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EVALUATION OF NEW ENGLAND SALT MARSH SUPPORT OF THE AMERICAN EEL, ANGUILLA ROSTRATA, AND THE IMPACTS OF HYDROLOGIC RESTRICTION

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EVALUATION OF NEW ENGLAND SALT MARSH SUPPORT OF THE AMERICAN EEL, *ANGUILLA ROSTRATA,* AND THE IMPACTS OF HYDROLOGIC RESTRICTION

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Dedication

This dissertation is dedicated to two strong women who have provided me with unwavering support – my grandmother, Josephine Lipinski, and my mother, Carol Eberhardt.

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ABSTRACT

American eels are frequently captured in saltmarsh habitats yet little is known about eel use of these systems. Coastal systems such as salt marshes are vulnerable to habitat impacts due to high rates of coastal development, including impacts from undersized culverts that result in tidally restricted systems upstream. Given the decline in the American eel population, a need exists for a clearer understanding of the functional difference of hydrologically restricted and unrestricted salt marshes in the support of eels. To address data gaps and inform saltmarsh management to support eels, laboratory and field experiments were employed that assessed the value of saltmarsh habitats to the life history of the American eel. Eels held in the laboratory were subject to a diet switch to determine the rates at which eel mucus, fin, muscle, and liver tissue assimilate the carbon and nitrogen isotope values of the diet (turnover rate) and the level of discrimination in each tissue relative to the diet (trophic discrimination factors). These data were used to inform the interpretation of data collected from hydrologically restricted and unrestricted creeks in three New England estuaries. Isotope data indicated strong evidence of salt marsh primary producers contributing to the basal diet of eels as well as consumption of marsh resident secondary consumers. Eel gut contents also contained abundant saltmarsh secondary consumers, confirming that eels serve as top predators and are residents in salt marshes. Greater eel trophic position measured upstream of reference creeks indicates that tidal restrictions may result in an altered food web in the tidally restricted marsh. Models were developed for predicting muscle and liver $\delta^{15}N$ and $\delta^{13}C$ from mucus and fin to provide a non-lethal alternative for sampling yellow eels for stable isotope analysis. Trends in data from a telemetry study suggest that eels released upstream of an undersized culvert with a self-regulating tide gate travelled shorter distances than eels in the reference creeks and had delayed movements to downstream areas of

the marsh relative to eels in the reference creek. This study addresses a critical data need for the management of salt marshes to support eels. It provides evidence of eel use of salt marshes as important foraging resources, negative impacts of tidal restriction on trophic support and movement of eels, as well as important data to support future stable isotope analysis of eels. The cumulative impact of marsh loss and degradation, such as through tidal restriction, may be a contributing factor in the decline of eel populations. Conservation and restoration of salt marshes as habitat and management of marshes to maintain ecological integrity will provide critical trophic support and access to essential resources for the American eel population.

INTRODUCTION

The American eel, *Anguilla rostrata*, ranges throughout the western North Atlantic, from Greenland to northern South America, and is one of approximately eighteen Anguillid species worldwide. American eels are a panmictic species (Avise 2003) due to the fact that sexually mature "silver" eels migrate to the Sargasso Sea to form a single breeding population and then die (Schmidt 1925). Once eggs hatch into the larval "leptocephali" stage they are distributed by ocean currents throughout the western Atlantic, Gulf of Mexico, and Caribbean Sea (Tesch 2003). Upon reaching coastal waters as transparent "glass eels" or pigmented "elvers," eels remain in inshore habitats for up to 40 years in the juvenile "yellow" life stage (Jessop 1987; Tesch 2003). After reaching sexual maturity, they begin the spawning migration back to the Sargasso Sea.

Historically, American eels were abundant in the Gulf of Maine (Goode 2006) and served as an important source of income and sustenance throughout New England and Canada (Bolster 2002, SRSF 2002). However, eels are in decline over the entirety of their range (Haro et al. 2000) and as a result, were proposed for listing under the US Endangered Species Act (ESA). After review in 2007 and again in 2015, it was determined that protection under the ESA was not warranted given the robust genetic pool as a result of American eels constituting a single breeding population as well as more recent data that eels can complete their life cycle in marine and estuarine waters (50 CFR Part 17 2015). Potential causes of eel decline include migration barriers, hydro turbine mortality, overfishing, and habitat loss (Haro et al. 2000). Due to the lack of knowledge and potential severity of habitat loss impacts on eels, the Atlantic States

Marine Fisheries Commission (ASMFC) Fishery Management Plan for the American eel lists use of inshore habitat and impacts of habitat loss as high priority research needs (ASMFC 2006).

Due to the similarities among the frequently studied eel species, *Anguilla japonica, Anguilla anguilla*, and *A. rostrata*, findings on habitat use are typically applied across all three species (Tesch 2003). The conventional understanding of Anguillids' inshore habitat use has been obligate catadromy; in fact, eel reliance on freshwater habitats is so well accepted that its common name is the "freshwater eel." A commonly held paradigm was that eels exhibited a period of residency in estuarine habitats prior to migration upstream to freshwater habitats in which they remained for many years until the onset of sexual maturation and subsequent outmigration (Moriarty 1978; Helfman et al. 1987). However, in recent decades research examining the life history of Anguillids has supported alternative hypotheses. Brackish and marine habitats are emerging as more important in eel life history than originally thought. Tsukamoto et al. (1998) collected eels in freshwater habitats in Japan and Germany and marine habitats in the North and East China Seas. Through analysis of the strontium (Sr) to calcium (Ca) ratios in otoliths, Tsukamoto et al. (1998) found a high degree of eel residency in both fresh and marine/brackish environments. Eels captured in freshwater had moved into freshwater habitats and remained; similarly, they also found marine eels that never used freshwater and remained in marine habitats (Tsukamoto et al. 1998). Subsequent research has observed similar use of exclusively brackish and marine habitats by eels, providing further evidence of facultative, rather than obligate catadromy (Tsukamoto and Arai 2001; Jessop et al. 2002; Tsukamoto et al. 2002; Jessop et al. 2004). Based largely on otolith Sr:Ca research, three general life history strategies for yellow eels have emerged: 1. residency in freshwater (true catadromy) with no use of brackish or marine habitats; 2. residency in brackish or marine habitats and no use of freshwater

habitats; and 3. movement between estuarine and freshwater habitats (Tsukamoto and Arai 2001; Morrison and Secor 2003; Tzeng et al. 2003).

The diversity of life history strategies displayed by Anguillids may be explained in part by the hypothesis that eels at high latitudes rely more heavily on estuarine habitats (Tsuakamoto and Arai 2001; Tsukamoto et al. 2002). In support of this, Tzeng et al. (2002; 2003) observed *A. japonica* captured in China, Japan and Taiwan utilizing marine, fresh and estuarine habitats; however, the majority of eels used estuarine habitats. Tsukamoto and Arai (2001) speculate that the greater use of estuarine habitats may be due to more abundant food in estuaries relative to freshwater habitats in northern regions (Gross 1987). Furthermore, evidence exists for faster growth in estuarine habitats than in freshwater in northern latitudes for *A. rostrata* (Oliveira 1999, Jessop et al. 2004; Morrison and Secor 2003), *A. anguilla* (Fernandez-Delgado et al. 1989; Harrod et al. 2005), and *A. japonica* (Tzeng et al. 2003) supporting the hypothesis that some eels remain in estuarine habitats due to the more favorable habitat conditions. Based on the observed preference of *A. japonica* for estuarine habitats, Tsukamoto et al. (1998) and Tsukamoto and Arai (2001) concluded that eels from these habitats may provide the greatest contribution to coastal productivity and eel recruitment. Accordingly, there is a need for greater understanding of estuarine habitat use by eels.

Within estuaries, Anguillids are frequently captured in saltmarsh habitats (Laffaille et al. 2000; Kimball and Able 2007; Eberhardt et al. 2011) and in some systems comprise the majority of fish biomass (Dionne et al. 1999). Despite the abundance of eels in salt marshes, little is known about Anguillid use of these habitats. Evidence exists for a limited home range of approximately 1 kilometer (km) in salt marsh creeks (Ford and Mercer 1979, Helfman et al. 1983; Bozeman et al. 1985) suggesting that salt marshes provide sufficient trophic support for

eels so as to preclude the need for movement over large areas to forage (Bozeman et al. 1985). However, this hypothesis remains largely untested. Particularly in light of the potential for the yellow life stage to remain resident in estuarine habitats such as salt marshes for many years (Jessop 1987; Tsukamoto and Arai 2001), a need exists for greater understanding of eel use of salt marshes in terms of movements and trophic support.

Coastal habitats such as salt marshes are particularly vulnerable to habitat impacts due to high rates of coastal development and use of salt marshes as transportation corridors. Structures such as culverts are frequently installed to allow the tide to continue underneath roads and highways where they intersect salt marsh creeks. Historically, engineers designed these structures to allow water to drain under the road and little consideration was given to factors such as fish passage or tidal support of the upstream ecosystem. As a result, many culverts do not accommodate the full tidal regime, resulting in a tidally restricted system upstream. In hydrologically restricted salt marshes of New England, halophytic vegetation is often replaced by exotic species such as common reed, *Phragmites australis,* upstream of the restriction (Roman et al. 1984; Burdick et al. 1997). Colonization by invasive species as well as changes to the infaunal communities (Fell et al. 1991) can shift the food base of restricted salt marshes resulting in an altered food web. Furthermore, reduced flooding in hydrologically restricted marshes can limit fish access to food resources (Weisberg and Lotrich 1982). Decreased eel movement and their use of hydrologically restricted salt marshes may result in some marsh areas or habitats contributing disproportionately to fish populations (Gillanders 2005). Furthermore, for the conservation of Anguillids, it is important to understand the functional differences of hydrologically restricted and unrestricted salt marshes in the trophic support of eels.

Project goals

Given the decline in the American eel population, as well as recent understanding that some eels depend on estuarine systems, a need exists for a clearer understanding of eel reliance on estuarine habitats such as salt marshes. To address data gaps and inform salt marsh management to support eels, the overalls goals of this project were:

1. To improve understanding of the value of salt marsh habitats to the life history of the American eel, *Anguilla rostrata*, with respect to trophic support and movement
2. To evaluate the functional equivalency of both hydrologically restricted and unrestricted salt marshes in the support of *A. rostrata*

Overview of experiments

The following four chapters describe the results of experiments devised to address these goals. Chapter I, in preparation to submit to a peer reviewed journal, details a laboratory experiment designed to provide stable isotope data to inform field investigations. The nitrogen and carbon stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) of eel tissues were evaluated before and after a diet switch in a controlled laboratory experiment. Tissue turnover (time to assimilate the isotope values of the new diet) and trophic discrimination factors (difference in isotope values between the diet and eel) for several types of tissues were calculated. These data were used to interpret stable isotope data from eels collected in salt marshes for a more accurate understanding of eel resource use.

Chapter II is a field experiment designed to evaluate trophic support of American eels in salt marshes. Eels and their potential food sources were collected in three New England estuaries (the Webhannet Estuary, ME, the Hampton-Seabrook Estuary, NH, and the Parker River Estuary, MA) dominated by salt marsh. Within each estuary eels were collected from upstream

and downstream areas of two creeks – a creek with a tidal restriction and a reference creek with unaltered hydrology. Eels and potential food sources were analyzed for $\delta^{15}N$ and $\delta^{13}C$ to determine eel trophic use of saltmarsh habitats over time and to evaluate the functional equivalency of hydrologically restricted and unrestricted salt marshes in terms of eel trophic support. Eel gut contents were also analyzed to evaluate recent use of salt marshes as a foraging resource. Chapter II was published in 2015 in *Estuaries and Coasts* (Eberhardt et al. 2015).

Chapter III develops the foundational support to establish a nonlethal method of eel sample collection for $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis. Predictive models were developed from field collected data to evaluate the potential for eel mucus and fin tissue to serve as nonlethal surrogates for liver and muscle tissue. Models were subsequently validated with laboratory collected data. Chapter III is in preparation for submission to a peer-reviewed journal.

Chapter IV examines the small-scale movements of eels in saltmarsh creeks with acoustic telemetry. Nine acoustic receivers were positioned in the Webhannet Estuary, Wells, ME. Receivers were installed along a creek that contains a self-regulating tide gate (SRT) with an undersized culvert, a reference creek, and the area downstream of the two creeks. Nine eels were captured, implanted with acoustic transmitters, and released upstream of the reference creek or above the SRT. Telemetry data were downloaded after a month to examine eel movements in saltmarsh creeks and the influence of the SRT on eel movement. Chapter IV is formatted as a dissertation chapter due to the low sample size.

The final section provides a summary of the findings of Chapters I-IV and discusses how these findings address the overall goals of the project. Finally, based on the data presented, recommendations are provided for future research and for natural resource managers on how best to manage salt marshes in support of American eels.

CHAPTER I

Stable isotope discrimination and turnover in multiple tissues of the American eel, *Anguilla rostrata*

ABSTRACT

Stable isotopes of carbon and nitrogen are frequently used to gain insight into organism feeding and movement patterns. Interpretation of isotope data is improved with species and tissue specific data on the rate and magnitude of isotope assimilation into tissues, particularly when the target species is a slow-growing omnivore. Stable carbon and nitrogen isotopes were evaluated in multiple tissues (fin, muscle, liver, and mucus) of yellow stage American eels (*Anguilla rostrata*) subject to a diet-switch experiment to determine isotopic turnover rates and discrimination. Trophic discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$; differences in isotope values between the diet and tissue) varied significantly among tissues for ^{15}N but not for ^{13}C. The $\Delta^{15}N$ values were greatest for liver, while fin and mucus were intermediate and equal, and muscle was lowest. Turnover of ^{15}N was fastest in mucus (half-life = 67 days) followed by fin (90 days), liver (97 days), and muscle tissue (191 days). Carbon half-lives for all tissues were longer than could be determined from the 35-day experimental period. The faster turnover of nitrogen in eel mucus renders it a useful indicator of short-term diet while muscle tissue can indicate long-term diets in yellow eels. Mucus also provides a non-invasive option for stable isotope analysis that is of particular importance for species in decline such as the American eel. By providing species and tissue specific trophic discrimination factors for carbon and nitrogen, as well as nitrogen

turnover estimates, this study addresses a critical data need for increasing the accuracy of estimates of trophic position, movement, and basal food sources for the American eel.

