.58 – 0.70, while the Distell fat meter had mean lipid values between 2.3 – 6.85%. In addition, for muscle tissues the C:N ratios for each river all fell within 3.20 – 3.37 which are typical ranges for smolt.

3.4: DISCUSSION

The goal of this study was to gain ecological knowledge from euthanized salmon and examine shifts in resource use from the parr to smolt phases prior to passing through smolt wheels/fish counting fences. Additionally, comparing the isotopic signatures and body size trends of smolt from multiple rivers in Eastern Canada can provide insight into the spatial variations of resource use, highlighting rivers that may offer more favourable conditions for growth in smolts.

Individual specialization

Individual resource use was examined to determine if rivers showed dietary consistency among individual smolts. Across all rivers high correlations were found between fin – muscle, muscle – liver, and fin – liver for both $\delta^{13}C$ and $\delta^{15}N$. The only exception was seen in Trinité for $\delta^{15}N$. This may suggest individual diet variability occurring in Trinité for trophic level, whereas all other rivers were found to be comprised of individual specialists throughout their long- and short-term diet.

River wide resource use

On a population level, only Conne River and Northwest Miramichi River were found to have significantly different $\delta^{13}C$ values between fin and muscle tissues. All other rivers showed no difference in $\delta^{13}C$ between tissues which suggests smolts analyzed in this study are not dramatically changing their prey selection from overwinter to capture in the smolt wheels (Hayden *et al.,* 2015). Additionally, given the observed decrease in metabolic rates of juvenile salmonids during the overwintering period, it is likely that their energy sources remain consistent from the fall months (Cunjak *et al*., 1998). Although linear patterns were seen for $\delta^{15}N$ between tissues, the depleted $\delta^{15}N$

from fin to muscle to liver pattern was consistent across all rivers, which could suggest a change to smaller prey closer to their capture date (Fry, 2006).

Fins have been observed to have similar turnover rates to muscle tissue with the turnover rate in fish being 2-5 months (Vander Zanden *et al.,* 2015; Dvergedal *et al*., 2019). The relationship between fin and muscle stable isotope values in fish is species specific (Willis *et al.,* 2013) and has been observed for multiple salmonid species to determine if caudal fin is a useful non-lethal surrogate for muscle stable isotope analysis (Hanish *et al.,* 2010). Hanish *et al.,* (2010) found little variation between fin and muscle tissues for most salmonid species with the exception of $\delta^{13}C$ in lake trout (*Salvelinus namaycush*). Moreover, studies focused on Atlantic salmon have found minimal variation in both $\delta^{13}C$ and $\delta^{15}N$ in fin and muscle tissues between smolts (Jardine *et al.,* 2005) and adults (Dempson and Power, 2004), although statistical significance was present in the adult study.

The present study shows consistent variation in $\delta^{15}N$ between fin and muscle tissues with muscle being depleted in $\delta^{15}N$, contrary to previous studies (Jardine *et al.,* 2005). The variation between fin and muscle seen in this study could be caused by fin tissues having different turnover rates than previously thought (Hayden *et al.,* 2017; Vander Zanden *et al.,* 2015) or may be due to the composition of the tissues and how they reflect their $\delta^{15}N$ values (Hayden *et al.,* 2015). Fin tissues may be enriched due to their heterogeneous structure, comprised of membrane and ray tissues (Hayden *et al.,* 2015). In this study, the tip to mid sections of the fin were taken which includes a high to moderate proportion of membrane to ray tissues (Hayden *et al.,* 2015). The tip section has previously been found to be more $\delta^{15}N$ depleted compared to base sections and may

be linked to a shorter-term diet due to the fast growth and tissue regeneration (Hayden *et al.,* 2015). Although fin has been used as the long-term diet tracer in past studies, more research would be needed to understand the turnover rate of different sections of the fin, especially on fast growing juvenile smolts.

If the tip of the fins is associated with the growth and regeneration of the smolt it could suggest that the fin sampled from this study are showing enriched $\delta^{15}N$ due an enriched dietary source and may be more indicative of a short-term diet (Hayden *et al.,* 2015; Heady and Moore, 2013). A laboratory study conducted on rainbow trout (*Oncorhynchus mykiss*) tissue turnover rates found that the fin tissue showed a diet switch at twelve days, however, the authors concluded this was unusually fast (Heady and Moore, 2013). Smolt would exhibit increased gape size compared to their parr counterpart which would allow them to increase the size of their prey and ultimately their $\delta^{15}N$ values (Hayden *et al.,* 2015). If the fin is more indicative of a short-term diet it may suggest the enriched $\delta^{15}N$ values, are due to an increase in trophic position from feeding on larger prey compared to their parr counterparts.

