EVALUATION OF THE INFLUENCE OF AMERICAN EEL (*ANGUILLA ROSTRATA*) TROPHIC INTERACTIONS ON *ANGUILLICOLA CRASSUS* INFECTION AND NEW METHODS FOR STABLE ISOTOPE ANALYSIS OF EELS

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ABSTRACT

The American eel, *Anguilla rostrata,* is a culturally, commercially, and ecologically important species in eastern North American river systems. They are currently facing several threats, one of which is the parasitic nematode *Anguillicola crassus.* My research sought to improve understanding on how *A. crassus* is transmitted in a river system using stable isotope analysis, as well as improve methods for isotope analysis of both eels and *A. crassus* nematodes. I found that trophic position in conjunction with condition factor is associated with higher *A. crassus* infection intensity in yellow eels. Additionally, I discovered that $\delta^{15}N$ of *A. crassus* nematodes is significantly affected by the presence of host material in the digestive tract. I also devised a lipid estimation model for eel muscle tissue which can be used to lipid correct muscle samples, as well as demonstrated the viability of eel caudal fins as a nonlethal surrogate tissue for isotope analysis.

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INTRODUCTION

Overview of the Status of Anguillid Eels

Across the globe, wild fish stocks are under increased pressure due to anthropogenic stressors such as overfishing and habitat alteration/destruction. However, studying the effects of these stressors can be challenging because many fish that we depend on for food are migratory, and their habitat and resource use changes over time. An excellent example of this is the American eel (*Anguilla rostrata*), a species of anguillid eel native to the eastern Americas, ranging from Venezuela to Greenland (Avise et al., 1986). Eels of the family Anguillidae act as both umbrella (species which conservation efforts towards will benefit large numbers of co-occurring species) and indicator (species health is indicative of environment quality) species (Itakura et al., 2020). As well, American eels are culturally significant to many Indigenous peoples (Kuhnlein and Humphries, 2017). They are also a prominent species for commercial fishing (Fisheries and Oceans Canada, 2018), as are anguillid eel species that occur outside of the Americas. Commercial fishing is done at multiple eel life stages with Japanese eels (*A. japonica*), European eels (*A. anguilla*), and American eels (*A. rostrata*) being the primary commercial targets. Japanese and American eels are both listed as endangered on the International Union for Conservation of Nature (IUCN) Red List (Jacoby et al., 2017; Pike et al., 2020b), while European eels are listed as critically endangered (Pike et al., 2020a) and currently are at risk of population collapse (Sonne et al., 2021).

American Eel Life Cycle

Eels typically live most of their life in a river system as "yellow eels," the sexually immature life stage characterized by yellow pigmentation on their abdomen (Tesch & Greenwood, 1977). Although many yellow eels inhabit freshwater rivers, lakes, and streams, they often live in estuaries of river systems or even coastal marine environments with some migrating between these habitats during this stage of their life (Jessop et al., 2008). Once they begin to sexually mature, they lose this yellow abdominal pigmentation and gain a white/silver pigmentation. These are known as "silver eels," and they migrate to spawn in an area off the North American continental shelf referred to as the Sargasso Sea (Béguer-Pon et al., 2015). Adult eels die after spawning, and larvae called leptocephali hatch from eggs and migrate back towards the continental shelf using the gulf stream current. Leptocephali metamorphize into the translucent juvenile "glass eels" as they begin entering coastal environments and river systems, after which they become pigmented and are known as elvers (Tesch & Greenwood, 1977; Jessop et al., 2008).

Overview of *Anguillicola crassus*

The parasite *Anguillicola crassus* is a major threat to American eels. *Anguillicola crassus* is an invasive nematode that infects the swimbladder of *Anguilla* eels. Having evolved to infect Japanese eels (*Anguilla japonica*), the swimbladder nematode was first recorded in wild American eels in 1996 in South Carolina (Fries et al., 1996). The parasite spread throughout the Atlantic coast of the eastern United States, eventually reported in the Canadian provinces of New Brunswick and Nova Scotia in 2007 (Aieta and Oliviera, 2009). It is hypothesized that rapid spread of *A. crassus* is attributed to

infected intermediate hosts (hosts which facilitate parasite development but do not host the final sexually mature stage) being translocated to non-endemic areas via ballast water of ships, since copepods are reported commonly in ship ballast water (Rockwell et al., 2009; Denny et al., 2013).

Anguillicola crassus begins its life cycle in the swimbladder lumen of an anguillid eel, the definitive host in which the adult nematodes reproduce sexually (De Charleroy et al., 1990). Females release eggs containing second stage (L_2) larvae that exit the eel swimbladder via the pneumatic duct, and travel through the GI tract until they are excreted with faeces by the eel. Larvae hatch within hours of contacting freshwater and lose their L_1 cuticle shortly after hatch (De Charleroy et al., 1990). The *A. crassus* life cycle continues when L_2 larvae are consumed by copepods and ostracods that serve as intermediate hosts where the parasites undergo required developmental processes within the haemocoel to become third stage (L3) larvae that are infective to eels (De Charleroy et al., 1990). *Anguillicola crassus* commonly infects freshwater invertebrate intermediate hosts, but some estuarine copepods are also suitable hosts, with *Eurytemora affinis* being a notable example and one very common in estuaries in the northern hemisphere (Kirk et al., 2000a). In addition to direct transmission from intermediate to the eel definitive host, infective L_3 larvae can also use various fishes as paratenic hosts (El-Shehabi et al., 2018). No essential development occurs in a paratenic host but rather it promotes transmission back to the definitive host because large eels are piscivorous (Kirk et al., 2000b). Once an eel consumes an infected paratenic host, they infect the eel to mature and complete their life cycle. Within the eel host, parasite larvae burrow through the intestinal wall, enter the body cavity of the eel, and migrate to the swimbladder. Larval parasites burrow into the

swimbladder submucosa where they moult to the L_4 stage and present with a darkened intestinal due to accumulation of heme following consumption eel blood (De Charleroy et al., 1990). Larvae then enter the swimbladder lumen, moult a final time to their adult stage, and reproduce (De Charleroy et al., 1990).

Presence of both larval stages and adult *Anguillicola crassus* in the swimbladder can cause damage to the swimbladder of American eels and European eels (*Anguilla anguilla*). This includes thickening of the swimbladder wall due to fibrosis, blood vessel inflammation and hemorrhage, as well as reduction of gas capacity in the swimbladder lumen (Lefebvre et al., 2011). *Anguillicola crassus* negatively effects buoyancy of sexually mature silver stage European eels (Palstra et al., 2007), and it is hypothesized that damage to the swimbladder can impair a diel vertical swimming that occurs during migration of eels heading to spawn (Sjöberg et al., 2009). The parasite can also cause adverse pathological effects in the elver and yellow eel life stages of American eels with 24% reduction in survival reported for 2-year-old eels (Warshafsky, 2017).

Research Objectives

The primary goals of my research are to: 1) gain a better understanding of how *A. crassus* is transmitted in populations of American eel in the freshwater and estuary parts of a river system using stable isotope analysis; and, 2) to improve methods and accuracy in interpreting stable isotope data on anguillid eels and the parasitic swimbladder nematodes that they host. The first goal is addressed in Chapter 1, where I use stable carbon (^{13}C) and nitrogen (^{15}N) ratios as well as host physical attributes (length and weight) to determine how feeding habits of eels might affect prevalence of *A. crassus* among the population and the intensity of parasite load in an individual eel. The second

goal is addressed in both Chapters 2 and 3. Chapter 2 is concerned with the trophic discrimination of the *A. crassus* nematode and how host materials within the parasite digestive tract might affect isotopic values. Chapter 3 analyzes effectiveness of non-lethal tissue sampling for stable isotope analyses and I develop a new eel-specific lipid estimation model to improve lipid $\delta^{13}C$ correction of anguillid eel muscle tissue.

CHAPTER 1: Trophic position and condition factor influence *A. crassus* intensity in American eel, *Anguilla rostrata*

1.1 Introduction

Research on *Anguillicola crassus* has increased dramatically in North America and Europe in response to both the rapid spread of the parasite as well as the declining populations of American eel and European eel (Sonne et al., 2021; Pike et al., 2020a; Pike et al., 2020b; Jacoby et al., 2017). The threat of *A. crassus* in limiting populations of these anguillid eels has been known for well over a decade (Lefebvre et al., 2011; Sjöberg et al., 2009; Palstra et al., 2007). Studies on *A. crassus* transmission have been conducted throughout endemic areas, some with particular focus on the role for paratenic hosts (Barry et al., 2017; Li et al., 2015; Pegg et al., 2015). Some sought to identify the diversity of animals that *A. crassus* can use as paratenic hosts (Li et al., 2015; Moravec & Skoríkková, 1998; Rolbiecki, 2002); this was accomplished by analyzing various aquatic and semi-aquatic organisms including fish, gastropods, and amphibians for the presence of *A. crassus*.

Two studies used stable isotope analysis to help determine dietary habits regarding prey species (i.e., types of animal hosts) that might be more important for *A. crassus* transmission (Barry et al., 2017; Pegg et al., 2015). Both of these studies used analysis of stable isotopes of carbon (^{13}C) and nitrogen (^{15}N), which are among the most common employed in research involving food webs (Vander Zanden et al., 1999). Carbon-13 experiences relatively little enrichment with trophic level (Post, 2002; Vander Zanden et al., 1999) and is used to determine the photosynthetic energy sources of organisms in a food web and to trace nutrient pathways within an ecosystem (Hayden et al., 2019; Kiljunen et al., 2006; Post, 2002). Conversely, Nitrogen-15 is enriched in a predictable

amount (~3.4‰) each time one organism consumes another has great utility in determining trophic position of an animal (Vander Zanden & Rasmussen, 2001; Post, 2002). Accordingly, stable isotope analysis has become increasingly popular in research investigating host-parasite interactions (Thieltges et al., 2019).

There were conflicting results in two studies regarding *A. crassus* infection status and prey species utilization by European eels (Pegg et al., 2015; Barry et al., 2017). According to the findings of Barry et al. (2017), smaller eels (elvers) that feed primarily on invertebrates had both higher prevalence (% of individuals in a population infected) and intensity (# of parasites in an individual) in Scotland than their larger counterparts. However, the findings of Pegg et al. (2015) concluded that large piscivorous eels in England had both higher prevalence and intensity (Pegg et al., 2015) than smaller eels. Little research has been conducted to determine effects the feeding habits (i.e. resource use & trophic position) of American eels on *A. crassus* infection rates/intensity in eastern Canadian river systems.