INTRODUCTION

American eels are a highly migratory species that move from their birthplace in the Sargasso Sea to inshore waters throughout the western North Atlantic where they remain for as many as 40 years before migrating back to the Sargasso Sea to spawn (Helfman et al. 1987; Jessop 1987; Tesch 2003). After eggs hatch, eels drift along ocean currents as leptocephali, and then metamorphose to transparent "glass eels" and then pigmented "elvers" as they enter coastal waters. Eels continue to gain pigment and grow as they move into the "yellow" life stage (Jessop 1987; Tesch 2003) followed by a final metamorphosis to "silver eel" prior to migrating back to the Sargasso Sea. Eels spend the majority of their lives in the yellow life stage and exhibit great plasticity in habitat use, moving among freshwater, estuarine, and marine habitats (Jessop et al. 2008). The large migrations as leptocephali and silver eels as well as the potential use of many inshore habitats as elvers and yellow eels results in exposure to many anthropogenic impacts and, as a result, the eel population is considered to be in decline (Haro et al. 2011). American eels were proposed for listing under the US Endangered Species Act (ESA) and after review in 2007 and again in 2015 it was determined that protection under the ESA was not warranted.

The use of many habitats and omnivorous diet of eels results in the potential for consuming a wide variety of prey (Tesch 2003). As a result, understanding the relative value of eel habitats can be difficult with traditional approaches such as gut content analysis that provide information on only the most recent meal. Stable isotopes hold great promise for understanding resource use over different time scales and at different life stages.

Carbon and nitrogen stable isotopes are commonly used in trophic ecology investigations to discern trends in feeding (e.g., Gaye-Siessegger et al. 2007; Andvik et al. 2010; McMahon et al. 2013; O'Brien 2015; Willis et al. 2017). Isotope values of a consumer can vary according to the proportion of heavy to light isotopes in food sources at the base of the food web, changes in isotope ratios as prey is assimilated into consumer tissues (i.e., discrimination) and variation in the rate at which the diet is assimilated (i.e., turnover; Fry 2006). With an understanding of both isotopic discrimination and turnover rates, the food sources and trophic position of a consumer can be more clearly estimated (Gannes et al. 1997; Post 2002).

Many food web investigations using stable isotopes have relied on trophic discrimination factors (TDFs) documented in the literature. The most commonly used TDFs are 3.4‰ for $\delta^{15}N$ (DeNiro and Epstein 1981; Minigawa and Wada 1984; Peterson and Fry 1987; Post 2002) and 0-1‰ for $\delta^{13}C$ (DeNiro and Epstein 1978; Fry and Sherr 1984). However, the magnitude of isotope enrichment between consumer and prey can vary widely (Vander Zanden and Rasmussen 2001; Bosley et al. 2002; Logan et al. 2006). Discrimination estimates may vary among species (Hobson and Clark 1992a; Vander Zanden and Rasmusssen 2001) as well as among tissue types within an individual consumer (Pinnegar and Polunin 1999). Various tissues are made up of different compounds, each of which fractionates carbon and nitrogen isotopes differently. Additionally, the biochemical makeup of the diet and the pathways by which it is assimilated, plays a role in determining the isotopic composition of consumer tissues (McMahon et al. 2010; Bloomfield et al. 2011). For example, dietary proteins can be routed to animal proteins with minimal isotopic fractionation while animal proteins synthesized from a carbohydrate rich diet will undergo fractionation because the carbohydrates must be catabolized and synthesized into amino acids. This process is known as nutrient or isotopic routing (Schwarcz 1991).

Turnover rate can vary by tissue (Sweeting et al. 2004); as such, by determining the $\delta^{13}C$ and $\delta^{15}N$ turnover times of various tissues, a change in diet of an organism can be inferred over different time periods. Tissues with a fast metabolic rate or high lipid content (e.g., liver) typically have faster turnover than tissues with lower lipid content or that are less metabolically active (e.g., muscle; Tieszen et al. 1983; Hobson and Clark 1992b; MacNeil et al. 2006). Turnover rates can vary by both species and life stage in fishes (Weidel et al. 2011). Quickly growing organisms tend to have faster turnover rates than organisms growing more slowly (Fry and Arnold 1982; Bosley et al. 2002). Evaluation of tissues with varying turnover rates allows greater insight into consumer diets, allowing for the understanding of more recent diet (rapid turnover) and long-term diet (slow turnover tissues). For example, fish muscle in slow growing species can have a turnover rate of many months (e.g., Miller 2006) to a year whereas the higher metabolic activity of the liver results in more rapid turnover of isotope values that can serve as an indicator of short-term diet such as weeks to months (Tieszen et al. 1983; Hobson and Clark 1992b).

Fish diets vary according to ontogeny, amount and quality of food available, and movement among habitats characterized by different food sources (Helfman et al. 1997; McMahon et al. 2016). If the diet of a consumer changes over time, such as with opportunistic feeders like the American eel, it is important to know the time it takes for the isotope values of the eel tissues to equilibrate to the isotope values of its food sources (i.e., turnover rate; Carter et al. 2019). For accurate interpretation of field collected data, more information on the timing of isotope turnover patterns and timing in eel tissues is needed.

To determine TDFs and the rates of isotopic turnover between eels and their diet, eels were held in a controlled laboratory experiment and fed two diets, each distinct in carbon and

nitrogen stable isotope values. Eel tissues were sampled for $\delta^{13}C$ and $\delta^{15}N$ before and then periodically after a change in diet, to estimate the rate at which each tissue assimilates the isotope values of the new diet. Specifically, the objective of this research was to determine the rates at which eel mucus, fin, muscle, and liver assimilate the carbon and nitrogen isotope values of its diet (turnover rate) and the level of enrichment in individual tissues relative to the diet (trophic discrimination factor) to provide critical information to inform field study of eel trophic ecology.

METHODS

Fish feeding experiment

Eels were obtained from an aquaculture facility where fish were fed a standard pelletized diet ensuring homogeneity of baseline stable isotope values. Yellow eels of a similar size (30-45 cm) were selected to standardize growth rates. Fifty-three eels were measured, weighed, and marked via subcutaneous injection of acrylic paint to allow for identification of individuals for growth measurements. Eels were held in an aquarium (1115 Liter capacity) equipped with a flow through seawater system to provide sufficient aeration and water exchange. Tanks and plumbing output and intake pipes were covered with mesh to prevent eel escape.

Eels were fed the same pellet food they received at the aquaculture facility ("control diet") for two weeks to aid in acclimation. Nine eels were sacrificed prior to the diet switch (Day 0) to serve as a baseline; the remaining 43 eels were switched to a treatment diet of labeled earthworms. Eels were fed 2% of their combined body weight each day (Arai 1987). The $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions of the control diet were identified and based on the results, a second diet ("treatment diet") was cultured to be isotopically distinct. Earthworms (*Eisenia foetida*) were fed corn husks enriched with 99.9% ^{15}N ammonium chloride (based on the

methods of MacNeil et al. 2006) to provide a food source enriched in both ^{13}C and ^{15}N relative to the control diet. Corn was selected for culture of the treatment diet to create a food source more enriched in ^{13}C than the control. Corn utilizes a C4 photosynthetic pathway resulting in $\delta^{13}C$ values more enriched than primary producers utilizing C3 photosynthesis (Bender 1968; Smith and Epstein 1971). The dietary components of the control and treatment diets were similar where the control diet was comprised of 11% carbohydrate, 48% protein, and 28% lipid (Biomar for DAN-EX 2848) and the treatment diet was comprised of approximately 15% carbohydrate, 59% protein, and 9% lipid (Tacon et al. 1983).

Five grams of ^{15}N ammonium chloride were added to a 1 Liter mixture of corn slurry and deionized water. The solution was mixed daily to ensure equal distribution of the label. The corn slurry was incubated for eight days to allow for bacteria within the slurry to take up the ^{15}N enriched ammonium and then was added to 108 grams of shredded, dampened corn husks. Four kilograms of earthworms were added to the corn slurry-husk mixture. After three weeks, nine earthworms were sampled to determine $\delta^{13}C$ and $\delta^{15}N$ and ensure that isotopically enriched bacteria were assimilated (Appendix A). Once treatment and control diets were confirmed to be isotopically distinct (Table 1), the remaining earthworms were harvested and frozen.

Table 1. Carbon and nitrogen stable isotope means (± standard error) for the control (eel feed) and treatment (earthworm) diets and results of unequal variances t-test ($\delta^{15}N$) and ANOVA ($\delta^{13}C$) for differences between the dietary composition of each diet.

	eel feed (control)	earthworm (treatment)	t/F	p
$\delta^{15}N$ (‰)	9.95 ± 0.13	108.09 ± 28.77	3.41	0.0052
$\delta^{13}C$ (‰)	-23.94 ± 0.07	-16.57 ± 0.13	652.51	0.0001

Fish were sacrificed for analysis 0, 1, 4, 7, 11, 14, 17, 21, 28, and 35 days relative to the diet switch. Five fish were sacrificed at each time with the exception of day 0 (n=9). Prior to culling, fish were immediately placed in water containing carbon dioxide for anesthetization. Carbon dioxide was selected due to the decreased potential for chemical alteration of fish tissues for isotope analysis relative to other commonly used fish anesthetics (e.g., MS-222, clove oil). Fish were identified by their mark, measured for length and mass and then sacrificed by way of decapitation in accordance with the American Veterinary Medical Association Panel on Euthanasia (2001) and following a protocol approved by the University of New Hampshire Institutional Animal Care and Use Committee (IACUC # 070702; Appendix B).

Sample collection and isotope analysis

Although mucus is not a tissue, and fin clips are comprised of multiple tissues (e.g., skin, bone, and muscle if clipped close to the body), for the simplicity of referring to all sample types in aggregate, mucus and fin will each be referred to as a tissue. Eels were rinsed with distilled water to remove surface debris and mucus samples were collected from the skin surface by gently scraping with the blunt edge of a scalpel. Each mucus sample was placed on a tin tray for drying. Fish were frozen prior to dissection of muscle, liver and fin tissue for stable isotope analysis. All samples were held in a drying oven at 60^{o}C to achieve constant weight, ground with a mortar and pestle, weighed to the nearest microgram into a tin capsule, and then compacted into a small cube. Tools and work surfaces were cleaned with 99.5% ethanol and Kimwipes® between processing each sample to prevent cross-contamination. Samples were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes at the University of New Hampshire Stable Isotope Laboratory with a Costech ECS4010 Elemental Analyzer coupled to a Delta Plus XP mass spectrometer (Thermo Finnigan). Stable isotope ratios are reported in delta (δ) notation per

mil units (‰) as follows:

$\delta X = (R_{sample}/R_{standard} - 1) \times 1000$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Stable isotope ratios were determined using Vienna Pee Dee Belemnite (VPDB) as the reference material for carbon and atmospheric N_2 (air) for nitrogen. Repeated analyses of laboratory standards (tuna muscle) varied less than 0.15 per mil for both $\delta^{15}N$ and $\delta^{13}C$.

Data analysis

To address unequal variances in diet data for $\delta^{15}N$, values for control and treatment diets were tested with an unequal variances t-test. Differences between $\delta^{13}C$ values for control and treatment diets were tested with a one-way analysis of variance (ANOVA). Tropic discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) were calculated for each tissue as the difference between the $\delta^{15}N$ and $\delta^{13}C$ values of the control diet and the baseline eels sacrificed at day 0 (i.e., $\Delta = \delta_{tissue} - \delta_{diet}$).

Turnover data were modeled as a function of time since the diet switch. Data fit a logistic curve: $y = a/(1+(a-n_o/n_o)*e^{(-rt)})$ where $y = \delta^{15}N$ of the tissue at a given time since the diet switch and a=the asymptote, defined as the maximum $\delta^{15}N$ value for the treatment diet plus the tissue specific trophic discrimination factor, n_o=the expected $\delta^{15}N$ value of y at time 0, r=the turnover rate, and t=time. To determine half-lives $\delta^{15}N$ data were logit transformed where logit $\delta^{15}N = \ln((y/a)/(1-y/a))$. Logit transformed data have a linear relationship with predictor values; therefore, logit $\delta^{15}N$ data were modeled against time with linear regression where logit $\delta^{15}N = \alpha+\beta t$. Half-life was calculated as $-\alpha/\beta$. Non-transformed data are presented in figures. Because

data for $\delta^{13}C$ did not fit a logistic model, data were analyzed with a two-way ANOVA with time, tissue type, and their interaction as factors.

RESULTS

Fish feeding and growth

Earthworm culture in the laboratory was successful in creating a treatment diet with a significantly different isotopic composition from that of the control for both $\delta^{13}C$ and $\delta^{15}N$ (Table 1). Mean $\delta^{13}C$ and $\delta^{15}N$ values for the control diet were -23.94±0.07‰ and 9.95±0.13‰ (mean ± standard error), respectively. Cultured earthworm $\delta^{15}N$ values were high and variable (108.09 ±28.77) as has been observed with other efforts to culture diets using isotope labeling (e.g., MacNeil et al. 2006). However, the variability within the treatment diet was negligible relative to the overall difference between control and treatment diet means. Earthworm $\delta^{13}C$ values were also sufficiently distinct with a difference of more than 7‰ from the control diet achieved through a natural abundance diet (i.e., without isotopic labeling; Table 1).

Eels held in the laboratory maintained high feeding vigor with both control and treatment diets. Eels appeared healthy and active; no signs of distress were present, and no mortality was observed for the length of the experiment. Worms remained in the tank for over 24 hours immediately following the diet switch, presumably as eels adjusted from a pellet-based diet to worms. After that all food was consumed within 24 hours of being fed. Although feed amounts were calculated to promote maintenance and growth, eels lost mass over the course of the experiment; mean eel mass decreased from 91.2 ± 23.6 grams (g) to 82.1 ± 21.9 g and was variable among individuals ranging from 0 to 24 g. Mean eel length did not change from 37.5 ± 3.7 centimeters (cm).