Even with the uncertainty in turnover rates for fin, the turnover rate between muscle and liver is well studied in fish and the turnover rate of liver is faster than that of muscle (Logan *et al.,* 2006). The consistent decrease from muscle to liver in $\delta^{15}N$ across all rivers suggests from the fall/winter months to a few weeks before capture at the smolt wheels, smolt may be experiencing a shift in trophic positions. A decrease of 1 – 1.5 ‰ in $\delta^{15}N$ wouldn't suggest a full shift in trophic levels; however, it may suggest smolt are feeding on different sized prey as they are migrating (Fry, 2006). Salmon are generalist feeders, and although as reduced food consumption has been observed in smolt

(McCormick and Saunders, 1987; Stefansson *et al*., 2003), they could be allocating most of their energy on the migration and passively feed drifting nearby prey. Moreover, the depletion of $\delta^{15}N$ provides evidence that smolt are feeding prior to their migration and are not experiencing food deprivation prior to capture at the smolt wheel (Doucett *et al.,* 1999). We would see an increase in $\delta^{15}N$ with a prolonged starvation period which has been established in previous studies focused on the proportion of food in the stomach of smolts (Larsson *et al.,* 2011).

While enriched $\delta^{15}N$ in the fin and muscle tissues might suggest starvation overwinter, previous research has documented depleted $\delta^{15}N$ values in the liver compared to those of muscle in Atlantic salmon (Jardine *et al.,* 2005). The consistent $\delta^{15}N$ depletion in liver tissues across various rivers and studies (i.e., Peterson *et al.,* 1993; Pinnegar and Polunin, 1999; Johnson *et al.,* 2002; Bunn *et al.,* 2003; Jardine, 2005) implies that this is a normal characteristic of fish liver, rather than evidence of a dietary shift. A potential reason could be the trophic amino acid composition of the tissues (Stephens *et al.,* 2023; Whiteman *et al.,* 2019), therefore the slight depletion in $\delta^{15}N$ may be caused by the structure of the tissue more so than a shift in prey (Hayden *et al.,* 2015).

Niche width

There was no trend observed for niche width size between tissues in each river. We expected to see a decrease in niche width in liver compared to muscle and fin as we expected the smolt to feed on a smaller pool of prey as they migrate towards the ocean from various freshwater habitats. However, this was only observed in Conne River and Kedgwick River. Over half of the rivers displayed an increase in niche width for liver

which may be due to smolt utilizing different energy sources in the new habitat and may be feeding on both larger and smaller prey to optimize energy (Larson *et al.,* 2011; Mikheev, 1984).

The Margaree had much larger niche widths for all three tissues compared to all other rivers. The Margaree River consists of two main river branches: the southwest Margaree and the northwest Margaree. The southwest has a large lake and is surrounded by farmland while the northwest is fed by many brooks and also passes through farmlands (Belding and Clark, 1938). Smolt coming from two river branches with separate habitats could cause a partition in resources (Hobson, 1999), particularly as smolt originating from the southwest developed in a lacustrine habitat compared to a riverine.

River comparisons

We expected salmon groups from similar groups to share similarities in their stable isotope composition because they are close spatially (Glibert *et al.,* 2019; Hobson, 1999). For carbon without the baseline corrections applied, rivers from the Southern Gulf of St. Lawrence and Cape Breton group, Nova Scotia Southern Upland – East group, and South Newfoundland - West group had the most enriched $\delta^{13}C$ values followed by Quebec Western North shore and then Gaspé group. This pattern stayed relatively similar with the baseline corrected rivers; however, West River switched to having more similar carbon values to the Gaspé rivers.