I studied American eels in the Wolastoq | Saint John River (W|SJR) below the Mactaquac Generating Station and sampled individuals from both freshwater and lower estuary populations. I anticipated that my results would reflect the results of Pegg et al. (2015) with an increase in prevalence and intensity of *A. crassus* among eels with higher trophic position. However, because *A. crassus* larval survival and transmission is impaired with increasing salinity (Kirk et al., 2000), I expect eels with higher marine resource use to have lower prevalence and intensity of infection by *A. crassus* either because they inhabit environments where transmission is lower, or they are consuming diadromous species which migrate from areas that *A. crassus* is not present. I used stable isotope analysis to

determine whether resource use and trophic position of *A. rostrata* affects the prevalence and/or infection intensity of the swimbladder parasite, *A. crassus*.

1.2 Methods

From July to October 2019, 120 yellow eels from sites in the Saint John Harbour, New Brunswick (45.26931, -66.09318 – Reversing Falls; 45.28158, -66.04850 – Mouth of Marsh Creek) and 50 yellow eels from the Wolastoq | Saint John River (W|SJR) near Fredericton, New Brunswick (45.95946, -66.82628 – Mckinley Ferry Site) following approval by Fisheries and Oceans Canada (Section 52 Permit Number 355911). These were captured with the aid of St. Mary's First Nations fishery (Fredericton), Atlantic Coastal Action Project (ACAP) Saint John, and commercial fisheries (Saint John). Eels were fished using baited fyke nets and eel traps.

After being captured, eels were held captive in the field prior to processing using 60L plastic barrels filled with water from the local environment. The water was aerated and kept cool with ice bags, which also helped induce torpor in the eels to reduce their activity (Walsh et al., 1983), making them easier to handle safely. Eels were exposed to an overdose of anaesthetic (100mg/L clove oil) by bath immersion. Following total loss of reactivity and near-cessation of opercular movement, we performed physical euthanasia. Euthanasia of eels consisted splitting of the skull using garden shears and homogenizing the brain and proximal spinal cord. Severing of the proximal spinal column is effective for most vertebrates; however, eels are very resilient, and show motility even after proximal spinal cord severance (Verheijen and Flight, 1997), hence their brain and proximal spinal cord must be homogenized as well.

Prior to final necropsy, the eels were weighed to the nearest 10th of a gram, and their lengths measured to the nearest 10th of a centimetre. Swimbladders were collected during necropst for parasite diagnostics and muscle samples and whole livers were collected for stable isotope analysis. Due to the quicker turnover rate, isotope ratios in liver are indicative of a shorter time frame (typically 2-3 weeks) and may better reveal temporal dietary shifts in the population; muscle typically has a slower turnover rate, meaning that its isotope ratios are indicative of a longer time frame (typically 3-4 months) and dietary profile (Tiezsen et al., 1983).

I also collected various species of invertebrates and small fish to acquire stable isotope data from potential prey species in the estuarine site to provide a reference point to compare eel isotope data. From the Saint John Harbour in October, I collected 5-7 of each of the following species for analysis: *Littorina littorea* (Common Periwinkle), *Mytilus edulis* (Blue Mussel), *Menidia menidia* (Atlantic Silverside), *Crangon spp.* (Sand Shrimp), *Gammarus spp.* (Amphipod). Atlantic Silversides and Sand Shrimp were captured via beach seine, while Periwinkles, Mussels, and Amphipods were gathered via rock-picking by hand. These species were all caught in areas that eels were captured previously. I euthanized Atlantic Silversides in a similar two-step fashion to eels, starting with overdose in anaesthetic (100mg/L clove oil) and followed by severing of the proximal spinal column. I euthanized all invertebrates via exposure to water >60ºC. Once euthanized, all species were placed on ice and transported back to UNB. In the lab, I carefully removed muscle tissue from the mussels, snails, and silversides, ensuring that I did not also collect organ tissue. Sand Shrimp and amphipods were left whole due to their small size. Isotope data from small freshwater fish from the W|SJR near Fredericton from

a recent study (Hayden et al., 2019) were used to provide a reference point to compare eel isotope data.

I dried muscle, whole livers, and whole organism samples in an oven at 60ºC: 48hrs for muscle tissues and whole organisms; and, 72 hours for livers. After drying, I homogenized dried tissues by grinding it into a powder using a mortar and pestle. At this point, all the samples were ready to be weighed except for livers, which underwent lipid extraction. Lipids consist primarily of long carbon chains and are relatively depleted in carbon-13 (Kiljunen et al., 2006), meaning that the $\delta^{13}C$ values of samples will be skewed unless lipids are corrected for or removed. Muscle samples from eels and Atlantic silversides were corrected for using mathematical models (Kiljunen et al., 2006), but models developed specifically for fish muscle tissues, livers require lipid extraction. This is accomplished using a series of washes with 2:1 chloroform:methanol solution until the washes ran clear, at which point they were washed once more with distilled water and placed in the oven at 60ºC for another 24hrs.

Stable isotope analysis of animal tissue requires 1.00mg - 1.20mg of material per sample for most accurate results. I accomplished this using a microgram-sensitive balance and small tin capsules as a vessel for the dried tissue powder/pieces. To submit the samples for analysis, the tin capsules must be folded and compressed into small cubes. Each sample was placed into an autosampler tray immediately after weighing and the weight recorded. The tray of samples was submitted to the UNB SINLAB for carbon-13 and nitrogen-15 analysis.

I used generalized linear models accounting for negative binomial distribution of parasites using Modern Applied Statistics with S., or MASS, in R (R Core Team, 2021; Venables & Ripley, 2002) to see if there was dependency between $\delta^{13}C$ and parasite intensity. I then examined how proportional usage of estuary versus freshwater resources differed among eels by creating a mixing model using Stable Isotope Mixing Models in R, or SIMMR (R Core Team, 2021; Parnell, 2021).

For the mixing model, I sorted eels into four different categories of infection: Uninfected; Low (1-5 parasites); Mid (6-15 parasites); and, High (15+ parasites). Infection category is based on the percentage of required swimbladder volume to maintain neutral buoyancy of the average eel which would be occupied by that number of adult *Anguillicola crassus*; I categorized Low as less than 10% total volume occupied, Mid as between 10 and 25% occupied, and High as more than 25% occupied. The volume required for neutral buoyancy is approximately 50ml/kg (Alexander, 1966; Alexander, 1971), and the average mass of the eels from our study was 227g, for which the required volume for neutral buoyancy is 11.35ml. Effective volume of adult *Anguillicola crassus* was calculated using the equation volume = $\pi r^2 h$, where r is the radius of the coiled nematode (the position most nematodes were naturally found in) and h is the cross-sectional diameter of the nematode at its widest point. Mean effective volume of *Anguillicola crassus* was calculated to be 0.18ml; therefore, a swimbladder with 11.35ml of volume would be filled by approximately 63 nematodes. 6 nematodes is approximately 10% of this number (minimum number to be classified as Mid), and 16 is approximately 25% (minimum number to be classified as High).

My next question was whether there were correlations between the $\delta^{15}N$ values within populations and number of parasites per eel to determine whether trophic position affected *A. crassus* infection significantly. For this, I calculated trophic position relative to *Gammarus spp.*, which was found in both freshwater and estuarine environments, then I created generalized linear models accounting for a negative binomial distribution using MASS. I then visualized this model using predicted values derived from the generalized linear model. Other factors included in these analyses were condition factor and capture month, both of which I included when creating generalized linear models for both $\delta^{13}C$ and $\delta^{15}N$.

Additionally, I created a carbon-nitrogen biplot separating eels by location and by month using ggplot2 in R (R Core Team, 2021; Wickham, 2016). I also analyzed whether there existed any other significant relationships between $\delta^{13}C$, $\delta^{15}N$, and other factors (capture month and condition factor) using linear regression models generated in R.

1.3 Results

At the freshwater Fredericton site in October, we were only able to capture 2 eels. No significant temporal differences were observed for liver $\delta^{13}C$ in either eel group However, liver $\delta^{15}N$ differs significantly by month for both groups (Figure 1). Eels from Fredericton sampling became depleted in $\delta^{15}N$ starting in September, and more so in October. Eels from the Saint John group only become depleted in $\delta^{15}N$ in October. At one of the 2 sample sites in Saint John (45.28158, -66.04850), known as Marsh Creek, most eels captured in September and October had uncharacteristically low $\delta^{13}C$ values for an estuary system; for the purposes of comparison, all eels captured there during those

months (18 eels) were sorted under a separate location group. Based on the $\delta^{13}C$ of these eels, they were excluded from any statistical analysis requiring locational baseline data as it was highly likely that these eels had migrated from further upstream in Marsh Creek