Trophic discrimination

Trophic discrimination factors were calculated for each tissue from eels sacrificed at day 0 (i.e., prior to the diet switch). Note that mucus will be referred to as a tissue for ease of discussing all samples collectively. TDFs ranged among tissues from 1.18‰ to 2.46‰ for $\Delta^{15}N$ and 1.99‰ to 3.11‰ for $\Delta^{13}C$ (Table 2). Discrimination for ^{15}N was significantly different among tissues (ANOVA; $F=16.9$; $p<0.001$) indicating that ^{15}N enrichment varies with tissue type for eels. A post-hoc test (Tukey-Kramer HSD) revealed that all tissue comparisons had significantly different $\Delta^{15}N$ values except for fin and mucus. TDFs for ^{15}N were significantly different from 3.4‰ for each tissue (t-test; muscle, $t=-16.0$, $p<0.0001$; fin, $t=-11.0$, $p<0.0001$; liver, $t=-7.52$, $p<0.0001$; mucus, $t=-27.7$, $p<0.0001$) suggesting that this commonly used discrimination value is not appropriate for eels. All eel tissue was more enriched (i.e., less negative) in ^{13}C than the diet (mean $\Delta^{13}C = 2.41$‰) and differences did not differ significantly among tissues ($F=1.70$; $p<0.182$; Table 2). Carbon discrimination for all tissues was significantly different from 0‰ (t-test; $t=11.4$, $p<0.0001$) and 1‰ ($t=6.7$, $p<0.0001$), indicating that carbon TDFs vary significantly from those commonly used in the literature (DeNiro and Epstein 1978; Fry and Sherr 1984).

Isotope turnover

The lack of eel growth during the course of the experiment was expected given the generally slow growth rate of eels and the variability in growth rates of cultured eels (Wickins 1987; Tesch 2003); therefore, growth-based models (e.g., Fry and Arnold 1982; Hesslein et al. 1993; MacNeil

Table 2. Trophic discrimination factors for American eel tissues (calculated as the difference between the $\delta^{13}C$ and $\delta^{15}N$ values of the control diet and the baseline eels sacrificed at day 0). Different letters indicate significant differences in discrimination of $\Delta^{15}N$ between tissues. Carbon discrimination values ($\Delta^{13}C$) for all tissues were not significantly different. The mean of $\Delta^{13}C$ for all tissues was 2.41‰.

Tissue	N	$\Delta^{15}N$ (SE)	$\Delta^{13}C$ (SE)	C:N
fin	10	1.86 ± 0.14^{b}	2.64 ± 0.63	5.23 ± 0.14
liver	10	2.46 ± 0.12^{a}	2.00 ± 0.17	5.90 ± 0.22
mucus	10	1.86 ± 0.06^{b}	3.11 ± 0.39	3.68 ± 0.03
muscle	10	1.18 ± 0.14^{c}	1.99 ± 0.38	4.67 ± 0.12

et al. 2006) were not appropriate for this dataset and isotope data were modeled against time. For all tissues, $\delta^{15}N$ values remained relatively constant for 11 days before increasing; as a result, data best fit a logistic model (Figure 1a-d). Liver tissue showed the greatest increase over the course of the experiment reaching a mean $\delta^{15}N$ value of 202.7‰ by day 35 (Fig. 1b); the half-life of liver tissue was calculated as 97 days (Table 3). Mucus tissue had the fastest turnover rate (half-life=67 days; Table 3) and reached a mean $\delta^{15}N$ value of 107.3‰ by the end of the experiment (Figure 1d) followed by fin (mean $\delta^{15}N$ of 69.6‰; half-life=90 days; Figure 1a; Table 3). Muscle had the slowest nitrogen turnover rate of the tissues measured with a mean $\delta^{15}N$ of 28.4‰ and half-life of 191 days (Figure 1c; Table 3).

No significant increase in $\delta^{13}C$ values was measured after the diet switch in each eel tissue throughout the 35 day project (Figs. 2a-d); thus, neither growth-based nor logistic models could be used for analysis of $\delta^{13}C$ data for turnover rates. It is possible that carbon turnover for

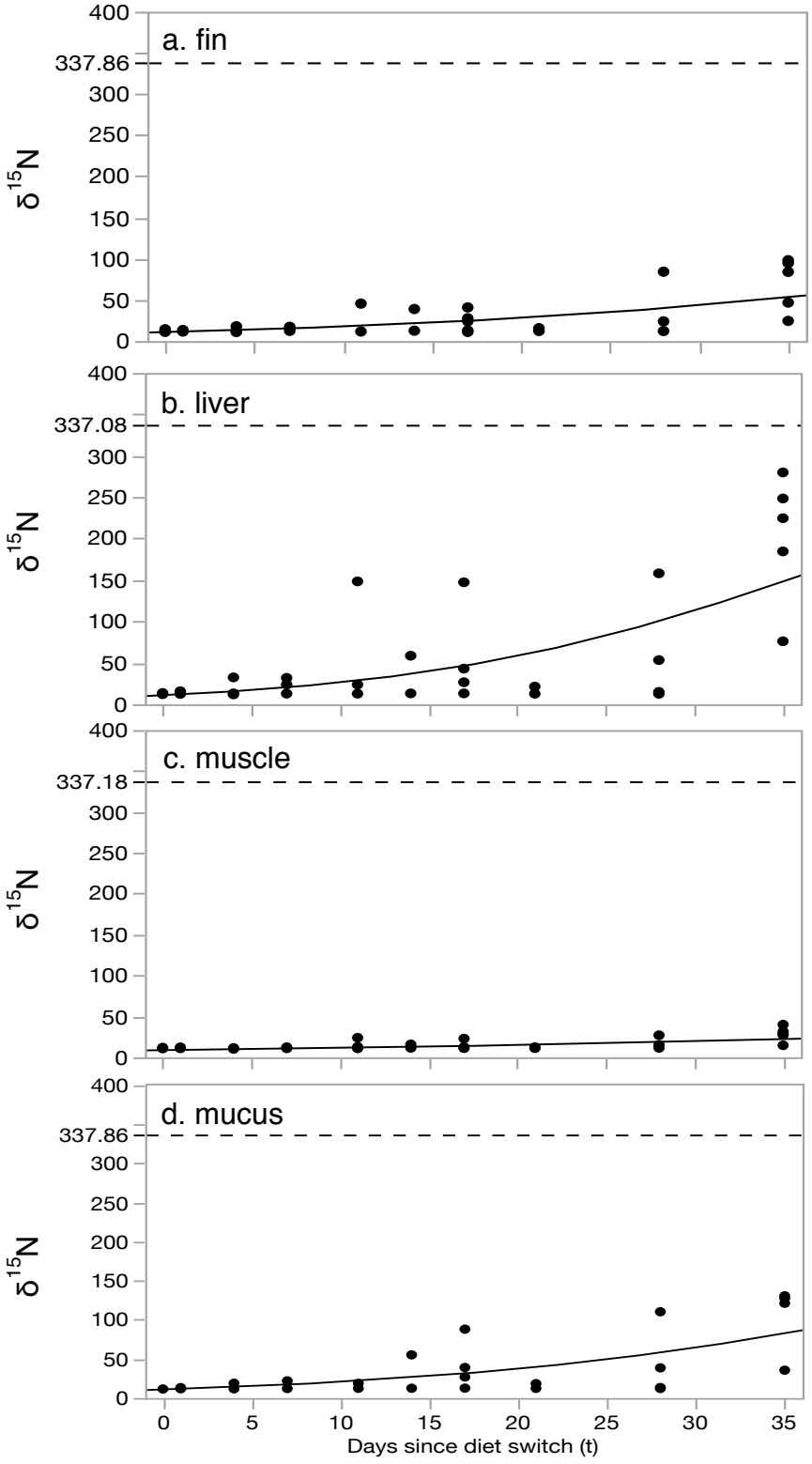

Figure 1. Nitrogen isotope values of eel a.) fin, b.) liver, c.) muscle, and d.) mucus after a diet switch. The horizontal dashed line represents the maximum $\delta^{15}N$ observed in the experiment + the tissue specific trophic discrimination factor ($\Delta^{15}N$; Table 2).

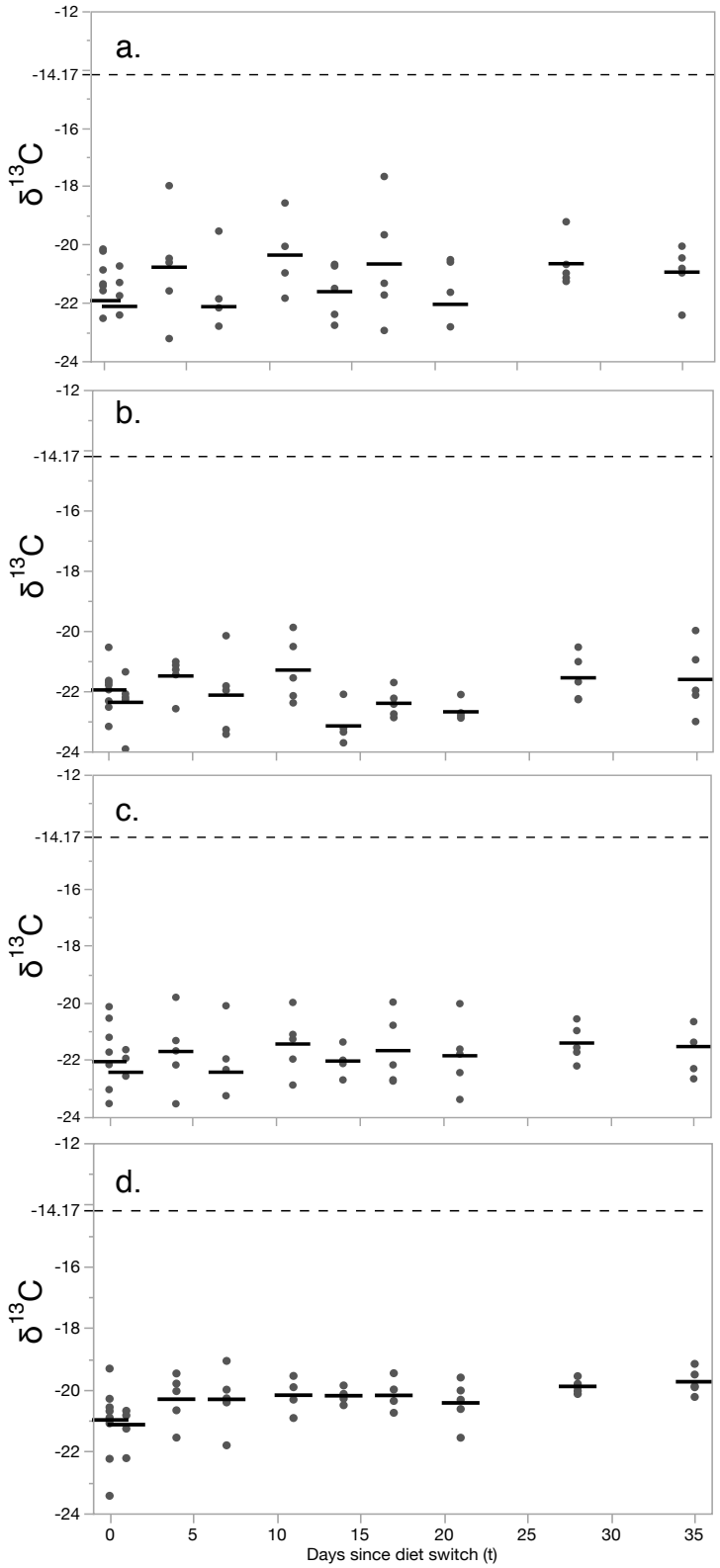

Figure 2. Carbon isotope values in eel a.) fin, b.) liver, c.) muscle, and d.) mucus after a diet switch. The horizontal line represents the equilibrium $\delta^{13}C$ value calculated as the mean $\delta^{13}C$ for the experimental diet ($\delta^{13}C$=-16.58‰) + the mean trophic discrimination factor ($\Delta^{13}C$=2.41‰).

Table 3. Half-lives of nitrogen turnover in eel tissues

Tissue	Regression equation	n	r^2	Half-life (days)
fin	logit ^{15}N=-3.40+0.0377x	53	0.4	90.2
liver	logit ^{15}N=-3.47+0.0736x	53	0.43	97.1
mucus	logit ^{15}N=-3.46+0.0518x	53	0.47	66.8
muscle	logit ^{15}N=-3.43+0.0179x	54	0.38	191.1

all tissues exceeds 35 days and as a result tissue-specific turnover rates could not be quantified for $\delta^{13}C$ in this experiment. Alternatively, direct nutrient routing could explain the lack of $\delta^{13}C$ increase as similar dietary components in both the baseline (pellet) and experimental (earthworms) feeds could result in little discrimination between diet and consumer if routed directly to eel tissues.

DISCUSSION

Tissue specific trophic discrimination factors

TDFs of ^{15}N for all eel tissues were within the range previously reported in the literature for other species of fish. However, nitrogen enrichment was significantly lower than 3.4‰, a frequently used discrimination value for estimating trophic position (Minigawa and Wada 1984). Minigawa and Wada (1984) calculated their $\Delta^{15}N$ estimate of 3.4‰ as the mean from many animals representing different classes from both terrestrial and aquatic environments. In a review of factors affecting ^{15}N discrimination by Vanderklift and Ponsard (2003), the biochemical form of nitrogen excretion emerged as important. Organisms that secrete primarily ammonia (ammonotelic organisms) such as eels and most other teleost fish, exhibited significantly lower $\Delta^{15}N$ than organisms that secrete nitrogenous waste as primarily urea or uric acid (ureotelic and uricotelic organisms, respectively; Vanderklift and Ponsard 2003). The findings of this study are

similar to those in prior investigations that also identified low ^{15}N discrimination in ammonotelic fish species (McCutchan et al. 2003; Logan et al. 2006; Caut et al. 2009). Vanderklift and Ponsard (2003) speculate that lower $\Delta^{15}N$ in ammonotelic organisms is due to the fewer biochemical reactions required for ammonia production. As a result, the inclusion of ureotelic and uricotelic organisms in Minigawa and Wada's (1984) estimate may have resulted in an artificially high measure of $\Delta^{15}N$ and as such, is not appropriate for eels and likely other ammonotelic organisms as well.