This could suggest that the baselines we collected were reflective of the diets from smolt in the Matapedia, Kedgwick, and West River but not for those of the

Margaree or Northwest Miramichi rivers. Margaree and Northwest Miramichi smolt are showing a more enriched carbon signature compared to their collected prey when corrected. A corrected carbon value close to 0 indicates similarity in the consumer consuming the prey used in the correction formula (Olsson *et al.,* 2009). Similar BMIs were used in this calculation for each river (appendix B.1); however, Margaree and Northwest Miramichi are showing deviation from the 0 intercept on the axis. This suggests the prey collected for this study do not encompass the prey consumed in the Margaree and Northwest Miramichi, and that they are consuming prey enriched compared to the baselines collected.

Enriched carbon values could stem from multiple reasons, enriched carbon in aquatic systems is typically indicative of marine signatures for fish species (France, 1995). This suggests there may be some input from marine or estuarine prey. Enriched carbon or nitrogen may also be indicative of anthropogenic activity such as inputs from organic fertilizers from agriculture (Freedman *et al.,* 2012). Moreover, there could have been some seasonality effects on the prey collected, since the baselines were not collected at the same time as the smolt.

The baselines collected for Northwest Miramichi, Margaree and West rivers were collected in the fall, whereas the smolts were collected in the spring. Basal isotopic signatures in river environments are typically more enriched in both $\delta^{13}C$ and $\delta^{15}N$ signatures during the fall compared to the spring (Harmelin-Vivien *et al.,* 2010). Harmelin – Vivien *et al.* (2010) reported seasonality differences of approximately 3‰ for $\delta^{13}C$ and 1‰ for $\delta^{15}N$ when examining seasonal changes in particulate organic carbon and nitrogen in a river system. Accounting for this seasonal variation, it suggests that the

smolts from Margaree and Northwest Miramichi may still be feeding on prey more enriched than the BMIs collected for this study.

For $\delta^{15}N$ no apparent pattern was observed among groups spatially for the uncorrected and baseline corrected data. However, the corrected data showed similarities between Matapedia, Kedgwick and West River in trophic position. Given the similarities in both $\delta^{13}Cc$ and TP, the West River appears more similar to the Matapedia and Kedgwick rivers in terms of productivity and resource availability for smolt. Similarities in river characteristics between Matapedia, Kedgwick and West River should be examined to see if factors such as temperature, biodiversity, surrounding habitat, etc. may be driving the similarities between these rivers that are not geographically close.

Body condition and resource use

Between the nine rivers body condition measurements across length, weight, condition factor, and lipid composition varied with no apparent trends seen between rivers. Conne river consistently had the largest smolt for fork length as well as in weight, while St. Jean had the smallest smolt in fork length and for weight. The only trends observed for increased isotope ratios and smolt size were observed in Southwest Miramichi and Kedgwick rivers, two rivers with sample sizes less than ten. The condition factor within all rivers were found to be between 0.8-1.01, this range of condition factor is normal for smolt as they experience a rapid growth in length compared to weight during this stage (McCormick, 2012; Ravikumar *et al.*, 2023). No rivers were found to have very low condition factors (i.e. < 0.8) throughout this study; however, it is interesting to note that the rivers with higher condition (>0.95) were found

to come from the Nova Scotia Southern Upland - East, and the South Newfoundland - West Regions.

Two lipid measurements were considered in this study: bioelectrical impedance and Distell Fish Fat Meter. Smolts experience a decrease in lipid levels when changing from the parr to smolt phase therefore lipid values were expected to be low (Meador *et al.,* 2002). The BIM produced percent lipid values less than 1% at a range of -0.58 to 0.70%, these results were lower than expected as smolt have shown lipid values between 2 – 4% in previous studies (Steffanson *et al.,* 2012) which were more similar to the results produced by the Distell Fat Meter. Based on this study, the bioelectrical impedance meter produced unreliable results with showing negative total lipid contents whereas, showing the Distell Fat Meter may prove more useful for measuring the lipid content of euthanized salmon smolt.

We examined the combined $\delta^{13}C$ and $\delta^{15}N$ to fork length for each river to examine if fork length was a factor for driving increased stable isotope ratios in smolt or vice versa. Resource use is connected to body size as productive systems with favourable feeding conditions affect length positively (Armstrong *et al.,* 2018). Combining $\delta^{13}C$ and $\delta^{15}N$ suggests we are expecting smolts with increased $\delta^{13}C$ to be larger; however as stated in Chapter 1, $\delta^{13}C$ is indicative of energy source rather than resource abundance (Post, 2002). Therefore, examining the relationship between $\delta^{13}C$ and fork length may not be as useful as the relationship between $\delta^{15}N$ and size (DeNiro and Epstein, 1981; Jennings *et al.,* 2008). For the purposes of this study, however, $\delta^{13}C$ and $\delta^{15}N$ were combined to examine the relationship between increased size and isotopically enriched smolt, and only found two rivers where the relationship was apparent, both with low sample sizes.