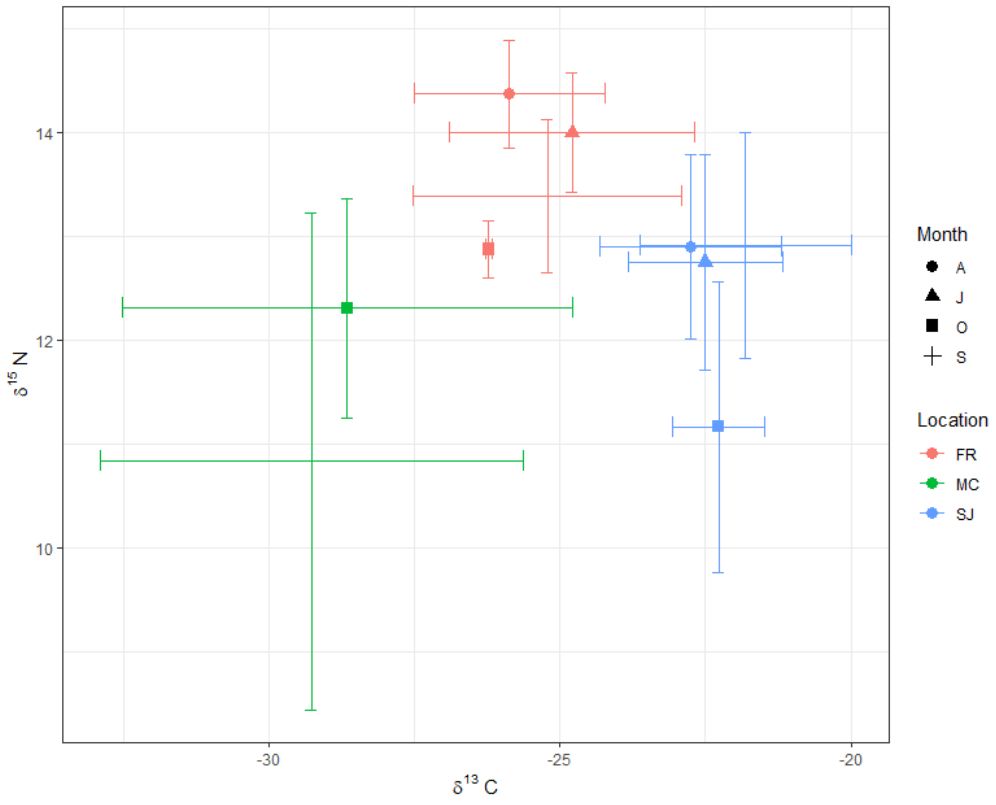

Figure 1: Carbon-nitrogen biplot from eel liver tissues, separated by both collection location (color) and month (shape). Timeframe for months was from July to October; each letter in legend corresponds to the first letter of the respective month. Location notations are as followed: FR = Fredericton group, MC = Marsh Creek group, SJ = Saint John group.

The condition factor of eels from the Saint John group was significantly positively correlated with condition factor ($p = 0.004$, $df = 80$). No significant relationship was

found between condition factor and $\delta^{15}N$; this lack of correlation held true when compared to trophic position as well.

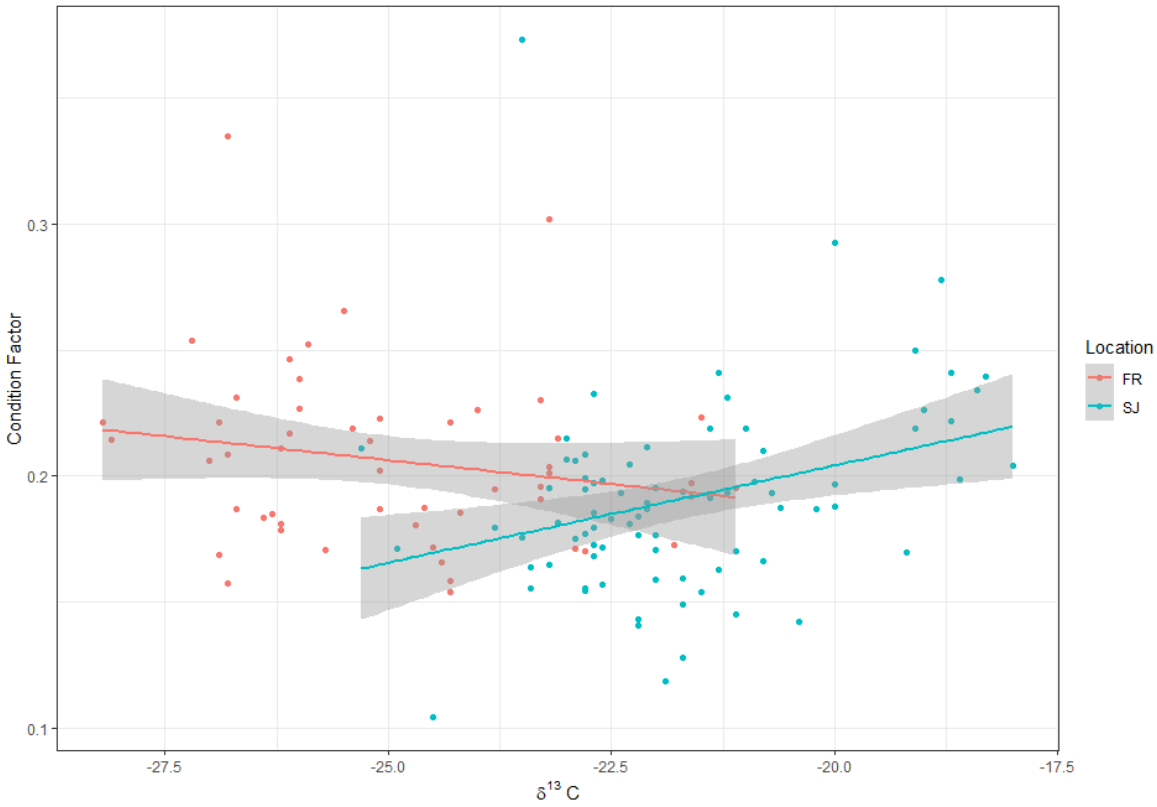

Figure 2: Regression of American eel condition factor against $\delta^{13}C$, separated by eel collection location. The slope of the line for the Fredericton group is -0.004 (r = 0.04), and the slope of the line for the Saint John group is 0.008 (r = 0.1).

When comparing groups, SIMMR calculates the probability of all possible configurations – that is, the order of greatest to least usage of a single specified resource – being true and plots the configuration with the highest probability. The calculated probability of the configuration of eel infection groups relative to freshwater resource usage seen in Figure 3 was 0.4169; the next highest probability configuration (0.1961) had the High group exhibiting higher freshwater resource use than the Mid group. The

third highest probability configuration (0.1714) placed the High group above the Low group for freshwater usage, but below the Mid group. Among the top 3 most probable configurations, the uninfected group always had the lowest freshwater usage. The combined probability of all groups in which the uninfected group had the lowest freshwater usage was 0.8536.

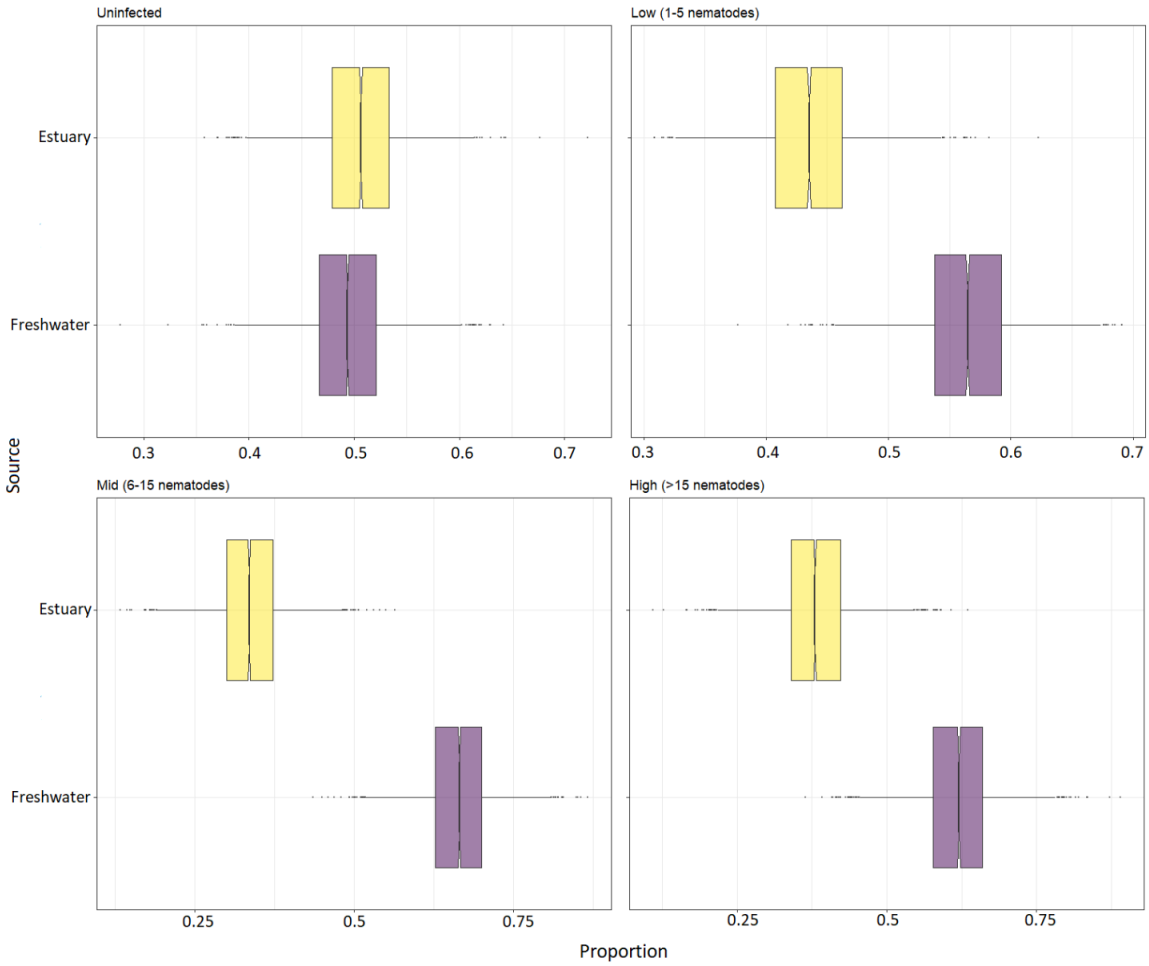

Figure 3: Stable isotope mixing model reflecting the proportional usage of two energy sources (freshwater & estuary) in the Wolastoq | Saint John River by American eels. Eels were sorted into 4 groups based on level of *A. crassus* infection intensity: Uninfected (0

nematodes in swimbladder); Low (1-5 nematodes in swimbladder); Mid (6-15 nematodes in swimbladder); and, High (>15 nematodes in swimbladder). Median (and 95% CI) of each group for estuary (E) and freshwater (F) usage are as follows: Uninfected - E = 0.51 (0.38-0.63), F = 0.49 (0.37-0.60); Low - E = 0.44 (0.32-0.52), F = 0.56 (0.47-0.67); Mid - E = 0.34 (0.20-0.48), F = 0.66 (0.54-0.82); High - E = 0.38 (0.21-0.53), F = 0.62 (0.45-0.79).