Isotope discrimination between diet and eels for ^{15}N was variable among tissues (Table 2). Eel liver was more enriched in ^{15}N than muscle, as has been observed in some studies of fish species including ocellate stingrays (*Potamotrygon motoro;* MacNeil et al. 2006) and mummichogs *(Fundulus heteroclitus*; Logan et al. 2006), but not others such as rainbow trout (*Onchorhynchus mykiss*, Pinnegar and Polunin 1999). Fin was more enriched in ^{15}N than muscle in this study, as was found in other studies comparing muscle and fin (Kelly et al. 2006; Sanderson et al. 2009; Andvik et al. 2010; Jardine et al. 2011). Mucus and fin were the only two tissues sampled that did not have statistically different levels of discrimination for nitrogen (Table 2). The similar TDFs observed here suggest that eel mucus may be as reliable as fin for ^{15}N dietary determinations.

Differences in TDFs among eel tissues (Table 1) can be attributed to nutrient routing as the biochemical components of the diet are differentially routed to each tissue. For instance, muscle tissue had the lowest TDF of the tissues measured, suggesting that more direct routing of dietary biochemical components (e.g., dietary protein routed to consumer muscle) occurred with muscle tissue than with liver, fin, or mucus, resulting in lower isotope discrimination in muscle

(Schwarcz 1991). The growing body of research in compound specific isotope analysis (CSIA; McMahon et al. 2019, Whiteman et al. 2019) suggests that the fate of specific monomers within the biochemical fractions (i.e., amino acids in proteins, fatty acids in lipids) is a determinant of the amount of isotope discrimination between diet and consumer. For instance, amino acids are identified as those that are directly routed to consumer tissue proteins (i.e., "source" amino acids) resulting in little to no nitrogen discrimination, or as those that are biochemically transformed as they are assimilated into consumer tissues (i.e., "trophic" amino acids) resulting in greater nitrogen isotope discrimination (O'Connell 2017). Because muscle tissue had a lower nitrogen discrimination value than the other eel tissues sampled, it may be that more source amino acids are being directly routed to muscle proteins than to liver, fin, or mucus. In addition to the potential effects of differential routing of source and trophic amino acids, the observed variation in TDFs among eel tissues may also be attributed to the metabolic activity of each tissue, where more metabolically active tissues, such as liver, exhibit higher levels of ^{15}N discrimination (Nuche-Pascual et al. 2018).

Eels are considered a "fatty" fish (Gallagher et al 1984). As a result, it is to be expected that eel tissues would generally be depleted in ^{13}C as lipid synthesis discriminates against ^{13}C (DeNiro and Epstein 1977). However, this was not the case. Within the range of values reported in the literature, the $\Delta^{13}C$ values measured for eels (1.99-3.11‰ across tissues; mean of 2.41‰) were generally higher than those observed in many other studies. Elsdon et al. (2010) found *F. heteroclitus* to be significantly more enriched in carbon relative to diet with $\Delta^{13}C$ ranging as high as 3.9‰. They attributed the variability observed in $\Delta^{13}C$ values to the plant-based diet resulting in higher $\Delta^{13}C$ values than animal-based diets (McCutchan et al. 2003). Building on this idea, Busst and Britton (2016) observed high variability in both carbon and nitrogen discrimination

across three tissue types in two omnivorous cyprinid species and generalized that higher TDFs occur with diets with large proportions of plant material. This may be driven by the fact that the $\delta^{13}C$ of complex compounds in plants that are difficult to break down can differ greatly from bulk carbon isotope values (Benner et al. 1987; Teece and Fogel 2007). Eels from which TDFs were determined in the present study were fed a protein rich diet developed from both fish and plant-based sources (Biomar DAN-EX fact sheet 2848). Differential routing of the biochemical factions to each tissue, in particular the non-essential amino acids (McMahon et al. 2010) associated with the plant-based components of the diet, may have resulted in the higher than expected $\Delta^{13}C$ measured for eels generally, and in particular for mucus.

The TDFs calculated for eels generally fell within the range reported in the literature; however, estimates among species and tissues are quite variable (Elsdon et al 2010; Busst and Britton 2016). The observed differences in TDFs among eel tissues, and between other species' estimates in the literature, may be attributed to the variation in composition and isotope values of the biochemical components of the diets and the varying efficiencies with which they are assimilated by each tissue (Martinez Del Rio et al. 2009; McMahon et al. 2015; Whiteman et al. 2019). This has great relevance to field-based studies of omnivorous species, particularly those that can exhibit amphidromous life history strategies, such as American eels, where differences in diet composition can result in variable discrimination and thus, errors in the interpretation of field collected tissue isotope values. For instance, an eel feeding in a brackish habitat on a protein rich diet will have tissues that reflect the isotope values of the diet as well as generally low TDFs as protein is routed directly to tissues with little discrimination. If the eel moves upstream to freshwater to a diet of primarily crustaceans and detritus, then over time tissues will reflect the isotope values of the new diet. Given the lower protein content of the new diet, it is

likely that some protein components are not directly routed and must be synthesized from carbohydrate fractions resulting in relatively larger TDFs. If a small TDF is applied to the freshwater dietary data it can result in artificially depleted isotope values and errors in estimates of dietary sources. Laboratory experiments designed to understand the efficiencies with which the tissues of omnivores take up diets of varying biochemical compositions will help identify variation in TDFs by macromolecule composition to improve modeling of field-based diets (McMahon et al. 2015; Busst and Britton 2016).

Estimates of isotopic turnover

Many studies of isotope turnover in fishes attempt to discern the proportion of turnover due to growth and/or metabolism; the lack of growth in the experimental period precludes these calculations in the present study. Previous studies of isotope turnover in fishes have identified dilution from growth rather than metabolism as the primary driver of isotope turnover in ectothermic fishes such as eels (Fry and Arnold 1982; Hesslein et al. 1993; Jardine et al. 2004). However, Martinez del Rio et al. (2009) suggest that the developmental stage of the organism, and thus, the rate at which they are growing is more important than the method of thermoregulation (i.e., ectotherm or endotherm) in determining the mechanism underlying isotopic turnover. Although the yellow life stage is the primary growth stage of the eel, growth occurs over many years so annual growth is slow (Jessop 1987; Poole and Reynolds 1996; Oliveira 1997; Oliveira and McCleave 2002). As a result, maintenance metabolism, rather than growth, is likely the primary mechanism governing isotope turnover in yellow eels.

The half-life values modeled for eels (Table 3) represent the slow turnover that is characteristic of sub-adult and adult fishes (Wiedel et al. 2011). Furthermore, the maximum $\delta^{15}N$ value, rather than the mean, was used as the asymptote in the logistic model to calculate turnover

rate due to $\delta^{15}N$ tissue values that exceeded the mean (Figure 1). As such, the half-lives presented here represent conservative estimates of eel tissue turnover. Eel mucus ^{15}N had a faster turnover rate than fin, liver, or muscle suggesting relatively faster rates of protein synthesis in mucus. Protein synthesis and catabolism rates are faster in liver tissue than in muscle tissue of eels (de la Higuera et al. 1999). De la Higuera et al. (1999) attributed the shorter half-life observed in eel liver protein relative to muscle protein to the continuous protein turnover in liver tissue (de la Higuera et al. 1999). Of the few studies evaluating nitrogen turnover of mucus, all have found faster turnover of mucus than muscle (Church et al. 2009; Heady and Moore 2012; Maruyama et al. 2015; Winter et al. 2019). Mucus $\delta^{15}N$ half-life in this experiment (67 days) was slower than the 36 days observed by Church et al. (2009) and the 17 days observed by Winter et al. (2019) for juvenile carp (*Cyprinus carpio*) fed a plant-based diet. Given that the rate of isotope incorporation can vary with body mass (Martinez del Rio et al. 2009), discrepancies between turnover time measured here and by Church et al. (2009) and Winter et al. (2019) may be due to the small size (mean mass at start of experiment was 18.4g and 8.0g, respectively) and faster growth rate of the young fishes used in their experiments relative to the larger size and slow growth of yellow eels used in this study (mean mass 91.4g). Smaller and relatively faster growing life stages of eels (i.e., leptocephali, glass eels, or elvers) should exhibit faster tissue turnover than the yellow eels measured here. Winter et al. (2019) examined ^{15}N turnover in carp fed either a plant or a fish-based diet and found slower nitrogen turnover in carp fed a protein-rich diet relative to a plant-based diet. The turnover estimate for carp fed the fish-based diet aligns closely with our mucus half-life estimate for eels despite large discrepancies in fish size. Both the carp (Winter et al. 2019) and eels were fed protein rich diets suggesting that diet composition may also influence tissue turnover rates. If diet is a primary driver of turnover rate,

than we would expect the turnover rates of Church et al. (2009)'s trout (that were fed a fish-based, and presumably protein-rich diet) to have similar turnover rates to the mucus estimates of other fish fed protein rich diets (Winter et al. 2019 and present study), but that was not the case. Future research to determine the relative importance of various factors (e.g., organism size, diet composition) to turnover rates will improve our understanding of nutrient assimilation in consumer tissues.

Carbon isotope values did not vary over time for any tissues measured (Figure 2) suggesting that the half-life of carbon in each of the tissues examined exceeds the 35-day experiment length and therefore, carbon turnover is slower than nitrogen for eels. Evidence exists for varying rates of turnover between carbon and nitrogen with data supporting faster rates in nitrogen in some studies (e.g., Jardine et al. 2004, Winter et al. 2019) and carbon in others (e.g., Johannsson et al. 2011) and varying relationships dependent on the tissues examined (e.g., Church et al. 2009). As was previously discussed with nitrogen, variability in carbon turnover rates may be attributed to nutrient routing, as the relative quantities of the biochemical fractions of the diet undergo different pathways of assimilation into consumer tissues (i.e., direct isotope routing or *de novo* synthesis; Gannes et al. 1998; Pinnegar and Polunin 1999; McMahon et al. 2010). High rates of carbon discrimination were observed between the baseline diet and eel tissues (Table 2). Non-essential amino acids require more biosynthetic steps to assimilate than essential amino acids which may also result in slower rates of assimilation (Whiteman et al. 2019). If eels relied on non-essential amino acids from the experimental diet throughout the course of the experiment, then the increased metabolic processing required could explain the slow rates of carbon turnover observed in all eel tissues.

However, an alternative explanation for the lack of carbon turnover exists. If the experimental diet closely met eel metabolic requirements, then the dietary components may have been directly routed to consumer tissues resulting in the observed lack of change in carbon isotopes among tissues (McMahon et al. 2015; Whiteman et al. 2019).

Potential for nutritional stress

Loss of mass observed in eels after the diet switch suggests that the earthworm diet provided was inadequate to promote growth. Although eels have been successfully sustained on worm diets in previous studies (Aston et al. 1982), a similar loss of mass in captive eels fed an earthworm diet ad libitum was observed by Lovern (1939). Evidence exists for low feeding rates of a subset of eels held in captivity due to inferior competitive ability (Peters et al. 1980). The lack of growth in many animals may be due to competition for food as evidenced by the variation in loss of mass among eels. Furthermore, liver tissue for one eel showed significant ^{15}N enrichment after 11 days of the diet switch while others exhibited minimal enrichment by day 28. However, all eels showed substantial enrichment by day 35.

Eels are biochemically well suited to periods of starvation (Dave et al. 1975). Under natural conditions yellow eels in temperate climates undergo seasonal starvation as food resources decrease in winter (Hopkirk et al. 1975) as well as ontogenetic starvation as they sexually mature to the silver stage and begin their spawning migration (Pankhurst and Sorensen 1984). Unlike many fishes that catabolize lipids first (Love 1970), eels in the initial stages of fasting deplete protein reserves more quickly than lipids (Lewis and Epple 1984; Boetius and Boetius 1985). Lovern (1939) observed that European eels lost weight due to loss of proteins only, so that the relative lipid content increased over time. Eels in the current experiment that lost the most mass had the highest C:N values in liver tissue (Figure 3) likely due to the reduction in

nitrogen as nonessential amino acids are lost (Saglio and Faunconneau 1988), particularly those with low C:N. Therefore, evidence exists for nutritional stress in eels fed the treatment diet.

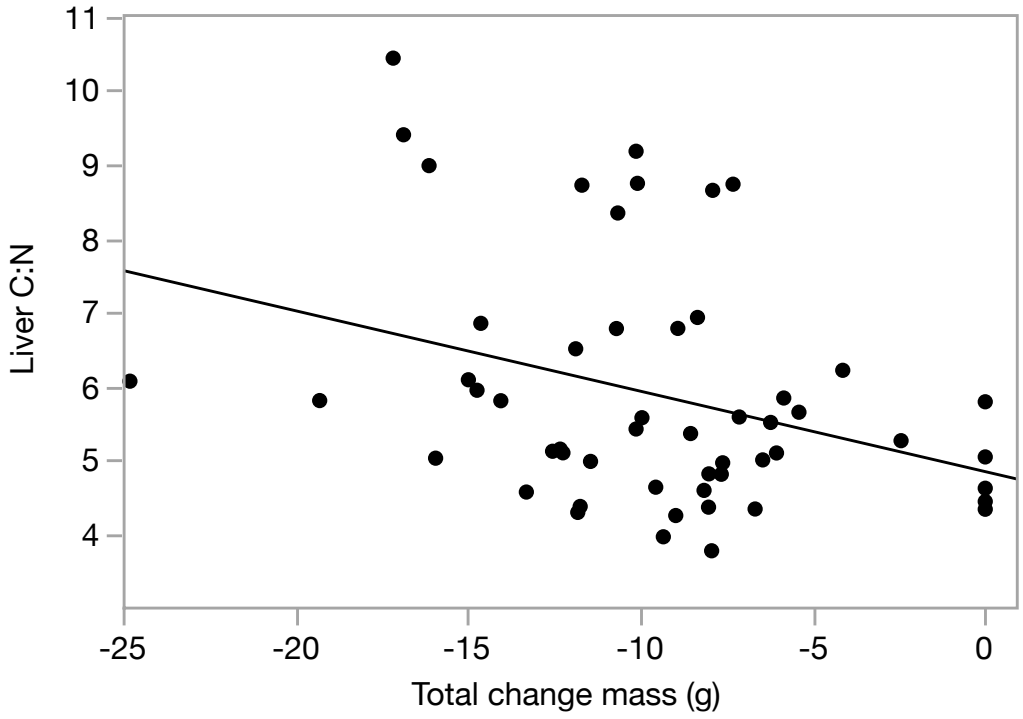

Figure 3. Relationship between liver C:N and change in eel mass; y=4.86-0.11x, $p<0.01$, $r=0.35$

Previous studies have documented changes to stable isotope values in fishes due to nutritional stress and have observed enrichment in the range of 1-2.5‰ for $\delta^{13}C$ and ≤1 for $\delta^{15}N$ (Doucett et al. 1999; Gaye-Seissegger et al. 2004). Furthermore, multiple experiments have documented no enrichment in carbon or nitrogen in fishes due to fasting (e.g., Herzka and Holt 2000; Jardine et al. 2004; Gaye-Siessegger et al. 2007). Due to the high level of enrichment in $\delta^{15}N$ in the experimental diet, the uncertainty of enrichment effects due to starvation, and the small degree of potential enrichment due to fasting relative to enrichment due to the ammonium chloride label, it is unlikely that the loss of eel mass had any magnifying effect on the nitrogen turnover data. If enrichment due to nutritional stress did occur, progressive ^{13}C enrichment may

have been observed in the diet switch data (Oelbermann and Sheu 2002; Gay-Seissegger et al. 2007) and artificially fast turnover rates may have been recorded. Because no statistically discernible change in $\delta^{13}C$ isotope values was observed in any tissues measured, isotope enrichment due to stress is likely not skewing the data.