Conclusion

This study provides additional information on the feeding habits of smolts prior to their out-migration and found that smolts have high individual diet specialization as well as consistency within their energy source between tissues. A decrease in $\delta^{15}N$ was seen from long to short term tissues which could be indicative of a downwards trophic shift prior to capture at the smolt wheel or the chemical composition of the tissues. Future lab-based studies on the turnover rate of fin for juvenile salmonids may allow us to correctly place the isotopic values of fin on an isotopic clock.

Not all smolts that were genetically assigned to the same groups exhibited similar or distinct resource use. Smolts from the Margaree and Northwest Miramichi rivers had significantly different $\delta^{15}N$ values, while those from West River showed resource use patterns similar to the Gaspé rivers. Group assignment of smolt may not be driving resource use and it looks to be more river specific, other than Margaree which is experiencing a much larger niche width than all other rivers. Moreover, no specific trends were observed with increasing $\delta^{13}C$: $\delta^{15}N$ values for fork lengths, with correlations found only in rivers with small sample sizes which provides evidence that enriched $\delta^{13}C$ resource use and increased trophic level are not good indicators for smolt body size.

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APPENDIX B

Table B. Benthic macroinvertebrates (BMIs) collected for Kedgwick, Matapedia, Margaree, Northwest Miramichi, and West River for baseline corrections. Species order name, common name, and sample size are listed. Brackets after a species order name represent the number of subsamples provided for stable isotope analysis. BMIs were selected based on dietary selections of smolt and representative BMIs for baseline carbon in the system.

River	Order	Common name	Sample size
Kedgwick	Hydropsoptera (2)	Caddisflies	5
	Trichoptera	Caddisflies	1
	Tipulidoptera	Crane flies	1
	Odonata (2)	Dragonflies	2
	Odonataoptera (2)	Dragonflies	2
	Soptera	Gastropods	1
	Ephemeroptera (4)	Mayflies	7
	Heptageniidae	Mayflies	3
	Leptohyoptera	Mayflies	1
	Ephemereilidae	Mayflies	2
	Baetiscidae	Mayflies	1
	Plecoptoptera	Stoneflies	10
Margaree	Hydropsoptera (3)	Caddisflies	14
	Ephemeroptera (6)	Mayflies	24
	Odonata	Dragonflies	1
	Heptageniidae	Mayflies	4
	Perlidae (5)	Stoneflies	11
	Rhyacophiidae	Stoneflies	3
	Perlidaoptera	Stoneflies	1
Matapedia	Trichoptera (4)	Caddisflies	27
	Diptera	Crane flies	2
	Odonata (3)	Dragonflies	5
	Gastropoda	Gastropods	4
	Ephemeroptera (4)	Mayflies	13
	Plecoptera (5)	Stoneflies	14
	Psephenidae	Water penny beetles	4
Northwest Miramichi	Philopotamidae	Caddisflies	6
	Hydropsoptera	Caddisflies	4
	Tipulidae	Crane flies	4
	Corydalidae	Dobsonflies	1
	Heptageniidae (2)	Mayflies	5
	Ephemeroptera (5)	Mayflies	55
	Perlidae (3)	Stoneflies	6
	Pteronarcyidae	Stoneflies	1
West River	Philopotamidae	Caddisflies	6
	Hydropsoptera (3)	Caddisflies	8

	Tipulidae	Crane flies	2
	Corydalidae (3)	Dobsonflies	4
	Odonata (5)	Dragonflies	8
	Heptageniidae	Mayflies	2
	Perlidae (5)	Stoneflies	8
	Psephenidae	Water penny beetles	1