Infected eels had significantly lower mean $\delta^{13}C$ and significantly higher usage of freshwater energy sources compared to uninfected eels. Other than this, no significant trend was found between parasite load and eel $\delta^{13}C$. In the highest probability mixing model, usage of estuary and freshwater energy source by uninfected eels was near-equal, with estuary usage being slightly higher. Proportion of freshwater usage is higher among eels that are infected. The 95% confidence interval (95% CI) of freshwater energy usage for uninfected eels is 37-60% (Figure 3). Eels classified under low infection intensity (1-5 nematodes in swimbladder) show an 95% CI of freshwater energy usage from 47-67% (Figure 3). For eels classified under mid infection intensity (6-15 nematodes in swimbladder), the 95% CI of freshwater energy usage is from 54-82% (Figure 3). For eels classified under high infection intensity (>15 nematodes), the 95% CI of freshwater energy usage is from 45-79% (Figure 3).

When analyzed using generalized linear models, condition factor has a significant relationship with parasite intensity ($p = 0.015$, *df* 133)., as does the interaction of trophic position with condition factor ($p = 0.011$, *df* 133). On its own, trophic position does not meet the threshold to be considered significant ($p = 0.071$, *df* 133); however, when analyzed along with condition factor, a significant relationship was revealed. Eels with

relatively low condition factor and trophic position have the lowest parasite intensity, and eels with relatively high values for both have highest parasite intensity. A significant positive relationship with parasite intensity and $\delta^{13}C$ was also detected, though only in eels sampled in Saint John.

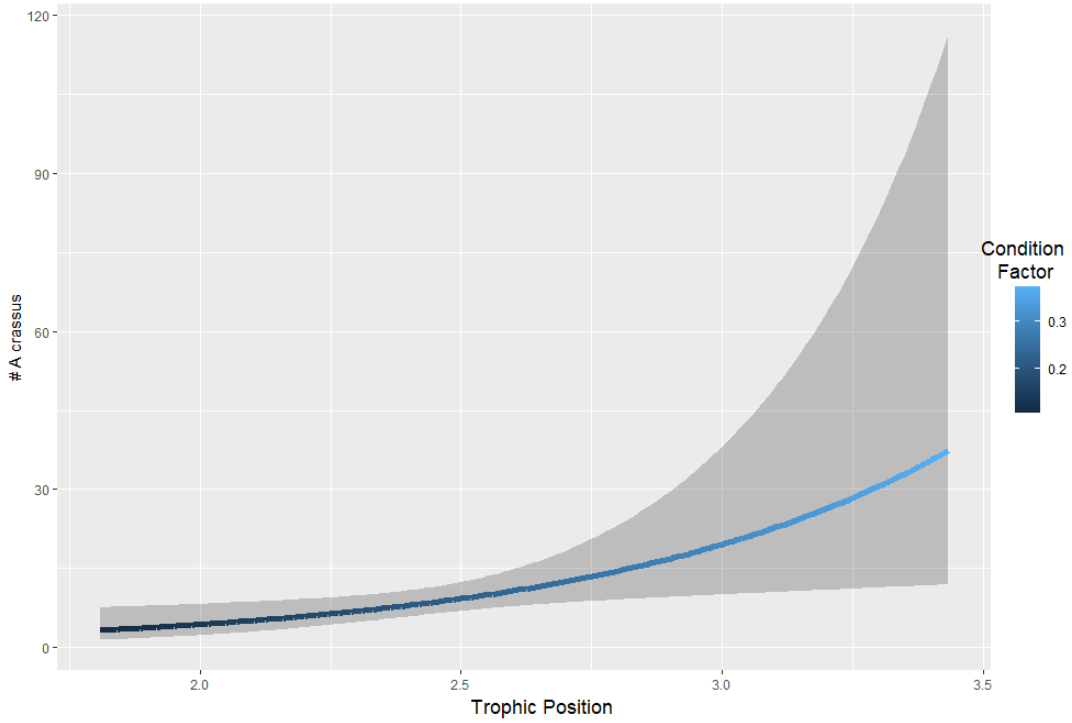

Figure 4: *Anguillicola crassus* parasite intensity prediction model from American eel trophic position and condition factor, derived from a generalized linear model for data with negative binomial distribution. Prediction has low standard error for eels with a trophic position less than 2.8 and condition factor less than 0.26; values above these have high standard error.

1.4 Discussion

In combination with the high probability that uninfected American eels had lowest freshwater usage of all four groups, significantly higher mean $\delta^{13}C$ in the

uninfected group demonstrates that the freshwater nutrient pathway results in more successful parasite transmission. This was expected because prevalence of the parasite is typically lower in estuaries than in freshwater systems as freshwater systems have a wider selection of intermediate hosts and longer larval survival time in the water (Kirk et al., 2000a; Kirk et al., 2000b). Also, the Wolastoq has numerous diadromous fish runs which influences $\delta^{13}C$ of organisms consuming marine-derived nutrients (Samways et al., 2015); these fishes are unlikely to have the parasite as they only enter the river system to spawn, therefore eels in freshwater environments with higher usage of these marine-derived nutrients will be less likely to be infected. Surprisingly, there were no significant trends between infection intensity and $\delta^{13}C$, and resource usage was similar among all infected eel groups in the mixing model. Whereas eels in the estuary are not as likely to be infected, they generally end up with similar parasite loads as compared to their freshwater counterparts.

Another interesting result is that Saint John eels with lower $\delta^{13}C$ had lower mean condition factor. A possible explanation is that these eels are recent migrants from the upper reaches of the estuary and that associated stressors with changing environments causes a slight decrease in condition factor. The change in salinity would result in changing osmoregulatory patterns which may result in higher energy expenditure. As well, an estuary model proposed by Whitfield et al. (2012), which adapts the Remane (1934) diagram to estuary systems, suggests that the upper estuary where the freshwater-saltwater gradient begins has lower species diversity than do either freshwater or lower estuarine environments; the lower species abundance may result in less food for migrating eels which may result in lower condition factor.

The generalized linear model for *A. crassus* load in relation to condition factor and trophic position are similar to previous studies on the European eel (Lefebvre et al., 2013; Pegg et al., 2015). Pegg et al. (2015) found that primarily piscivorous eels were more likely to be infected, however we found that trophic position did not affect the likelihood of infection. Trophic position did, however, play a role in predicting the number of *A. crassus* in the swimbladder, along with condition factor; similarly, Lefebvre et al. (2013) found that eels with greater mass and body length for their age were more intensely infected by *A.* crassus.

With trophic position of an eel being a factor in predicting *A. crassus* load, this suggests that the primary mode of transmission of *A. crassus* to yellow eels in the Wolastoq system are various fishes that serve as paratenic hosts. It is highly unlikely that this eel life stage is feeding on small pelagic zooplankton simply due to their size differential. An eel with a lower trophic position is likely feeding on a higher proportion of benthic macroinvertebrates. It may be that macroinvertebrates, despite being plausible hosts of *A. crassus* (Moravec & Skoríkková, 1998), are less effective as paratenic hosts for *A. crassus*. This would explain higher trophic position being a predictor of higher *A. crassus* loads in American eels. Furthermore, Pegg et al. (2015) speculate that the greater energy intake that is also associated with piscivory might explain the positive correlation between fast growth rates and high *A. crassus* infection intensity (Lefebvre et al. 2013). This would explain my observation of American eel condition factor being a significant factor in predicting parasite load.

The objectives of this study were to determine interrelationships between resource use, trophic position, and prevalence and intensity of *A. crassus* infections in American

eels for comparisons with previous studies on European eels. I determined that the research of Pegg et al. (2015) is most applicable to my observations from the W|SJR system due to their findings that trophic position and piscivory are positively correlated with both prevalence and intensity of *A. crassus* infection. A notable difference found in my research is that neither trophic position nor condition factor are good predictors of whether an eel becomes infected in the first place, yet they have strong influence on the parasite intensity of eels that become infected. Resource use was a better predictor regarding whether an eel will become infected, with uninfected eels showing much higher estuary usage than freshwater usage.

CHAPTER 2: Trophic discrimination of *Anguillicola crassus* is skewed by host material present in the digestive tract

2.1 Introduction

Stable carbon and nitrogen isotope ratios are used extensively in ecological studies (Boecklen et al., 2011; Post, 2002; Rubenstein & Hobson, 2004; Vander Zanden et al., 1999). However, despite use in some parasitological research (Thieltges et al., 2019), there are still relatively few studies that focus on interactions between hosts and their parasites (Nachev et al., 2017). Furthermore, studies on trophic enrichment of $\delta^{13}C$ and $\delta^{15}N$ between an endoparasite and its host have shown large variations in how enriched (or depleted) parasites are, relative to the host on which they feed (Nachev et al., 2017; Thieltges et al., 2019).

Not all endoparasites show the typical 3-3.4‰ enrichment in $\delta^{15}N$ relative to their host (Pinnegar et al., 2001; Thieltges et al., 2019). Many endoparasites are equal in $\delta^{13}C$ and $\delta^{15}N$, while others are depleted in $\delta^{13}C$ and $\delta^{15}N$ relative to their host (Nachev et al., 2017; Thieltges et al., 2019). Some parasites use paratenic hosts in their life cycle; in said hosts, parasites do not undergo growth or development and are thus not actively consuming and incorporating material from the host; these parasites are often equal to or depleted in $\delta^{13}C$ and $\delta^{15}N$ relative to their host as a result (Gilbert et al., 2020).

Other parasites are thought to feed on metabolites from nutrients already processed by the host which would make them lighter isotopically (Nachev et al., 2017). Some parasites are thought to have selective metabolism which promotes uptake of nutrients which are more depleted in $\delta^{13}C$ and $\delta^{15}N$ than others, resulting in the parasite

being equal to or depleted in $\delta^{13}C$ and $\delta^{15}N$ relative to their host (Thieltges et al., 2019). Another hypothesis regarding parasites that are equal to their host in $\delta^{13}C$ and $\delta^{15}N$ is that rather than actively feeding on host tissue, they passively feed on nutrients derived from host-consumed material in the host's digestive system (Goedknegt et al., 2018); because they derive nutrients from the exact same source that the host does, they have an extremely similar isotopic profile to their host.