Applications to Future Research

This study provides the first tissue specific TDFs ($\Delta^{15}N$ and $\Delta^{13}C$) for the American eel, a critical step for increasing the accuracy of eel isotope data interpretation. The use of carbon and nitrogen isotopes as a tool for understanding diet has increased over the last few decades, and so has our understanding of the data needs for diet reconstruction. Mixing models have become a powerful tool for estimating prey contribution to consumer diet (e.g., Phillips and Gregg 2001; Moore and Semmens 2008) but they are limited by a lack of taxon-specific discrimination data for the tissues being studied (Johnson et al. 1998; Bond and Diamond 2011). Results from this study provide a critical data need for increasing the accuracy of estimates of trophic position, movement, and basal food sources for the American eel by providing TDFs for liver, muscle, fin, and mucus.

In addition, we provide estimates of $\delta^{15}N$ turnover rates in four eel tissues. Although muscle is commonly used in stable isotope examinations of fish trophic ecology, our data suggest that it may not be the most informative indicator of diet for slow-growing, omnivorous species that rely on varied food sources such as eels. Due to the slow turnover rate measured for yellow eels, muscle tissue of eels in the wild is likely not at equilibrium with the diet unless yellow eel diet is generally consistent over the course of many months to a year. Thus, the slow isotopic turnover renders isotope analysis of eel muscle a better indicator of longer-term trophic

interactions and integrated dietary habits rather than short-term dietary shifts or movements among habitats.

Mucus had a shorter half-life than all other tissues measured and is collected with a non-invasive method, allowing for collection of repeated samples over time. Mucus is particularly valuable for understanding short term changes in slow growing fishes where muscle tissue turnover can be quite slow such as with the eel. While some tissues measured in the literature may exhibit faster turnover (e.g., blood plasma, Vander Zanden et al. 2015), mucus presents a relatively short-term indicator that is easily sampled from living fishes by applying the methods used by Church et al (2009) and in this study. A non-invasive sampling method for stable isotope analysis is of particular importance for species with populations in decline such as the American eel (ASMFC 2012). As such, this contribution strengthens a growing body of literature that proposes mucus as a viable method for evaluating short term $\delta^{15}N$ changes in fishes (e.g., 67 days for yellow eels in the present study; 26 days for juvenile steelhead trout (Church et al. 2009), 95 days for stone moroko (Shigeta et al. 2017); 144 days for Amur minnow (Shigeta et al. 2017)).

Understanding tissue specific isotopic turnover is important for answering ecological questions for species with complex life histories such as the American eel. Eels exploit a wide range of habitats and are unique in that their movements are not strictly aligned with seasonal or reproductive changes such as with many other migratory species (Arai and Chino 2012). Rather, eels move among freshwater, marine, and estuarine habitats over varying temporal scales (Jessop et al. 2008) and can exhibit catadromous, amphidromous, or marine/estuarine life history patterns. The lack of predictability can make eel movements among habitats difficult to trace. Comparison of tissues with different turnover times can be used to detect dietary shifts that can

indicate habitat use trends. Our results suggest that eel mucus can serve as a short-term indicator representative of seasonal change (approximately 3 months to reach equilibrium with diet), liver and fin as a midterm indicator (approximately 6 months), and muscle as a long-term indicator (approximately 1 year). Therefore, the present study provides important information for reconstructing eel resource use and migration patterns.

The eels in the present study exhibited faster turnover and lower discrimination for nitrogen than for carbon. Compound specific isotope analysis could provide more insight into the mechanisms driving these differences. For instance, eels may have been relying heavily on dietary glycine or serine. These two amino acids are non-essential, resulting in carbon fractionation between diet and consumer, and despite undergoing transamination, exhibit patterns similar to source amino acids resulting in little to no nitrogen fractionation (Whiteman et al. 2019). To truly understand the mechanisms driving isotopic shifts between diet and eel tissue, further research is needed to examine the composition, isotope values, and fate of specific biochemical fractions (e.g., carbohydrates, lipids and proteins) and their monomers (i.e., monossacharrides, fatty acids, and amino acids) between diet and consumer (Schwarcz 1991; Ambrose and Norr 1993; McMahon et al. 2010; Whiteman et al. 2019).

CHAPTER II

Rethinking the freshwater eel: Saltmarsh trophic support of the American eel, *Anguilla rostrata*

Note: This chapter was published in the journal Estuaries and Coasts. The original publication is available at https://link.springer.com/article/10.1007/s12237-015-9960-4. Differences in text citations and use of scientific rather than common names throughout the manuscript reflect the journal requirements.

ABSTRACT

Despite the fact that American eel (*Anguilla rostrata*) are frequently captured in salt marshes, their role in saltmarsh food webs and the influence of human impacts, such as tidal restrictions, on this role remains unclear. To better understand saltmarsh trophic support of *A. rostrata*, eels were collected from tidally restricted and unrestricted saltmarsh creeks within three New England estuaries. Gut contents were examined, and eel muscle tissue was analyzed for carbon and nitrogen stable isotope values and entered into MixSir mixing models to understand if saltmarsh food sources are important contributors to eel diet. Data suggest that eel prey rely heavily on saltmarsh organic matter and eels utilize saltmarsh secondary production as an energetic resource over time, and thus, can be considered saltmarsh residents. Gut contents indicate that *A. rostrata* function as top predators, feeding primarily on secondary consumers including other fish species, crustaceans and polychaetes. Higher *A. rostrata* trophic position measured upstream of reference creeks suggests that severe tidal restrictions may result in altered food webs, but it is not clear how this impacts the overall fitness of *A. rostrata* populations in New England salt marshes.

INTRODUCTION

The American eel, *Anguilla rostrata*, ranges throughout the western North Atlantic and has a unique life history where juvenile eels remain inshore in estuaries and freshwater habitats in the "yellow" life stage before undergoing a spawning migration to the Sargasso Sea up to 20 years later (Jessop 1987; Tesch 2003). Historically, *A. rostrata* was abundant in the Gulf of Maine (Goode 2006) and served as an important source of income and sustenance throughout northern New England and Canada (Bolster 2002; SRSF 2002). While eels are not highly valued in the United States as a food source, increasing demand for American eels for overseas aquaculture operations has resulted in an increase in both fishing pressure and the economic value of the commercial fishery (Haro et al. 2000; Jessop 1997). However, *A. rostrata* is in decline over the entirety of its range (Haro et al. 2000). Potential causes include migration barriers, hydroelectric turbine mortality, overfishing, and habitat loss (Haro et al. 2000). Due to the lack of knowledge on eel ecology in estuaries and potential severity of habitat loss impacts on *A. rostrata*, the Atlantic States Marine Fisheries Commission Interstate Fishery Management Plan for the American eel lists use of inshore habitat and impacts of habitat loss as high priority research needs (ASMFC 2000).

The conventional understanding of Anguillids' inshore habitat use has been obligate catadromy; however, a high degree of residency (Jessop et al. 2002; Jessop et al. 2004; Tsukamoto and Arai 2001; Tsukamoto et al. 1998; Tsukamoto et al. 2002) and faster growth in estuaries at higher latitudes (Jessop et al. 2004; Morrison et al. 2003; Oliveira 1999) suggests that northern estuarine habitats may be favored more than freshwater habitats. For example, *A. rostrata* are frequently captured in New England saltmarsh habitats (e.g., Dionne et al. 1999; Eberhardt et al. 2011; Nixon and Oviatt 1973) and in some studies were found to comprise the

majority of fish biomass (Dionne et al. 1999). Despite the abundance of *A. rostrata* in northeast salt marshes, little is known about their use of these habitats. Evidence exists for a limited home range of approximately 1 kilometer in saltmarsh creeks (Bozeman et al. 1985; Ford and Mercer 1986; Helfman et al. 1983) suggesting that salt marshes provide sufficient trophic support for *A. rostrata*. However, with few exceptions (e.g., Wenner and Musick 1975), the majority of knowledge of yellow eel foraging ecology comes from freshwater habitats (Aoyama and Miller 2003; Tesch 2003). In light of the potential for the yellow life stage to remain resident in estuaries for many years (Jessop 1987; Tsukamoto and Arai 2001) and the habitat value that intact salt marshes provide, a need exists for greater understanding of *A. rostrata* use of salt marshes in terms of trophic support.

Coastal habitats such as salt marshes are particularly vulnerable to habitat impacts due to high rates of coastal development and their use as transportation corridors. Structures such as culverts are frequently installed to provide varying degrees of tidal connectivity where roadways intersect saltmarsh creeks but often have negative impacts on intact marsh ecosystems and the many ecological values that they provide (Roman and Burdick 2012). Many culverts do not accommodate the full tidal regime, resulting in a tidally restricted system upstream where halophytic vegetation is replaced by invasive species such as *Phragmites australis* (common reed; Burdick et al. 1997; Chambers et al. 2012; Roman et al. 1984). Colonization by invasive species as well as changes to the infaunal communities (Fell et al. 1991) may shift the food-base of tidally restricted salt marshes resulting in an altered food web. Furthermore, decreased flooding and accelerated water velocity through undersized culverts can limit fish movement and access to food resources (Eberhardt et al. 2011; Weisberg and Lotrich 1982). Such barriers may result in changes to *A. rostrata* or prey movement as well as habitat degradation upstream and as

a result, some marsh areas may contribute disproportionately to fish populations within larger estuaries (Gillanders 2005). In turn, this may limit the export of marsh production to open water habitats via fish migration (Kneib 1997). Examining the functional differences of tidally restricted and unrestricted salt marshes in the trophic support of *A. rostrata* will improve our understanding of how eels use tidal marshes and how human influence alters the habitat value salt marshes provide for eels.

Stable isotope and gut content analyses were used to evaluate the functional equivalency of both tidally restricted and unrestricted salt marshes in the trophic support of *A. rostrata* in three New England (USA) estuaries. *A. rostrata* and their potential food resources were collected from three estuaries to test the hypotheses that 1. Saltmarsh primary and secondary production serve as important energetic resources for *A. rostrata* and its prey; 2. Trophic position of *A. rostrata* is altered in tidally restricted salt marshes relative to unrestricted systems; and 3. *A. rostrata* nutritional sources continue to be represented by saltmarsh sources over time suggesting that eels are resident in salt marshes.

METHODS

To evaluate the foraging ecology of *A. rostrata* in saltmarsh habitats, three estuaries containing extensive marsh complexes were selected: the Webhannet Estuary, Maine (WEB); the Hampton-Seabrook Estuary, New Hampshire (HSE); and the Parker River Estuary, Massachusetts (PRE; Figure 4; Table 4). Within each marsh, one tidally restricted and one reference creek were sampled for a total of six creeks (n=3 for each hydrology treatment). Creeks were selected to represent similar characteristics such as size and availability of intertidal and subtidal habitats. Samples were collected from locations upstream and downstream of the culvert in tidally restricted creeks, and in comparable upstream and downstream sections of reference

creeks to examine foraging patterns; only upstream data were analyzed to test for effects of tidal restriction. *A. rostrata* were collected from each creek using eel pots and were measured for length to the nearest millimeter (mm). Captured eels were anesthetized, sacrificed, and frozen according to a protocol approved by the Institutional Animal Care and Use Committee of the University of New Hampshire (IACUC permit 070702). In the laboratory, fish muscle tissue was dissected and dried for 48 hours at 60ºC to achieve constant weight. Primary consumers representing potential prey species were also collected from the marsh to provide baseline data for the calculation of *A. rostrata* trophic position. Gastropods were removed from the shell, rinsed with distilled water and analyzed whole. The adductor muscle was dissected out for analysis of stable isotope values of bivalve species.

To determine the relative contribution of saltmarsh primary production to *A. rostrata* diet, organic matter was collected from restricted and references creeks from within each estuary. Samples of the most abundant species were collected including the C3 plants *P. australis* and the cattail species *Typha latifolia* and *Typha angustifolia* (hereafter collectively referred to as "*Typha*"), and the C4 plants *Spartina alterniflora* (cordgrass) and *Spartina patens* (salt hay). Leaves from plants of each species were rinsed with distilled water, scraped for removal of epiphytes, and dried for 48 hours at 60ºC to achieve constant weight. Nekton and vegetation samples were ground using a coffee grinder and weighed into aluminum tins in preparation for stable isotope analysis.