APPENDIX C

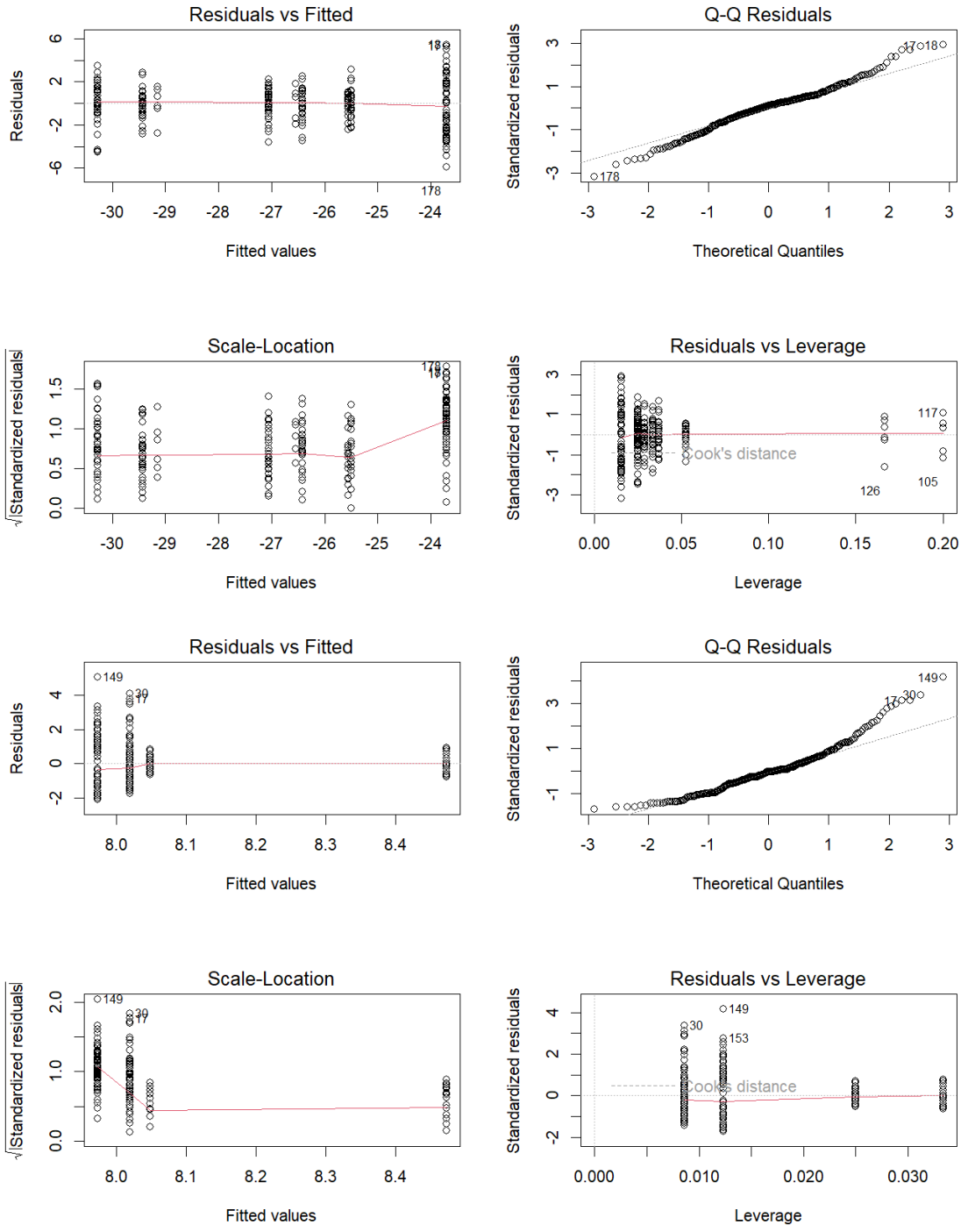

Figure C. Residual plots for ANOVA comparing $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) between the uncorrected stable isotope data for all nine rivers. $\delta^{15}N$ violates assumptions of normality (Q-Q plot) and homoscedasticity (Scale-Location) therefore a Kruskal-Wallis test was performed for $\delta^{15}N$ on the uncorrected rivers.