Anguillicola crassus is an active blood feeder that gains a dark brown/black pigmentation in its digestive tract due to accumulation of heme following degradation of haemoglobin (De Charleroy et al., 1990). Accordingly, it is expected that they will have slight $\delta^{13}C$ enrichment (<1‰) as well as $\delta^{15}N$ enrichment (~3.4‰) relative to their host (Post, 2002; Nachev et al., 2017). However, because of the presence of host tissue material that accumulates within the parasite digestive tract, I expect that isotope values derived from intact parasites will be negatively skewed as this material will either be undigested and still reflect the host's isotopic profile, or it will be digested and lighter isotopically due to incorporation of heavier isotopes by the nematode. Both would result in lower $\delta^{13}C$ and $\delta^{15}N$ values that do not actually reflect their growth and development. To test this hypothesis, I sought to compare pairs of adult *A. crassus* nematodes from infected American eels, with one nematode left whole and the other having the contents of its digestive tract removed via microdissection. The $\delta^{13}C$ and $\delta^{15}N$ values from these paired parasite samples would then be compared to their host isotope profiles to determine whether host material in the parasite digestive tract skews the isotope ratios of the *A. crassus* swimbladder parasite.

2.2 Methods

From June – October 2021, yellow stage American eels were captured from the Wolastoq | Saint John River near Fredericton with assistance from St. Mary's First Nations fishery (Fredericton). Eels were similarly collected by a commercial fisherman (Deadeye Bait Company) from a downstream tributary of the W|SJR (i.e., Jemseg River). The eels were held in containers filled with water from the local environment. The water was aerated and kept cool with ice bags, which also helped induce torpor in the eels to reduce their activity (Walsh et al., 1983), making them easier to handle safely. Eels were exposed to an overdose of anaesthetic (100mg/L clove oil) by bath immersion. Following total loss of reactivity and near-cessation of opercular movement, we performed physical euthanasia. Euthanasia of eels consisted splitting of the skull using garden shears and homogenizing the brain and proximal spinal cord.

Eels were weighed (0.1g) and their lengths measured (mm) prior to necropsy. The swimbladder was removed for parasite collection, and eel muscle tissue collected from post-anal epaxial musculature (easiest area to get boneless muscle) for stable isotope analysis. Since not every infected eel had parasites large enough for dissection to eliminate intestinal contents but,19 eels yielded adult *A. crassus* pairs of sufficient size. One eel yielded two pairs of large parasites, resulting in 20 pairs of *A. crassus* collected in total. Collection of worm pairs permitted comparison of isotopic profiles from a dissected nematode and a whole nematode acquired from the same host.

Nematode dissection involved making a small perforation near either the head or tail of the nematode, gently grasping the parasite gastrointestinal (GI) tract with a pair of fine forceps, and gentle pulling to remove the GI tract containing host materials. This

process was effective only when using parasites greater than 2cm due to difficulty with dissections of smaller parasites and the yielding of too little material for stable isotope analysis. Both whole and dissected nematodes were dried in a 60˚C oven for a minimum of 24hrs; eel muscle was dried at 60˚C for a minimum of 48hrs. I homogenized dried muscle via mortar & pestle, and nematode samples using a blunt probe inside the sample tube to reduce loss of material. For each sample, I weighed 1.000mg – 1.200mg of sample material using a microgram-sensitive balance and small tin capsules as a vessel for the dried tissue powder/pieces and placed these in an autosampler tray. Once finished, I submitted the tray to the UNB SINLAB for carbon-13 and nitrogen-15 analysis.

My data analysis consisted of taking the differences between the $\delta^{13}C$ and $\delta^{15}N$ of the host and both the whole and dissected *A. crassus* parasites then performing a t-test between the data differences for whole and dissected nematodes. I summarized the data in a biplot using ggplot2 in R (R Core Team, 2021; Wickham, 2016). Parasite data are provided relative to their specific hosts but the data were separated by host sampling location to better reflect the data because isotopic profiles of eel hosts show regional variation. To test whether *A. crassus* feed exclusively on host blood cells, I mathematically corrected for lipid content of the host eel and compared the parasite $\delta^{13}C$ values to both uncorrected and corrected host values using the lipid-correction model outlined in Kiljunen et al. (2006).

2.3 Results

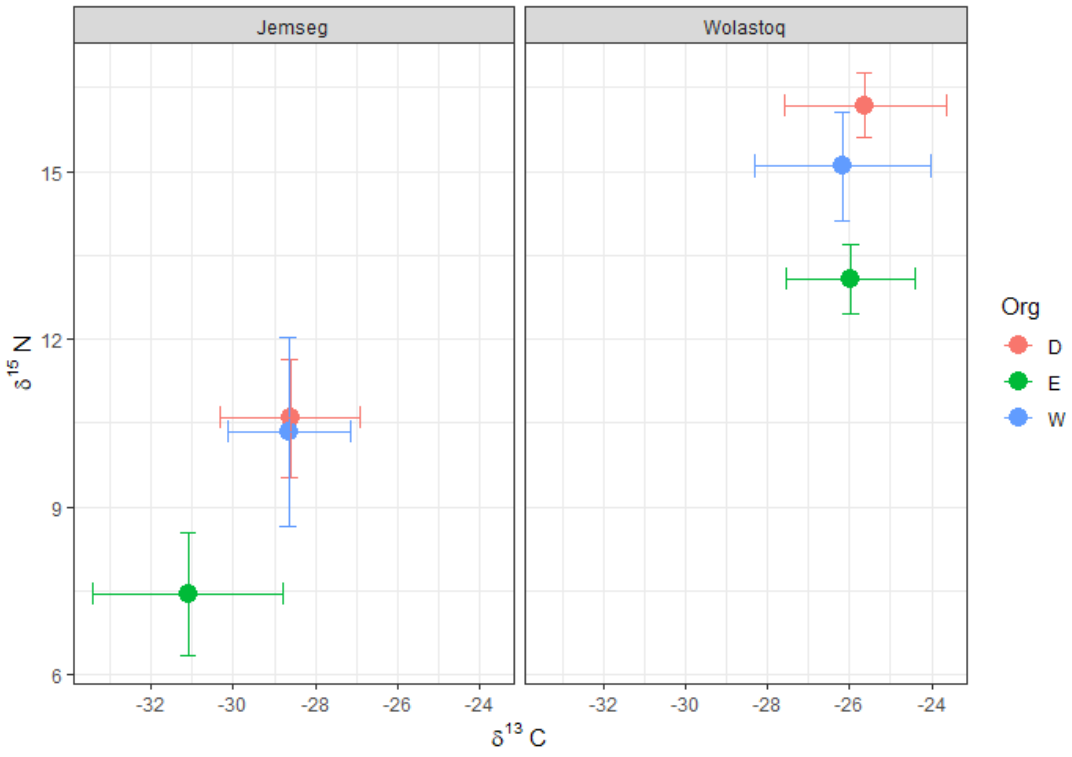

Figure 5: Biplot comparing $\delta^{13}C$ and $\delta^{15}N$ values of whole (W) and dissected (D) *A. crassus* nematodes to host eel muscle tissue (E). Plot is separated by location: On the left, organisms captured at the Grand Lake-Jemseg River confluence (6 eels + 1 eel with 2 parasite pairs removed for 7 comparisons total), NB; on the right, organisms captured in the Wolastoq | Saint John River (W|SJR), NB, approx. 3km below the Mactaquac Generating Station (13 eels for 13 comparisons total).

On average, both whole and dissected *A. crassus* are slightly enriched in ^{13}C relative to their host eel (Figure 5). The difference between the mean enrichment of the two groups is not significant ($p = 0.480$; Figure 6). Mean ^{13}C enrichment of whole nematodes is 0.50‰ (s.d. ± 1.86‰), and 0.90‰ (s.d. ± 1.46‰) for dissected nematodes. Furthermore, there is no correlation between $\delta^{13}C$ and C:N ratios of either group of

nematodes. Whole and dissected *A. crassus* are also both enriched in $\delta^{15}N$ (Figure 5), with dissected nematodes being significantly more enriched relative to the host than whole nematodes ($p < 0.001$; Figure 6). Mean ^{15}N enrichment was 2.19‰ (s.d. ± 1.29‰) for whole nematodes and 3.03‰ (s.d. ± 0.72‰) for dissected nematodes.

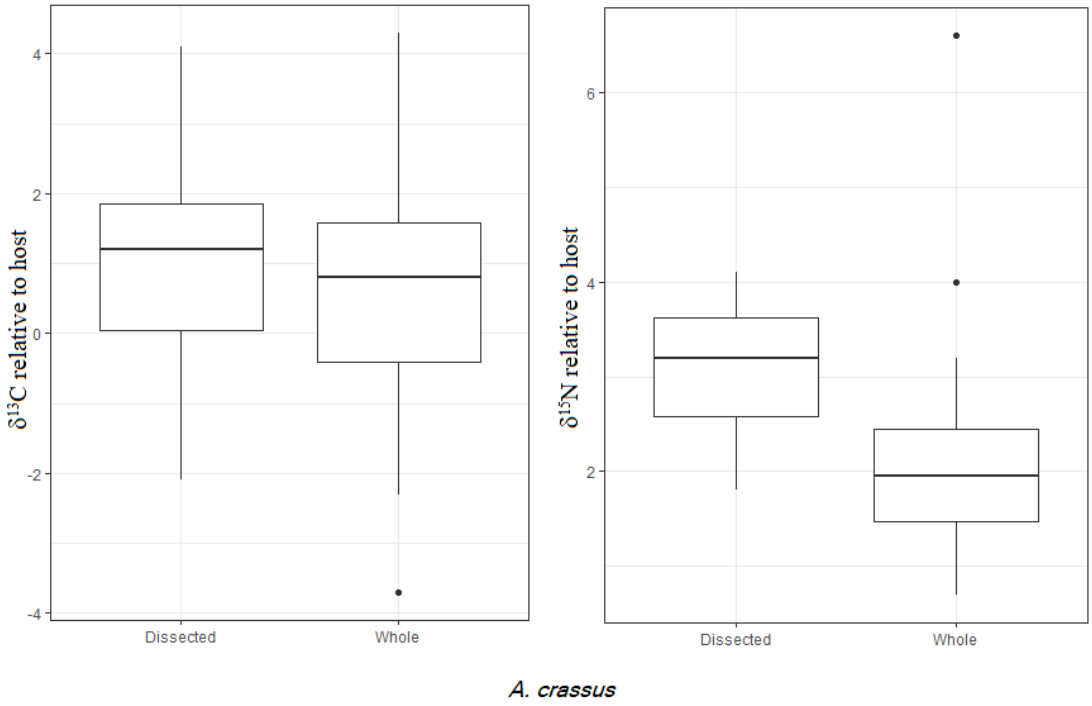

Figure 6: Boxplots of $\delta^{13}C$ (left) and $\delta^{15}N$ (right) of dissected and whole *A. crassus* nematodes compared to muscle tissue values of the host eel. Median (M) and 95% Confidence Interval (95% CI) for $\delta^{13}C$ values relative to host are as follows: Dissected - M = 1.2‰, 95% CI = -2.0‰ – 4.0‰; Whole - M = 0.8‰, 95% CI = -2.2‰ – 4.2‰. Median (M) and 95% CI for $\delta^{15}N$ values relative to host are as follows: Dissected - M = 3.2‰, 95% CI = 1.8‰ – 4.2‰; Whole - M = 2.0‰, 95% CI = 0.5‰ – 3.2‰.