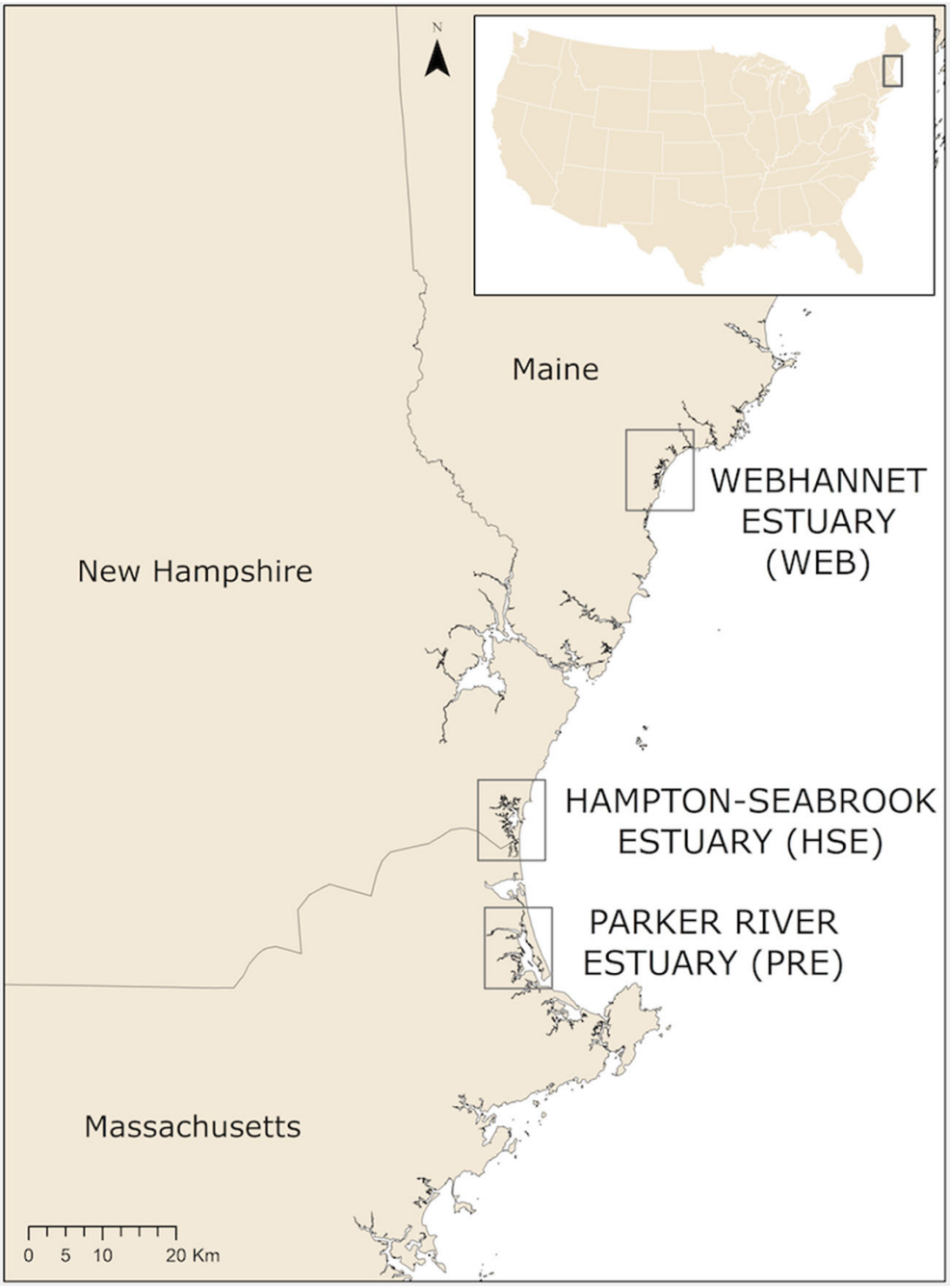

Figure 4. Location of sampling sites for stable isotope and gut content sampling collection. A tidally restricted and unrestricted creek were each sampled within the Webhannet Estuary (Maine), the Hampton-Seabrook Estuary (New Hampshire) and the Parker River Estuary (Massachusetts).

Table 4. Sampling site characteristics for restricted and reference creeks in each estuary. Sources of data for salinity and tide height are: Webhannet Estuary (Adamowicz and O'Brien 2012 for the restricted creek tide height data; Burdick et al. 1999 for the reference creek tide heights and well salinity data); Hampton-Seabrook Estuary (Burdick et al. 2010 for well salinity and restricted tide range; predicted tide range in estuary from NOAA for same collection days): Parker River Estuary (Burdick, unpublished data).

	Webhannet Estuary		Hampton-Seabrook Estuary		Parker River Estuary	
Variables	Restricted	Reference	Restricted	Reference	Restricted	Reference
Salinity (ppt; pore water)	22.8	27.3	15	29	31.4	28.5
Tide Range (cm)	64	210	30	294	145	166

Benthic microalgae were collected on a 210 micron (μm) mesh screen according to the protocol outlined by Levin and Currin (2012). At the start of the ebb tide, ashed silica was sprinkled on the sediment. The mesh screen was sprayed with filtered seawater, placed over the silica layer, and air bubbles were removed with a plastic spatula. An additional layer of silica was sprinkled on top of the mesh screen and then a layer of fiberglass screen elevated off the substrate with a foam ring was installed to provide shade and prevent desiccation. After several hours, the screen was removed, rinsed with distilled water and stored on ice. In the laboratory samples were decanted and filtered onto precombusted (450^{o}C for 4 hours) 47 mm glass fiber filters (GF/F) using a low-pressure vacuum pump filtration system. Filters were examined under a microscope to remove debris and then dried at 60^{o}C until a constant weight was reached.

Particulate organic matter (POM) was collected from the restricted and reference creeks in each estuary by filtering 4 liters of water through a 64 μm mesh. Samples were filtered through ashed GF/F at low pressure in the laboratory and dried at 60^{o}C until a constant weight was reached. Filters for both benthic microalgae and POM were stored in a desiccator prior to analysis. Material was removed from each filter using forceps and placed into tin capsules for analysis of stable isotopes. Stable isotope values for primary producers in adjacent habitats (i.e.,

terrestrial and marine) were taken from the literature to serve as end members in mixing models. Data were obtained from projects that occurred in the same estuaries (i.e., WEB and PRE) to represent marine (marine POM values; Deegan 2004) and terrestrial (*Quercus rubra*, Deegan 2004; Vincent, unpublished data) sources of primary production.

All primary producer, invertebrate, and nekton samples were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes at the University of New Hampshire Stable Isotope Laboratory with a Costech ECS4010 Elemental Analyzer coupled to a Delta Plus XP mass spectrometer (Thermo Finnigan). Stable isotope ratios are reported in delta notation per mil units (‰) as:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000‰$$

Where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Stable isotope ratios were determined using Vienna Pee Dee Belemnite (VPDB) as the reference material for carbon and atmospheric N_2 (air) for nitrogen. Delta ^{15}N values are reported on the VPDB scale using International Atomic Energy Agency-N1 (IAEA; 0.4 per mil) and IAEA-N2 (20.3 per mil). Repeated analyses of laboratory standards (tuna for eels and invertebrates, and apple leaves for plants) varied less than 0.15‰ for both $\delta^{15}N$ and $\delta^{13}C$.

Carbon and nitrogen stable isotope values of *A. rostrata* captured in upstream regions were evaluated for differences between hydrologic regimes with an analysis of covariance (ANCOVA) using JMP statistical software (JMP 11.0; SAS Institute, Cary, North Carolina, USA). The hydrology of the creek (restricted or restored) served as the main factor with eel length as the covariate to account for ontogenetic change in diet (Ogden 1970; Facey and Labar 1981). The estuary was included as a block to remove the variability in *A. rostrata* diet associated with latitudinal differences among estuaries (as in Tesch 2003). Residuals were

examined for normality and homogeneity of variance; all data met the assumptions of the general linear model.

Mixing models were developed from stable isotope data with MixSir software (Moore and Semmens 2008) to examine the relative contributions of saltmarsh primary producers to *A. rostrata* diets in each marsh treatment (i.e., upstream restricted, downstream reference, etc.). The input parameters for MixSir include $\delta^{15}N$ and $\delta^{13}C$ data for individual *A. rostrata*, means and standard deviations for potential primary producer sources specific to each estuary, and tissue-diet discrimination factors and associated standard deviations. All mixing models met the diagnostic requirements of MixSir (i.e., posterior draws, duplicate draws and the ratio between the posterior at the best draw and the posterior density; Moore and Semmens 2008). However, it should be noted that low sample sizes for some treatment combinations (e.g., Webhannet/downstream/restricted and Webhannet/downstream/reference) decrease confidence in those results for inference to eel populations in general.

Estimates of contributions of prey items to consumer diets as well as consumer trophic position are subject to multiple sources of uncertainty (Moore and Semmens 2008; Vander Zanden and Rasmussen 2001), including changes in isotope ratios as prey are assimilated into consumer tissues (discrimination) and variation in the rate at which the diet is assimilated (turnover; Fry 2006). Many food web investigations using stable isotopes rely upon discrimination factors documented in the literature; however, evidence exists for species and tissue specific variability in both discrimination and turnover estimates (Hobson and Clark 1992a; Hobson and Clark 1992b; Logan et al. 2006; Pinnegar and Polunin 1999; Tieszen et al. 1983; Vander Zanden and Rasmussen 2001). To address these potential sources of uncertainty, *A. rostrata* discrimination factors and turnover rates were determined in a laboratory diet switch

experiment. *A. rostrata* were fed a cultured earthworm diet of known carbon and nitrogen isotope values and tissues were sampled over time to calculate discrimination and turnover. *A. rostrata* muscle turnover rate was estimated to be 191 days (Eberhardt, unpublished data). The discrimination factor was calculated as $\Delta^{15}N=1.18$ (±0.14) and $\Delta^{13}C=1.99$ (±0.38) for *A. rostrata* muscle (Eberhardt, unpublished data). Trophic position was calculated for *A. rostrata* (Vander Zanden and Rasmussen 1999) and estimated to be 4.0. Similarly, Persic et al. (2004) estimated yellow stage *Anguilla anguilla* to feed at a trophic level of 4.1. As such, discrimination estimates were adjusted to reflect eels feeding at the 4th trophic level in order to increase model robustness.

To evaluate impacts of restricted hydrology on *A. rostrata* diet, the trophic position of *A. rostrata* captured from upstream regions was calculated from $\delta^{15}N$ data for eels measuring between 20 and 40 cm. Trophic position was calculated as $TP_{eel} = (\delta^{15}N_{eel} - \delta^{15}N_{PC}/\Delta^{15}N_{eel})/2$ (Vander Zanden and Rasmussen 1999) where TP_{eel} is the trophic position of the eel, $\delta^{15}N_{eel}$ is the eel nitrogen isotope value, $\delta^{15}N_{PC}$ is the nitrogen isotope value of primary consumers, and $\Delta^{15}N_{eel}$ is the *A. rostrata* nitrogen isotope discrimination value for one trophic level. The model uses primary consumers as the baseline trophic level to account for variation in $\delta^{15}N$ values of basal resources. Stable isotope data for *Geukensia demissus* (ribbed mussel), *Mytilus edulis* (blue mussel), and *Littorina littorea* (common periwinkle) were collected and analyzed specific to each estuary and hydrology treatment for the calculation of trophic position (Appendix C). Data for trophic position were tested for effects of site, hydrology, and the interaction with a two-way ANOVA. Significant results for the interaction term were further evaluated with a Tukey-Kramer post hoc test.

Gut contents were analyzed from all captured eels to evaluate the importance of saltmarsh secondary production to eel diet. Guts were removed from eels and the relative fullness was estimated visually and assigned to one of three general categories: <50% full, 50-75% full or 75-100% full. Gut contents were examined with a dissecting microscope and identified to major taxon. To identify the contribution of each food type to the diet, the relative volume of each food type for each gut was calculated as the wet weight of each item as a proportion of the total gut contents (Hyslop 1980). Gut contents of *A. rostrata* with guts ≥50% full were compared among tidally restricted and reference saltmarsh creeks with a one-factor analysis of similarity (ANOSIM), a nonparametric equivalent to multivariate ANOVA, with PRIMER statistical software (Clarke and Green 1988).

Gut contents indicate intra-day diet. Using 2 half lives to define the period of integration for the isotope data, muscle data indicate *A. rostrata* diet integrated over approximately one year. While gut content data indicate what an animal has ingested hours before capture, stable isotope data provide information on what prey items an organism has assimilated over the past year. As such, proportions of prey items from gut contents were considered with isotope data to provide a time series of resource use by *A. rostrata*.