APPENDIX D

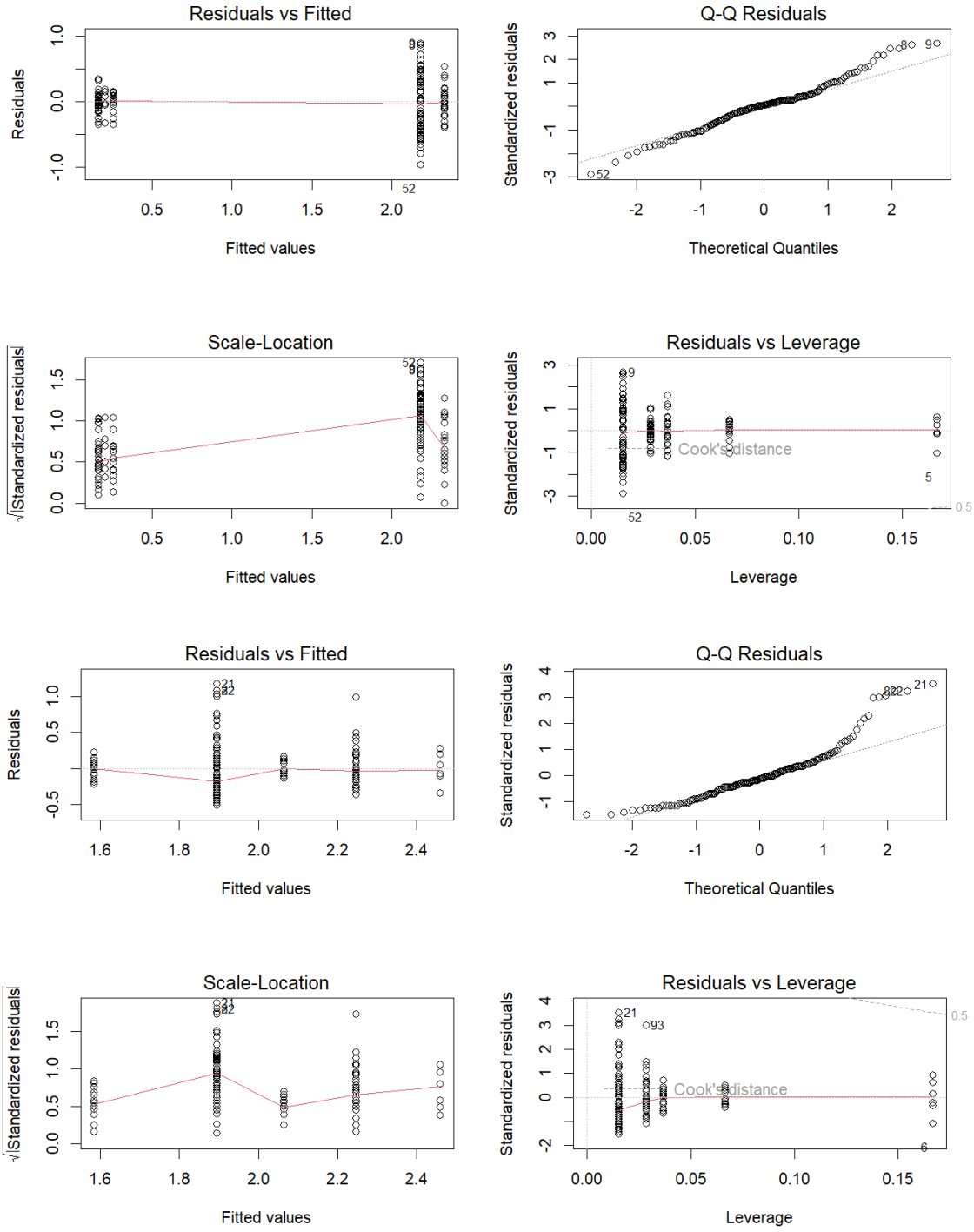

Figure D. Residual plots for ANOVA comparing $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) between the corrected stable isotope data for all five rivers. Both residual plots violate the assumption of heteroskedasticity (Scale – Location). $\delta^{15}N$ violates the assumption of linearity (Residual vs Fitted) and normality (Q-Q residual). Therefore, a Kruskall-Wallis test was performed for both $\delta^{13}C$ and $\delta^{15}N$ for the corrected data.