2.4 Discussion

The significant difference in $\delta^{15}N$ enrichment between whole and dissected nematodes shows that host molecules present in the parasite intestinal tract is indeed skewing the $\delta^{15}N$ profile of *A. crassus* nematodes. The mean enrichment $\delta^{15}N$ by 3.03‰ among dissected *A. crassus* is consistent with previous trophic discrimination studies (Post, 2002; Boecklen et al., 2011, Nachev el al., 2017). Despite host material in the digestive tract skewing the $\delta^{15}N$ value of *A. crassus* nematodes, there is no significant difference in $\delta^{13}C$ enrichment between whole and dissected nematodes; this suggests that host material is not skewing the $\delta^{13}C$ ratios in *A. crassus* nematodes to a significant degree.

The mean $\delta^{13}C$ values of *A. crassus* nematodes reflect what is expected: that they should be slightly enriched relative to the host eel (<1.0‰). However, some of the values for both whole and dissected nematodes are either depleted in $\delta^{13}C$ or enriched in $\delta^{13}C$ by >1.0‰ relative to host. Depletion in $\delta^{13}C$ is often caused by lipids, which tend to be isotopically lighter than protein (DeNiro and Epstein, 1977). Therefore, I hypothesized that female *A. crassus* have a higher lipid content due to egg production, since some nematode eggs have been found to have significantly higher lipid content than their adult male counterparts (Krusberg et al., 1973); however, I found no correlation between nematode $\delta^{13}C$ and nematode C:N ratios, which makes this explanation unlikely. It may be that the nematodes are taking in different amounts of lipids from the blood and thus incorporating more depleted $\delta^{13}C$ into their tissues. Although I found no correlation with eel C:N and nematode $\delta^{13}C$, this is not sufficient to rule out this explanation as the eel's blood lipid content may be different than its muscle lipid content. To properly test this

hypothesis, blood from infected eels would have to be analyzed for lipid content and compared to the C:N ratios of dissected *Anguillicola crassus* nematodes from said eel.

Further research into host-parasite isotopic discrimination of nematodes such as *A. crassus* would benefit from performing lipid extraction to determine if the cause for some nematodes being depleted in $\delta^{13}C$ relative to hosts is related to lipid content. However, because of the size requirements for effective dissection of *A. crassus* nematodes, wild eels should not be captured at random for the sole purpose of extracting and comparing *A. crassus* as many eels will not have large enough parasites for dissection. My study, for example, used eels which were already being captured for different research projects. Another possible solution to this issue could be to collect parasite L2 larvae from the host eel gastrointestinal tract. Presence of *A. crassus* L2 indicates that adult nematodes are most likely present (De Charleroy et al., 1990), which makes it possible to target specific eels for euthanasia and effective collection of sizeable parasites required for these investigations. Assessment of eels for L2 can be done by temporary holding of eels to collect faeces or by a saline enema delivered with blunt-probe dosing needle.

Initially, I predicted that host blood present in the digestive tract of *Anguillicola crassus* nematodes is likely skewing isotope values, and that by removing said contents that I would observe an increase in both the $\delta^{13}C$ and $\delta^{15}N$ of the nematodes. My research demonstrates that removal of the digestive tract of *A. crassus* significantly reduced negative skewing of the nematode $\delta^{15}N$ profile relative to the host eel, but that $\delta^{13}C$ was relatively unaffected. This has implications for any parasite species that is actively feeding on host materials, with the type and abundance of host molecules in its digestive

tract differentially skewing parasite $\delta^{15}N$ profiles if this material is not or cannot be removed. While this is likely not the only reason that parasites exhibit $\delta^{15}N$ discrimination that differs from the average 3.4‰ (Boecklen et al., 2011; Post, 2002; Thieltges et al., 2019), my results demonstrate that it can certainly be a significant factor, and that future research could benefit from removing host materials from the digestive tract wherever possible.

CHAPTER 3: Comparative estimates of trophic ecology of American eels inferred from stable isotope ratios of lethal and non-lethally sampled tissue

3.1 Introduction

With increasing use of stable isotope analysis in ecological research, interest has increased in the viability of non-lethal tissue sampling methods (Hanisch et al., 2010; Kelly et al., 2006). Most commonly, $\delta^{13}C$ and $\delta^{15}N$ values of non-lethal tissues are compared to $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissue, as muscle is most commonly used for isotope analysis (Winter et al., 2019). Lethal sampling methods are particularly undesirable for research involving threatened or endangered keystone, indicator, and umbrella species. Studies on the viability of non-lethal surrogate tissues have been performed across several taxonomic groups of fishes, including Salmonids, Cyprinids, and Esocids (Hanisch et al., 2010; Hayden et al., 2017; Winter et al., 2019); notably, anguillid eels have not received the same attention as these other groups with regard to family-specific non-lethal sampling methods.

Both research and conservation initiatives concerning anguillid eels could benefit from non-lethal stable isotope sampling. Species of the family Anguillidae act as both umbrella and indicator species (Itakura et al., 2020). In North America, the American eel (*Anguilla rostrata)* has a wide distribution, with their range also including southern Greenland and northern Venezuela (Avise et al., 1986). Across much of their range, American eels have experienced major population declines over the past few decades, resulting in this species being added to the IUCN's Seafood Red List (Jacoby et al., 2017). They are listed as "Threatened" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2012), but have yet to be classified under the Species At

Risk Act (SARA). Regional variation occurs, including areas of eastern Canada where eels are widespread but other areas show significant declines (i.e., Lake Ontario, upper St. Lawrence River; COSEWIC 2012).

My objective was to determine the viability of using American eel caudal fin clips as a surrogate tissue for future non-lethal stable isotope analysis. I compared $\delta^{13}C$ and $\delta^{15}N$ values in fin tissue to $\delta^{13}C$ and $\delta^{15}N$ values from both muscle and liver tissue, as they have different turnover rates, to determine which tissue fin was most analogous to. I tested a prediction that I would observe a strong correlation between both tissues, allowing me to conclude that non-lethal fin clip collections are a viable surrogate to avoid the requirement for future lethal sampling of tissues. A secondary objective was to try and improve lipid correction models for $\delta^{13}C$ in anguillid eel muscle tissue by creating an eel-specific lipid estimation model. Lipids typically are significantly depleted in $\delta^{13}C$ relative to other material in tissue (DeNiro and Epstein, 1977) and therefore must either be corrected for or chemically removed, with mathematical correction being much more cost-effective than chemical lipid extraction.

3.2 Methods

Sampling took place in New Brunswick, Eastern Canada. American eels were collected following approval by Fisheries and Oceans Canada (Section 52 Permit Number 355911) near Saint John Harbour (45.26931, -66.09318) and from the Wolastoq | St. John River near Fredericton (45.95946, -66.82628), New Brunswick with assistance from the Maliseet Nation Conservation Council and St. Mary's First Nations fishery (Fredericton), Atlantic Coastal Action Project (ACAP) Saint John, and commercial fishers (Saint John). Sampling took place between July and October 2019. Eels were captured using baited

fyke nets and baited eel pot traps. After capture, eels were euthanized using a two-step procedure. First, they were exposed to an overdose of anaesthetic (100 mg clove oil solution [1 part clove oil, 9 parts 95% ethanol] per liter of water). Following total loss of reactivity and cessation of opercular movement, the skull was split bilaterally, and the brain and proximal spinal cord disrupted via pithing.

I collected ~1g muscle samples, whole livers, and caudal fin clips (hereafter fin) from the 40 eels sampled for fin-muscle-liver analysis/comparison. 20g of muscle tissue from 93 eels was collected and submitted for lipid, protein, moisture and ash content analysis. Muscle tissue was dehydrated for 48 hours, livers for 72 hours, and fin clips for 24 hours in a 60°C oven. Dehydrated samples were ground into a homogenous powder. During this process, I performed lipid extraction on liver samples, for which we used a series of washes in a 2-part-chloroform:1-part-methanol solution, followed by a wash in distilled water. During isotope analysis, 2 fin samples and 1 muscle sample were physically dropped and lost by the automated mass spectrometer, therefore 38 liver-fin and 37 muscle-fin sample comparisons were completed. I corrected for high lipids in muscle samples (indicated by high C:N ratios) using mathematical models outlined by Kiljunen et al. (2006), with the McConnaughey & McRoy (1979) equation for estimating % lipid content being replaced with the following equation that I developed based on data for lipid, protein, moisture and ash content in American eel muscle tissue:

$$\text{Lipid\%} = 100[-0.1098(\frac{2337}{C{:}N^{3.6}+767} - \text{C:N} + 4.23\text{x}10^{-3})]$$

Equation 1

To construct this equation, I first converted protein weight and lipid weight from grams to percentage of dry weight. From there, I used these values, as well as the C:N values, to build an equation in which the C:N ratio could be deduced using both protein and lipid dry weight percentages. The equation is as follows:

$$C:N = 0.00423 + 3.196(Protein\%) + 9.1096(Lipid\%)$$

Equation 2

Protein % was determined from the C:N ratio by plotting the protein:lipid ratio values against the C:N ratio values and fitting a line to the data. The equation of that line is:

$$Protein:Lipid = 767.69 \times C:N^{-3.6}$$

Equation 3

Protein:Lipid ratio from C:N was used to determine the percent weight of protein. The average ash content (i.e., dry weight from muscle tissue that was not protein or fat) from eel muscle was 4.72%, meaning that 95.28% of the dry weight was protein and fat. Using the following system of equations, we were able to deduce an equation to solve for protein %:

$$Protein\% + Lipid\% = 0.9528$$

Equation 4

$$Protein \div Lipid = R$$

Equation 5

Solving R for protein (P) yielded the following equation:

$$R = (1250P) \div (1191 - 1250P)$$

Equation 6

$$P = (1191R) \div \{1250(R + 1)\}$$ Equation 6, rearranged for P

Theoretically, I could have also solved this equation for lipid content using the same approach but this yielded lower precision than both the final equation (Equation 1) and the original McConnaughey & McRoy (1979) equation. I believe this was due to high variability in the dry weight lipid%, which ranged from 1-50%. High variability in dry weight protein% necessitated incorporation of a prediction model into the final lipid equation rather than using the mean (as was done for ash content); however, variability was still low enough that the prediction model translated to provide a more accurate final equation.