RESULTS

The stable isotope values of *A. rostrata* muscle tissue ranged from 6.5 to 12.8 for $\delta^{15}N$ and from -22.3 to -13.0 for $\delta^{13}C$ (Figure 5). Both $\delta^{15}N$ and $\delta^{13}C$ values of *A. rostrata* muscle tissue were significantly different among estuaries but did not differ between restricted and reference creeks, by eel length, or the interaction term between hydrology and eel length (ANCOVA; Table 5). Potential sources of primary production included red oak, benthic microalgae, estuarine POM, saltmarsh grasses, brackish species and marine POM. Mean $\delta^{15}N$

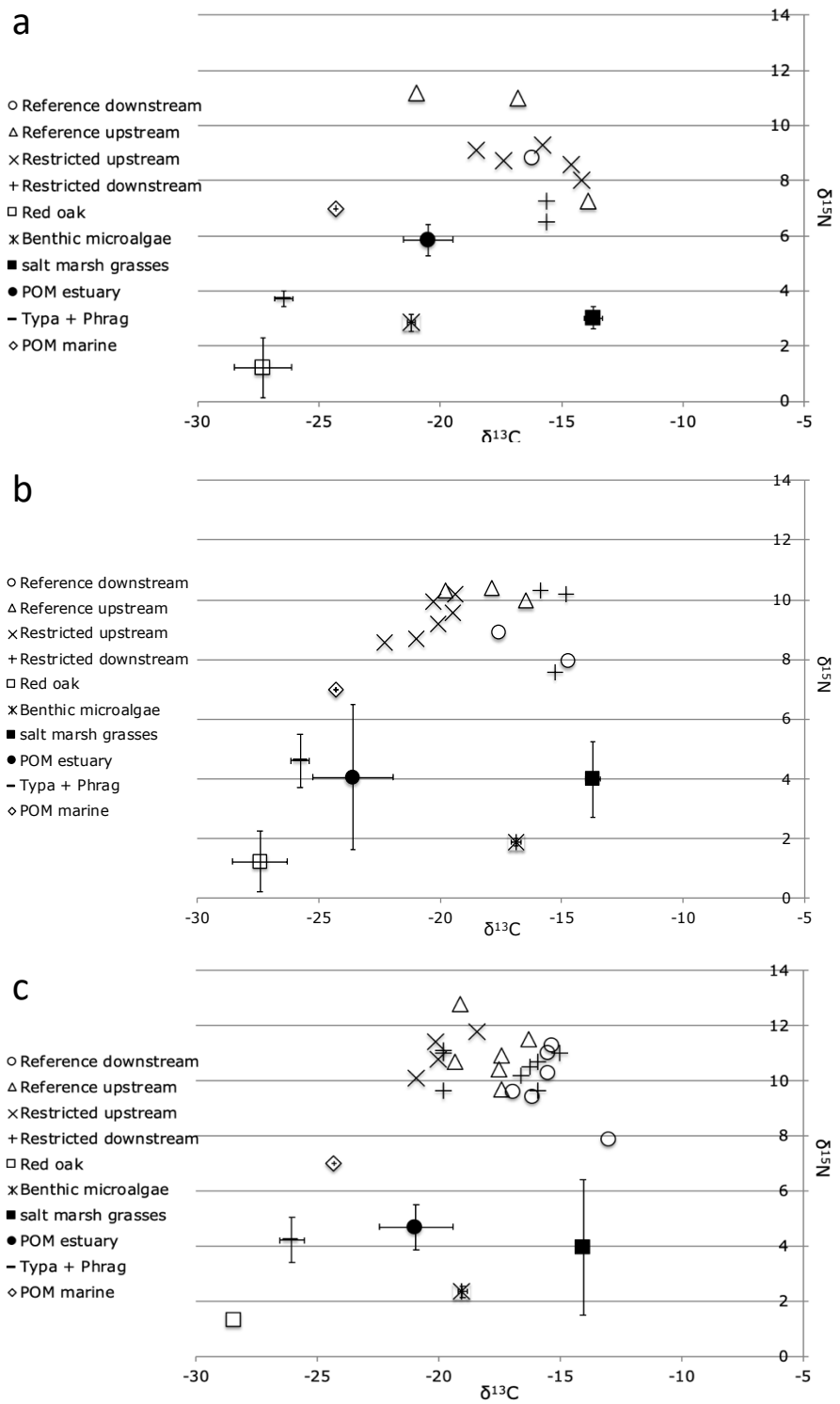

Figure 5. Biplots for carbon and nitrogen stable isotope values used in mixing models, including data for individual eels and the means (± standard deviation) of potential prey items. Data are presented for each of the three estuaries sampled: (a) Webhannet Estuary, Maine (WEB), (b) Hampton-Seabrook Estuary, NH (HSE) and (c) Parker River Estuary, Massachusetts (PRE)

Table 5. Results of analysis of covariance on $\delta^{15}N$ and $\delta^{13}C$ data for eel muscle tissue collected in upstream regions of tidally restricted and unrestricted creeks. Hydrology (tidally restricted or unrestricted) was analyzed as the main treatment with eel length as a covariate; site was included as a blocking factor. Site include the Parker River Estuary (MA), Hampton-Seabrook Estuary (NH) and the Webhannet Estuary (ME).

	df	F Ratio	p
$\delta^{15}N$			
Site	2	4.6	0.027*
Length	1	1	0.338
Hydrology	1	3.8	0.069
Hydrology:length	1	0.6	0.444
$\delta^{13}C$			
Site	2	8.2	0.003*
Length	1	1.9	0.182
Hydrology	1	0.2	0.703
Hydrology:length	1	0	0.935

*statistically significant at α=0.05

values for primary producers ranged from 1.2 to 7.0‰ and mean $\delta^{13}C$ ranged from -28.4 to -13.7‰ (Table 6).

Mixing model outputs are more robust when the number of sources is limited; similarly, source items with similar isotope ratios can confound modeled diet distributions (Moore and Semmens 2008). As a result, data were pooled where isotope values were statistically similar; data for the saltmarsh halophytes *S. alterniflora* and *S. patens* were not significantly different (one-way ANOVA) nor were the brackish/freshwater species *P. australis* and *Typha*, so they were pooled into "marsh grasses" and "brackish species" categories, respectively. Primary producer values specific to each estuary were used in mixing models.

Table 6. Mean primary producer $\delta^{15}N$ and $\delta^{13}C$ values (±SE) used in eel mixing models and mean *Anguilla rostrata* values (individual values were used in mixing models). Data for each of the three estuaries were pooled by species from similar habitats where statistically similar: Marsh grasses (ANOVA; PRE: $\delta^{15}N$ df=1, F=1.0, p<0.385, $\delta^{13}C$ df=1, F=9.4, p<0.037; HSE: $\delta^{15}N$ df=1, F=3.6, p<0.116, $\delta^{13}C$ df=1, F=0.8, p<0.422.) Note: Samples sizes were too small to statistically test marsh grasses from WEB and to test the brackish species for each estuary. Primary producers include red oak (*Quercus rubra*), benthic microalgae, saltmarsh greases (*Spartina alterniflora* and *S. patens*), estuarine particulate organic matter (estuarine POM), brackish species (*Phragmites australis*, *Typha latifolia*, and *Typha angustifolia*) and marine particulate organic matter (marine POM).

	Webhannet Estuary (WEB)			Hampton-Seabrook Estuary (HSE)			Parker River Estuary (PRE)		
	n	**$\delta^{15}N$**	**$\delta^{13}C$**	n	**$\delta^{15}N$**	**$\delta^{13}C$**	n	**$\delta^{15}N$**	**$\delta^{13}C$**
Primary producers									
Red oak	8	1.23 ± 1.09	-27.29 ± 1.16	9	1.24 ± 1.02	-27.42 ± 1.14	1	1.30	-28.40
Benthic microalgae	2	2.85 ± 0.30	-21.19 ± 0.14	2	1.88 ± 0.12	-16.87 ± 0.21	4	2.36 ± 0.21	-19.03 ± 0.18
Saltmarsh grasses	2	3.02 ± 0.40	-13.70 ± 0.38	7	4.00 ± 1.27	-13.73 ± 0.30	6	3.97 ± 2.45	-14.06 ± 0.19
Estuarine POM	12	5.86 ± 0.56	-20.50 ± 1.01	11	4.06 ± 2.42	-23.60 ± 1.67	12	4.70 ± 0.83	-20.94 ± 1.52
Brackish species	3	3.71 ± 0.27	-26.43 ± 0.37	4	4.61 ± 0.91	-25.77 ± 0.37	7	4.22 ± 0.82	-26.05 ± 0.49
Marine POM	1	7.00	-24.30	1	7.00	-24.30	1	7.00	-24.30
Anguilla rostrata									
Restricted creek	6	8.78 ± 0.45	-15.99 ± 1.65	6	9.35 ± 0.65	-20.43 ± 1.10	4	11.01 ± 0.73	-19.84 ± 1.06
Reference creek	3	9.82 ± 2.21	-17.22 ± 3.54	3	10.26 ± 0.21	-18.05 ± 1.64	6	10.99 ± 1.04	-17.81 ± 1.15

Mixing model outputs suggest that multiple sources of primary production support the diet of *A. rostrata* (Table 7). POM represented a common source of primary production to the prey of *A. rostrata*; evidence of trophic pathways based on POM from marine and/or estuarine sources was found at all sites. The contribution of saltmarsh macrophytes to eel basal diet was modeled in downstream regions of all estuaries (9-23% of diet) regardless of hydrology (with the exception of PRE/restricted/downstream); whereas, saltmarsh macrophtyes were not a primary contributor to eels captured in upstream locations. Model results suggest important differences in basal source contributions among estuaries. Marine POM was the dominant basal resource for *A. rostrata* captured from PRE (47 – 95% of diet). The northern two estuaries (WEB and HSE) were less saline (Table 4), with dietary contributions from primarily estuarine POM and saltmarsh grasses in addition to POM marine. In one case (HSE/restricted/upstream) the dominant basal source was modeled as brackish species (80% of diet) where the tide was restricted and brackish species dominate.

Comparison of mixing model outputs for upstream data between tidally restricted and reference creeks provides insight into the impact of tidal restriction on the trophic ecology of eels. Evidence for an effect of tidal restriction varied by estuary, perhaps due to the magnitude of the restriction. *A. rostrata* basal resource use in PRE and WEB did not differ between reference and restricted creeks within each estuary. However, the organic matter sources supporting *A. rostrata* captured upstream were different between restricted and reference creeks in HSE, the site with the greatest degree of restriction (Table 7). *A. rostrata* from the reference creek of HSE were supported by primarily marine POM, while *A. rostrata* from the restricted creek were supported by brackish/invasive plant production as the primary carbon source.

Table 7. Results (median proportion) of mixing models examining the relative contributions of primary producers to *Anguilla rostrata* basal diet. Primary producer sources constituting 10% or greater to eel basal diet are indicated in bold.

			n	Red oak	Benthic Microalgae	Saltmarsh Grasses	Estuarine POM	Brackish Species	Marine POM
Webhannet Estuary (WEB)	Restricted	Upstream	6	0.06	0.05	0.03	**0.65**	0.09	0.09
		Downstream	2	**0.19**	**0.22**	**0.22**	**0.10**	**0.12**	0.07
	Reference	Upstream	3	0.01	0.01	0.01	**0.90**	0.02	0.02
		Downstream	1	0.05	0.09	0.09	**0.26**	0.09	**0.36**
Hampton-Seabrook Estuary (HSE)	Restricted	Upstream	6	0.02	0.01	0.01	0.01	**0.80**	**0.13**
		Downstream	3	0.02	0.05	**0.18**	**0.56**	0.04	**0.11**
	Reference	Upstream	3	0.02	0.02	0.02	0.04	0.04	**0.83**
		Downstream	3	0.07	0.08	0.09	**0.40**	**0.10**	**0.16**
Parker River Estuary (PRE)	Restricted	Upstream	4	0.01	0.00	0.01	0.01	0.01	**0.95**
		Downstream	8	0.01	0.01	0.03	0.01	0.01	**0.91**
	Reference	Upstream	6	0.00	0.00	0.01	0.01	0.01	**0.95**
		Downstream	6	0.01	0.01	**0.23**	**0.22**	0.02	**0.47**

Gut content data were analyzed for *A. rostrata* that had guts at least 50% full (n=31). The guts were comprised primarily of species resident to saltmarsh and other estuarine habitats. The common saltmarsh resident fish *F. heteroclitus* and polychaete worms (*Nereid* species and Polynoidae) were the most abundant items in *A. rostrata* guts occupying 39% and 33% of guts, respectively (Fig. 6). Other species present included shrimp (*Crangon septemspinosa* and *Palaemonetes* species), *Gammarid* amphipods, fish species not resident to salt marshes (classified as such due to the size of scales, opercula, and pharyngeal jaws), as well as plant material (Fig. 6). The gut of one small eel (15.9 cm) contained 57 mosquito larvae. Gut content composition of *A. rostrata* collected upstream of tidal restrictions did not differ from those captured upstream in reference creeks (one-way ANOSIM, R= -0.06, p=0.89).

Trophic position was calculated for *A. rostrata* ranging from 20 to 40 cm using eel muscle $\delta^{15}N$ data (Table 8). The 20 to 40 cm size range was selected to analyze eels within the same feeding guild (Ogden 1970; Facey and Labar 1981; Tesch 2003) while maintaining an adequate sample size. The mean trophic position of *A. rostrata* varied between hydrology, sampling region and their interaction (two way ANOVA, df=2, F=4.0, $p<0.032$). A posterior test (Tukey's HSD) revealed that the trophic position of *A. rostrata* collected in upstream reference creeks was significantly greater than *A. rostrata* collected in upstream restricted creeks in two of the three sites sampled (WEB and HSE).

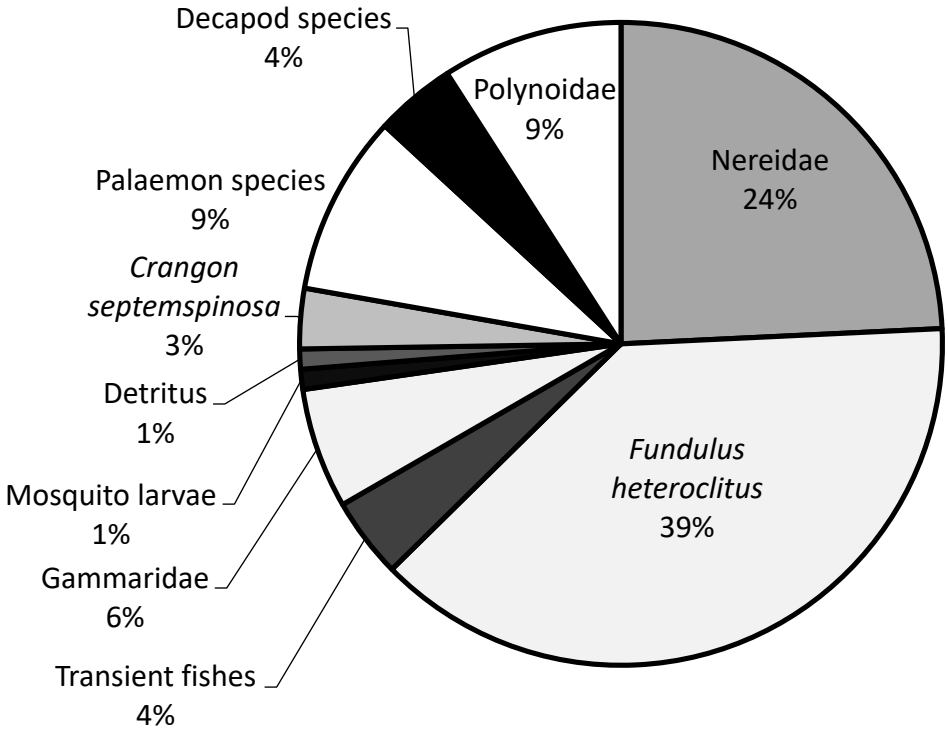

Figure 6. Summary of prey items found in the stomachs of Anguilla rostrata (n=31). Data are included for stomachs that were over 50% full and are expressed as the mean relative volume (calculated as the mean wet weight of each item as a proportion of the total gut contents).