4.0 Chapter 4: DISCUSSION

This thesis examined trends in the resource use and body size of Atlantic salmon smolt spatially and temporally from rivers across Eastern Canada. Carbon and nitrogen stable isotopes of smolt tissues were not found to be strong predictors for smolt body size over time and across rivers, and no trends were observed for increased $\delta^{13}C$ and $\delta^{15}N$ on fork length. Body size was found to be influenced more by river rather than resource use, therefore other factors within the freshwater environment may have a greater influence on smolt growth (Simmons *et al.*, 2022). Although resource use was not found to be a predictor in this study, resource use was only examined for a short part of the salmon's life. Future studies examining the effects of resource use and body size may want to examine earlier life stages, such as fry and parr to rule out the effects during the early freshwater stages.

Over the 16-year time series no strong increasing or decreasing trends were observed for smolt fork length, although fluctuations did occur within rivers Conne, Trinité and St. Jean rivers showed no significant decline in smolt length from 2000 – 2016. This study allowed us to examine a broader picture of smolt data from the last two decades, and do not indicate any major decreasing trends which have been observed in previous studies (i.e. Jutila *et al.*, 2006). However, just because a decreasing trend in size was not observed overtime across three rivers does not mean it is not occurring in other rivers, like those that are listed as a concern.

While the overall trends across the three rivers indicate stability, an in-depth analysis of individual rivers, such as the St. Jean, reveals important ecological dynamics. St. Jean was found to consistently be depleted in $\delta^{13}C$ and $\delta^{15}N$ and had the smallest fork length throughout the collection years. The stability of these isotopic values over time

may be influenced by factors such as in-river habitat characteristics, food availability, and surrounding environmental conditions (Fry, 2002; Glibert *et al.,* 2019; Hobson, 1999). Additionally, the smaller size of St. Jean smolt compared to those from other rivers may result in gape limitations (Galvan *et al.,* 2010). The extensive temporal dataset that has been compiled enables a comprehensive analysis, reducing the risk of misinterpreting short-term fluctuations that could arise from examining data over just a few years.

Building on these insights, the dataset created from this project offers valuable opportunities for future studies and long-term environmental monitoring. By expanding the research from three to nine rivers we were able to examine the influence of geographic range as well as connections between smolt genetically assigned groups (Bradbury *et al.,* 2021). This study alone revealed similarities between rivers in regard to resource use in rivers that were geographically separated. Similarities in isotopic values between separated sites could suggest similar prey availability, river chemistry as well as surrounding habitats, which may be useful for smolt management. Protocols applied to Matapedia and Kedgwick could have similar effects to the West River smolt because of their isotopic similarities.

4.1 IMPLICATIONS

This study allowed us to examine previously collected data and answer new ecological questions about a species at risk. Understanding the drivers of smolt condition prior to sea entry is useful for trying to increase survivability of out-migrating smolt and at sea. Few studies have been conducted on the diet of smolt during migration (i.e. Larsson *et al.*, 2011) and this study would lead to the hypothesis that feeding at the beginning of migration is important to smolt; however, prey selection does not have a

large impact on body condition. Moreover, this study provided data on the freshwater isotopic signatures of rivers in Eastern Canada which can be used to help assign geographic isotopic signatures in multiple ecological studies (i.e. Shipley *et al.*, 2024). This helps promote collaboration among the stable isotope and Atlantic salmon ecology communities and spark ideas to gain more widespread knowledge on these topics.

4.2 FUTURE WORK

Taking various tissues from the smolt for stable isotope analysis allowed us to further examine the variation between tissues; however, this method is not sustainable for monitoring live Atlantic salmon. Since taking fin clips is often the non-lethal method used in research as a surrogate for muscle tissue (Graham *et al.*, 2013; Jardine *et al.*, 2005), this study provides additional information on the variation between those tissues in juvenile fish. However, future studies may want to examine the difference between the tip section of the fin and muscle since this study included the mid section of the fin which is less practical for field sampling. Moreover, determining a more precise turnover rate for fin tissues for juvenile Atlantic salmon would offer a more reliable timeline for examining dietary changes over time.

To expand this research future studies should take baseline samples at smolt wheel sites so all isotopic data can be compared once corrected for geographic variability. Expanding the database by taking stable isotope data on smolt from other rivers in Eastern Canada may provide a better understanding of resource use across more genetically assigned groups. Moreover, identifying variables other than resource use within freshwater environments that could be driving parr and smolt growth is important for increasing the chances of smolt survivability (Simmons *et al.*, 2020).

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