Replacing R with Equation 3:

$$P = (1191(767.69 \times C{:}N^{-3.6}) \div \{1250((767.69 \times C{:}N^{-3.6}) + 1)\}$$

$$P = 731.455 \div (C{:}N^{3.6} + 767.69)$$

Equation 7

Now we have an equation (Equation 7) to determine the protein% of eel muscle using the C:N ratio, which we incorporate into equation 2:

$$C{:}N = 0.00423 + 3.196[731.455/(C{:}N^{3.6} + 767.69)] + 9.1096(\text{Lipid\%})$$

This equation, when rearranged for Lipid%, yields Equation 1. Regression plots for both the McConnaughey & McRoy (1979) and our Equation 1 plots are shown in Figure 7.

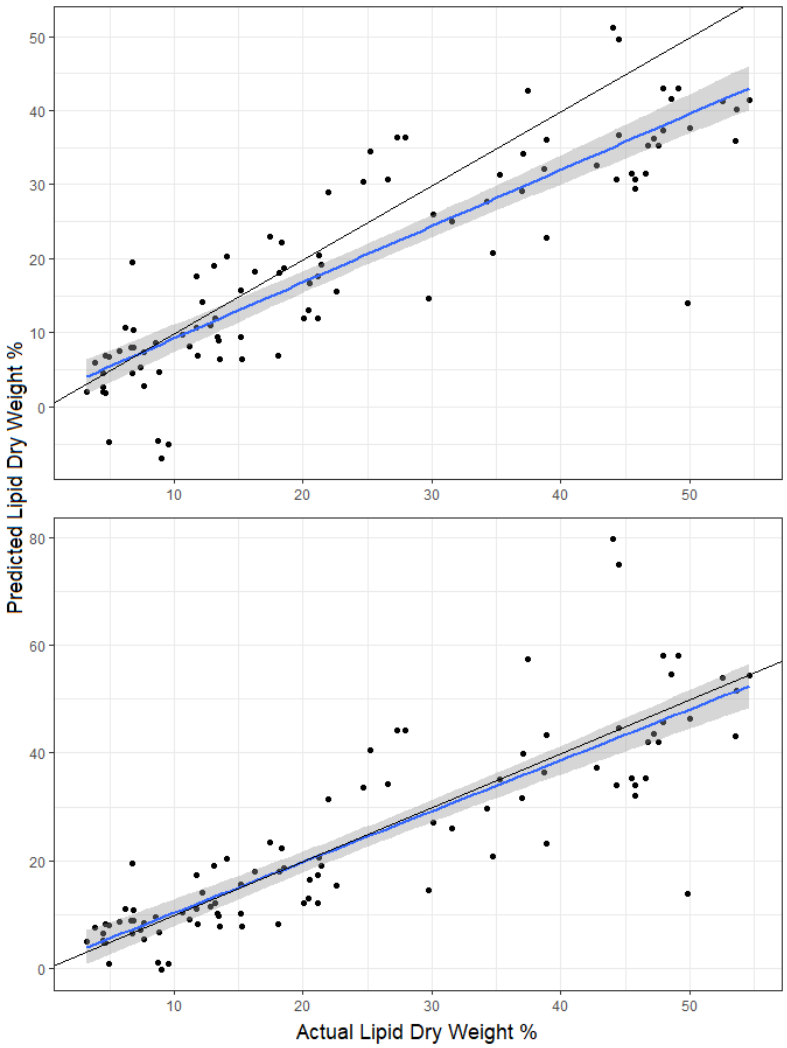

Figure 7: Predicted lipid dry weight percentage versus actual lipid dry weight percentage in American eel muscle tissue using two different mathematical models: the equation used to predict lipid content in McConnaughey & McRoy (1979) and Kiljunen et al. (2006) (Top); and, our Equation 1 (Bottom). Black lines represent the 1:1 line for reference, Blue lines represent the line of best fit for data using each model, and shading represents the 95% confidence interval for each regression line.

The new equation provides a better lipid estimation for American eels than the McConnaughey & McRoy (1979) equation (Table 1). Both r^2 values are comparable, but the resulting slope and intercept of predicted versus observed values are much closer to a

1:1 relationship when our Equation 1 is used; the root-mean-square-error is also lower. It should be noted that the lipid estimation is most accurate for muscle samples with C:N ratios < 6.5:1 eels; above this threshold, the model tends towards overestimation.

Table 1: Slopes, intercepts, r^2 and root-mean-square-error for predicted lipid content compared to observed lipid content in American eel muscle tissue. The two lipid models compared here are the McConnaughey & McRoy (1979) model used by Kiljunen et al. (2006), denoted as "M&M", and our equation 1, denoted as "Novel".

Model	Slope	Intercept	r^2	Root-mean-square-error
M&M	0.76	1.64	0.76	8.84
Novel	0.94	0.88	0.73	7.89

3.3 Results

$\delta^{13}C$ values for muscle tissue and fin were highly correlated (r^2 = 0.93, Figure 9) as were $\delta^{13}C$ of liver tissue and fin ($r^2 = 0.91$, Figure 9). The correlation in $\delta^{15}N$ for muscle tissue and fin is not as strong though was still highly correlated (r^2 = 0.82, Figure 7); this is also the case for the $\delta^{15}N$ correlation between liver tissue and caudal fin clips (r^2 = 0.78, Figure 9). For $\delta^{13}C$, fin is depleted by 1.0 ± 0.9‰ (mean ± SD) relative to muscle tissue and depleted by 0.4 ± 1.0‰ relative to liver tissue (Figure 8). Fin is enriched in $\delta^{15}N$ relative to muscle tissue by 0.9 ± 0.6‰ (Figure 8), and depleted relative to liver tissue by -0.9 ± 0.5‰ (Figure 8), putting $\delta^{15}N$ values for fin halfway between $\delta^{15}N$ for muscle and liver.

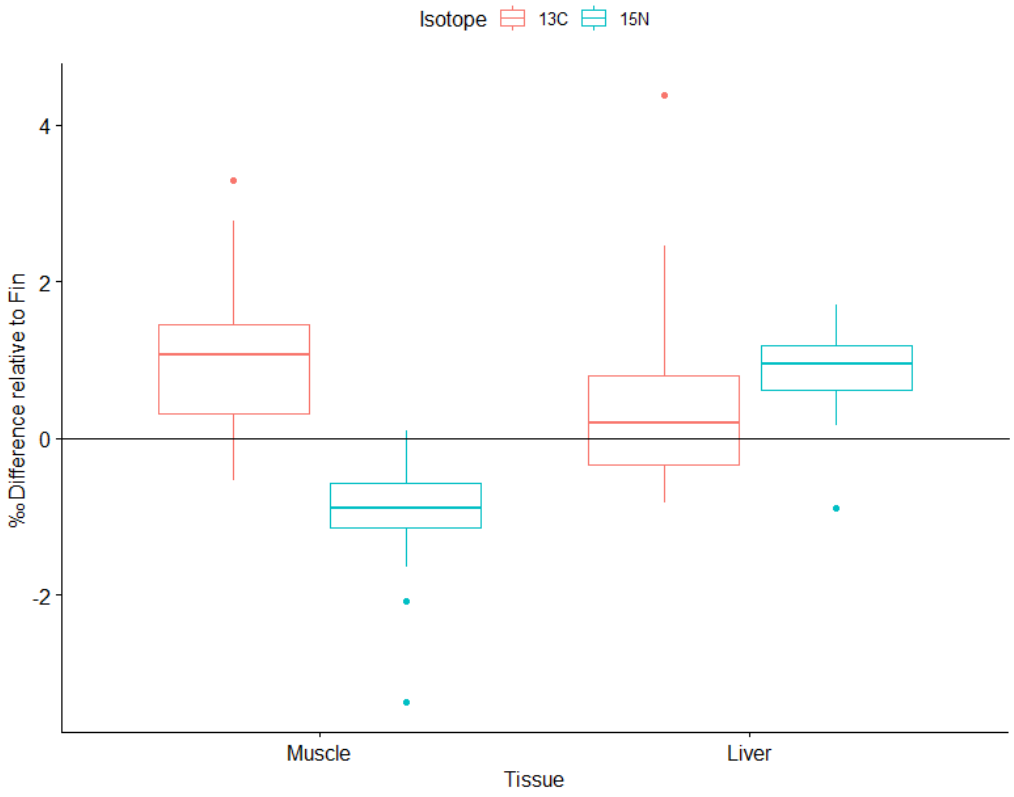

Figure 8: Boxplot comparison of $\delta^{13}C$ and $\delta^{15}N$ ‰ differences between fin and muscle tissue as well as fin and liver tissue. Positive values reflect enrichment relative to fin and negative values reflect depletion. Mean differences for $\delta^{13}C$ relative to fin is 1.0‰ (s.d. ± 0.9‰) for muscle and 0.4‰ (s.d. ± 1.0‰) for liver. Mean differences for $\delta^{15}N$ relative to fin is -0.9‰ (s.d. ± 0.6‰) for muscle and 0.9‰ (s.d. ± 0.5‰) for liver.

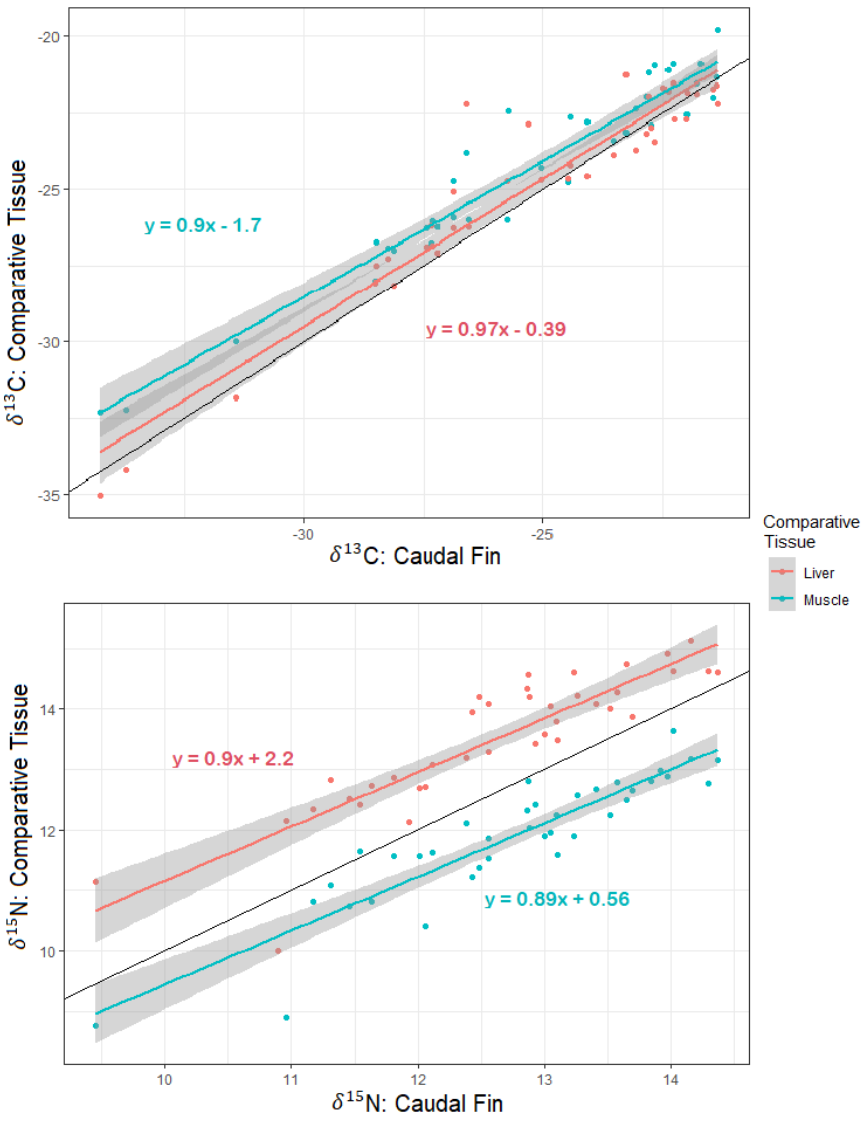

Figure 9: $\delta^{13}C$ and $\delta^{15}N$ values for caudal fin tissue regressed against $\delta^{13}C$ and $\delta^{15}N$ values for liver tissue and muscle tissue from American eels. Shading represents the 95% confidence interval for each regression line, and the black line is the 1:1 line for reference.

Of the slopes from the four comparisons, only one differed significantly from a slope of 1; the slope for fin-muscle $\delta^{13}C$ ($p < 0.05$, Table 2). The slope for fin-muscle $\delta^{15}N$ was only slightly above the significance threshold of 0.05 ($p = 0.054$, Table 2).

Table 2: Summary statistics for two-tailed t-tests of the linear slopes of $\delta^{13}C$ and $\delta^{15}N$ comparisons between tissues to a slope of 1.

Comparison	95% CI	df	*t*	*p*
fin-muscle $\delta^{13}C$	0.81, 0.96	36	2.38	0.011
fin-muscle $\delta^{15}N$	0.75, 1.02	36	1.65	0.054
fin-liver $\delta^{13}C$	0.86, 1.07	37	0.58	0.283
fin-liver $\delta^{15}N$	0.74, 1.06	37	1.27	0.106

3.4 Discussion

Similar to previous studies on both tropical and temperate fish species (Jardine et al., 2011; Kelly et al., 2006), $\delta^{13}C$ values for American eel muscle tissue and fin tissue are highly correlated. My data show that muscle is slightly enriched in $\delta^{13}C$ relative to fin. $\delta^{13}C$ values for liver tissue and fin are similarly correlated, but the mean difference between liver and fin clip $\delta^{13}C$ values (-0.4‰) is less than half of the difference between muscle and fin $\delta^{13}C$ values (-1‰). Additionally, the slopes for the fin-muscle and fin-liver $\delta^{13}C$ comparisons were tested for significant difference vs. a slope of 1; fin-muscle differed significantly, whereas fin-liver did not.

I also observed a strong correlation between $\delta^{15}N$ of muscle tissue and fin tissue and between liver tissue and fin tissue. As with $\delta^{13}C$, we also compared the $\delta^{15}N$ slopes

for fin-muscle and fin-liver to a slope of 1; neither differed significantly, however the *p*-value for fin-muscle, which was just above the 0.05 threshold, was nearly half that of fin-liver (fin-muscle $p = 0.054$, fin-liver $p = 0.106$; Table 2).

This closer match between caudal fin clips and liver tissue suggests that fin tissue has a quicker turnover rate than muscle tissue in American eels. Muscle tissue typically has a slower turnover rate than liver (Buchheister & Latour, 2010; Logan et al., 2006; Skinner et al., 2017). This would imply that fin $\delta^{13}C$ and $\delta^{15}N$ values in eels are more representative of liver than muscle and that fin clips are more well-suited to studies requiring shorter-term dietary indicators where liver tissue would typically be used. Further study on isotopic turnover in eel tissues could help confirm this.

Our results indicate that caudal fin clips are a viable non-lethal tissue surrogate for stable isotope analysis studies involving American eels, and that they appear to be more analogous to liver tissue than muscle. This utility of fins as a surrogate tissue is compatible with studies on a variety of fishes (Jardine et al., 2011; Kelly et al., 2006), suggesting that caudal fins are similarly useful as surrogate tissues for other anguillid eel species. This is important because anguillid eels are highly beneficial for studying ecosystems due to their wide distribution and their potential as both indicator and umbrella species (Itakura et al., 2020). Furthermore, two congeneric species are also classified as threatened by the IUCN, with Japanese eel being classified as Endangered (Kasai et al., 2021), and European eel classified as Critically Endangered (Bevacqua et al., 2015). Thus, it is beneficial for studies involving anguillid eel species to have opportunity for non-lethal tissue sampling for stable isotope analysis.

CONCLUSION

Summary of Research

My research sought to improve understanding of how *A. crassus* spreads in American eel populations in freshwater and estuary areas of a river system, which was achieved by the results of chapter 1; I discovered that higher trophic position and condition factor together played a role in influencing the number of *A. crassus* an individual eel is infected with. Along with this, I also sought to improve methods for isotopic analysis involving both anguillid eels as well as nematode parasites such as *A. crassus*; this was accomplished in chapters 2 and 3. Chapter 2 found that by removing material from the digestive tract of *A. crassus*, mean $\delta^{15}N$ of the nematodes increased to 3.03‰, which is closer to the theoretical trophic discrimination value of 3.4‰ (Post, 2002; Boecklen et al., 2011, Nachev el al., 2017). In chapter 3, I devised a mathematical equation for better estimating the lipid content of anguillid eels, which can be used in conjunction with the Kiljunen et al. (2006) lipid correction equation to better correct $\delta^{13}C$ in eel muscle. I also tested the viability of eel caudal fin clips vs muscle and liver tissue for use as a non-lethal surrogate for stable isotope analysis; fin tissue was found to be both viable and more analogous to liver tissue than muscle tissue.

Implications for Future Research

Both my research and the research of Pegg et al. (2015) have found evidence that within river systems, primarily piscivorous anguillid eels (i.e. eels with higher trophic position and condition factor) have greater *A. crassus* infection intensity. These studies, however, only looked at the yellow eel life stage. A good focus for future research on *A.*

crassus would be to try to understand how trophic interactions in the younger life stages (i.e. glass eel & elver) influence infection intensity of *A. crassus* during said life stages. It may be that eels experience greater infection intensity during these early life stages while they feed directly on the intermediate hosts of *A. crassus* (copepods & ostracods), followed by a decrease in infection intensity as they begin feeding on macroinvertebrates, followed once again by an increase in infection intensity when they become more piscivorous.

My research has also demonstrated that the digestive tract contents of *A. crassus* has a significant effect on the nematode's $\delta^{15}N$, and that future studies would benefit from the removal of said contents. Our methods used dissection which worked well for larger *A. crassus*. It may be possible to keep smaller parasites, which cannot be dissected easily, alive long enough for them to expel the contents of their digestive tracts; however, experimentation would need to be conducted on a case-by-case basis depending on the species of parasite to determine whether this would work.

Finally, not only have I demonstrated that nonlethal sampling of caudal fins is a viable method for research involving stable isotope analysis and anguillid eels, but I have also created a better method for estimating the lipid content of eel muscle tissue. Nonlethal sampling is incredibly important for research as the American eel is already listed as endangered by the IUCN and could potentially be listed under SARA in Canada. My lipid estimation model can be used in conjunction with the lipid correction model devised by Kiljunen et al. (2006) to better correct for lipid without having to perform lipid extraction on the samples.

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APPENDIX I

CURRICULUM VITAE

Ethan Norman Augustine

Universities Attended:

University of New Brunswick (2020) - Bachelor of Science

Publications:

N/A

Presentations:

Augustine, Ethan. Marine Block Guest Lecture, 2021. “Stable Isotope Analysis, American Eels and *A. crassus*”