Table 8. Mean trophic position (mean ±1 standard deviation) for *Anguilla rostrata*

			n	Trophic position
Webhannet Estuary (WEB)	Restricted	Upstream	2	2.2 ± 0.5
		Downstream	6	3.1 ± 0.4
	Reference	Upstream	1	4.0
		Downstream	3	4.8 ± 1.9
Hampton-Seabrook Estuary (HSE)	Restricted	Upstream	3	3.3 ± 1.3
		Downstream	6	3.6 ± 0.6
	Reference	Upstream	2	4.0 ± 0.6
		Downstream	3	5.5 ± 0.2
Parker River Estuary (PRE)	Restricted	Upstream	8	3.8 ± 0.5
		Downstream	4	5.0 ± 0.6
	Reference	Upstream	6	4.1 ± 1.1
		Downstream	6	5.0 ± 0.9

DISCUSSION

Mixing models indicate that *A. rostrata* foods webs are supported by a diversity of organic matter sources that vary across estuaries. While it is clear from the data that *A. rostrata* basal diet stems from many available sources (e.g., estuarine, marine, brackish/invasive species), strong evidence exists for *A. rostrata* support from saltmarsh primary production in the form of saltmarsh grasses and/or POM. The relative contribution of saltmarsh macrophyte detritus to POM can be quite variable depending on the coastal geomorphology of the system, recent meteorological events and the size and proximity of vegetated marsh habitat (Odum et al. 1979; Roman and Daiber 1989; Sullivan and Moncreiff 1990; McClelland and Valiela 1998). Due to considerable evidence for *S. alterniflora* detritus across saltmarsh dominated estuaries of the eastern United States (Teal 1962; Odum & de la Cruz 1967; Roman & Daiber 1989), it is reasonable to assume that assimilation of POM by *A. rostrata* and its prey represents, in part, use of saltmarsh production. It should be noted that *A. rostrata* are likely not grazing directly on saltmarsh macrophytes since direct herbivory is considered uncommon (Tesch 2003). Rather, *A. rostrata* may directly ingest small amounts of saltmarsh macrophytes as detritus while foraging for prey. More likely, the majority of the carbon and nitrogen fixed by vegetation is assimilated into *A. rostrata* diets indirectly as their prey digest vegetation tissue or bacteria and fungus growing on the vegetation (i.e., detrital food web).

A. rostrata are considered opportunistic omnivores (Tesch 2003) and the abundance of prey items identified in *A. rostrata* guts supports this. Secondary consumers were common in guts of *A. rostrata* and it is clear that *A. rostrata* are functioning as a top predator in saltmarsh food webs. The abundance of saltmarsh resident (*F. heteroclitus*) and dependent (e.g., *C. maenus*, *Palaemonetes* spp., *C. septemspinosa*) species in gut contents confirms that *A. rostrata*

diet relies heavily on saltmarsh secondary production. POM has been found to be an important energetic resource to saltmarsh infauna, shrimp and *F. heteroclitus* in New England salt marshes (Dibble and Meyerson 2013). Therefore, it is likely that the dominance of POM as a basal resource at most sites is due to eel foraging on suspension feeding primary consumers such as polychaetes resulting in trophic transfer of POM to *A. rostrata*.

Estuaries appeared to function differently in terms of the basal support of eels. Furthermore, evidence exists for impacts of tidal restriction in some systems, but not others. In the upstream area of the HSE restricted marsh, mixing model results indicate that trophic pathways were driven by *Typha* and *P. australis*, species common to tidally restricted systems (Chambers et al. 2012; Roman et al. 1984) whereas the reference creek in HSE was driven by marine POM. Additionally, eels from the restricted area of HSE fed at one trophic level lower than those captured in the reference creek of the same estuary. Of all estuaries sampled, the tidal restriction at HSE results in the greatest reduction in tidal amplitude (Table 4) and as a result, the upstream area is colonized by primarily invasive and brackish vegetation. Eels captured from WEB, the estuary with the intermediate degree of restriction of the estuaries sampled, were supported by primarily estuarine basal sources in the upstream regions of both the restricted and reference creeks; however, the reduced trophic position in eels captured from the restricted system (Table 8) suggest that, like in HSE, the structure of the restricted creek food web is altered relative to the reference creek. Finally, eels captured from PRE, the site with the most tidal flushing and smallest restriction, had a basal diet driven by marine organic matter sources in both reference and restricted creeks. Although the experiment was not designed to explicitly test for the degree of restriction, it appears that in creeks with severe tidal restrictions, more food is eaten locally and at a lower trophic level.

PRE is a well-mixed estuary and the undersized culvert in the restricted creek reduced the tidal regime only slightly (Table 4). As such, it is possible that *A. rostrata* captured in PRE had isotope signatures reflecting basal marine sources because either prey were relying on marine carbon sources or eels had recently moved from marine to estuarine habitats.

Evidence for *A. rostrata* feeding at a lower trophic level in the restricted creeks of two of the three estuaries sampled suggests that the trophic structure of highly restricted systems is altered. Although the food web structure of middle trophic levels in estuarine systems is highly complex and poorly understood (Douglass et al. 2011), changes to the infaunal community composition have been documented as a result of tidal restriction (Fell et al. 1991). Decreased prey diversity, and subsequently the loss of trophic levels have been documented in tidally restricted mangrove systems (Layman et al. 2007). Furthermore, decreased movement of important prey species (e.g., *F. heteroclitus*, *C. septemspinosa*) between upstream and downstream regions of tidally restricted marshes (Eberhardt et al. 2011) may result in a loss of prey resources for *A. rostrata* in some marsh areas. As a result, the observed lower trophic position of eels in highly restricted systems may indicate an altered trophic structure in marshes subject to reduced tidal flushing.

Little data exist regarding *A. rostrata* use of saltmarsh habitats as a food source (e.g., Wenner and Musik 1975) so our results provide important new information regarding the trophic support of *A. rostrata* in New England salt marshes. Data indicate that *A. rostrata* rely on saltmarsh primary production (isotope data) as a basal resource and secondary production (gut contents) to meet energetic demands. The spatial variation observed in basal food resources among estuaries supports previous research in New England marshes suggesting that upper trophic levels derive organic matter from local sources (Deegan and Garritt 1997) and also

suggests that *A. rostrata* are resident in these systems. Microchemical analysis of *A. rostrata* otoliths has documented use of estuarine habitats by eels (e.g., Tsukamoto and Arai 2001; Jessop et al. 2002), and our results provide compelling evidence that salt marshes serve as critical habitats in the life history of *A. rostrata* as a sustained foraging area over time. Prior to this study, little evidence existed for sustained use of New England salt marshes over time by *A. rostrata*. Most assessments of saltmarsh food webs go no higher on the trophic spectrum than *F. heteroclitus*, likely because higher trophic levels in northern New England salt marshes are thought to be primarily transient (Ayvazian et al. 1992). Our data indicate that *A. rostrata* can function as resident top predators in New England salt marshes.

Human impacts including habitat loss have resulted in a decline in *A. rostrata* populations (Haro et al. 2000). Given the clear importance of saltmarsh primary and secondary production to *A. rostrata* diet (observed through mixing models and gut contents) as well as the altered trophic support (as measured by trophic position) upstream of tidal restrictions, habitat functional impacts through tidal restrictions and other mechanisms have resulted in an alteration of trophic resources for eels. As 37% of New England salt marshes have been lost over the last 200 years (Bromberg and Bertness 2005), the cumulative impact of marsh loss is likely a contributing factor in the decline of *A. rostrata* populations.

Fish can play an important role in exporting energy and nutrients out of estuarine habitats to downstream open water (Kneib 1997). Considering *A. rostrata* are residents that exploit a diversity of food resources in the salt marsh, and that *A. rostrata* undergo a spawning migration of thousands of miles, the potential exists for saltmarsh derived production to support marine food webs as eels are preyed upon along their migratory paths (Beguer-Pon et al. 2012). Furthermore, as semelparous organisms, eels that complete the spawning migration will enter the

detrital food web of the Sargasso Sea. This potential mechanism represents a large-scale movement of nutrients and energy from the highly productive salt marshes of New England to distant open ocean habitats. Given the decline in *A. rostrata* populations and the important role that salt marshes play as a foraging resource, as well as the potential role of *A. rostrata* as a vector of trophic transfer of marsh production to open ocean habitats, it is critical to maintain the ecological integrity of salt marshes (e.g., through tidal restoration) in support of *A. rostrata* populations.

CHAPTER III

Evaluation of nonlethal sampling methods of *Anguilla rostrata* for stable isotope analysis

ABSTRACT

Stable isotope analysis is a valuable method for examining nutrient flow in ecosystems and the dynamics of trophic relationships. In most cases, animal tissue collection (typically muscle or liver) requires that the animal be sacrificed. The evidence that exists for non-lethal alternatives to sampling for stable isotope analysis suggests that species-specific data are required. A nondestructive method for collecting samples for stable isotope analysis is most critical for species with populations in decline, such as the American eel *Anguilla rostrata*. To evaluate the viability of non-lethal sampling methods as a replacement for methods that require animal sacrifices, liver and muscle tissues, fin clips, and mucus were sampled from American eels. All tissue pair models were significant suggesting eel fin and mucus have the potential to serve as nonlethal surrogates for muscle and liver for both $\delta^{15}N$ and $\delta^{13}C$; however, the longer turnover time of eel muscle must be accounted for when designing an experiment and interpreting data. Mucus, in particular, can have great utility as a source of isotope data given the potential for repeat sampling due to noninvasive methods and abundant source material. The predictive models developed here provide nonlethal alternatives for sampling yellow eel tissues for stable isotope analysis that will allow for the large-scale data collection efforts needed to improve our understanding of eel habitat use, movement, and trophic relationships.

INTRODUCTION

Stable isotope analysis (SIA) is an increasingly relied upon method for examining nutrient flow in ecosystems and the dynamics of trophic relationships. SIA has proven a valuable tool in fisheries research to examine questions focused on the impacts of non-native species (Cucherousset et al. 2012), dietary trends (MacAvoy et al. 2001; Willis et al. 2017), trophic relationships among organisms (Vander Zanden and Rasmussen 1999), and habitat use (Fantle et al. 1999; Vincent et al. 2015; Eberhardt et al. 2015) among others. SIA provides longer-term information (i.e., over a period of weeks, months, or years) on organism diet as opposed to traditional gut content analyses that provide information on what an organism has consumed in recent days (Gannes et al. 1997). Furthermore, SIA provides information on the components of the diet that are assimilated by the organism, whether catabolized for energy or synthesized into tissues, rather than simply what was ingested (Fry 2006).

Carbon and nitrogen are two commonly analyzed isotopes for understanding trophic dynamics and structure. Nitrogen isotopes are useful for understanding the trophic level at which an organism is feeding (Minagawa and Wada 1984; Peterson and Fry 1987) while carbon isotopes can be used to differentiate among organic matter sources at the base of the food web (Peterson et al. 1985; Deegan and Garritt 1997). In addition to dietary trends, carbon and nitrogen isotopes can be used to understand origins and migrations as organisms move among habitats with spatially distinct isotope values (Cunjak et al. 2005; Willis et al. 2013; McMahon and Newsome 2019).

Dietary stable isotope studies typically involve collecting samples of potential food sources as well as tissue from the target organism. Because stable isotopes are thought to move through food webs with predictable or little change in their values (Smith and Epstein 1970;

Minagawa and Wada 1984), food source values can be used to develop inferences about organism diet, trophic level, and movement. Muscle and liver are frequently sampled tissues for SIA of fishes to infer dietary trends. Muscle is the most commonly used tissue due to the abundance of material and the fact that it is easy to homogenize (Jardine and Cunjak 2005). Furthermore, the relatively low lipid and inorganic carbonate content of muscle tissue serves to limit the variability of isotope measurements (Pinnegar and Polunin 1999). Due to the higher metabolic rate of liver relative to muscle, liver isotope values can provide additional information about more recent dietary trends than muscle and so is also frequently sampled (Tieszen et al. 1983; Miller 2006).

In most cases, sampling muscle and liver tissue for SIA requires that the animal be sacrificed. Some evidence exists for the use of non-lethal alternatives to sampling fish for dietary stable isotope analysis, particularly the use of fin clips (Jardine et al. 2005; Kennedy et al. 2005; Kelly et al. 2006). Analysis of fish epidermal mucus is emerging as a promising indicator of muscle isotope values given significant relationships identified in multiple species (Church et al. 2009; Shigeta et al. 2017; Maruyama et al. 2017; Winter et al. 2019). Fish scales have also recorded similar isotope values to muscle tissue and present an additional non-lethal method that can be easy to sample (Kelly et al. 2006; Sanderson et al. 2009). Although each of these potential non-lethal sources has demonstrated significant relationships to muscle and/or liver (Fincel et al. 2012; McCloskey et al. 2018; Winter et al. 2019), the tissue pair relationships have not been consistent across species suggesting that species-specific data are required for developing non-lethal approaches for SIA (Galvan et al. 2015).

A nondestructive method for collecting samples for stable isotope analysis is most critical for species at risk, such as the American eel, *Anguilla rostrata*. The American eel is in decline

throughout its range (Castonguay et al. 1994; Haro et al. 2000). Potential causes include a variety of anthropogenic impacts including migration barriers and nearshore habitat degradation and loss (Haro et al. 2000). To inform management practices in support of the American eel population, the Atlantic States Marine Fisheries Commission (ASMFC) Fishery Management Plan for the American eel lists understanding the food habits of American eel in various habitats, movements of eels in the yellow life stage among habitats, habitat preferences and needs, and predator-prey relationships as high priority research needs (ASMFC 2000).

Stable isotopes provide an important mechanism for addressing the data needs identified by the ASMFC for management of the American eel, particularly with respect to eel reliance on foraging habitats, habitat preferences and movements among them, and predator-prey interactions. American eels range from Greenland to northern South America and constitute a single breeding population (Pujolar 2013). The wide geographic range and complex life history of the American eel requires trophic investigations that examine the potential for variation in resource use across habitats, latitudes, and life stages. Developing a body of research that accounts for these effects will require the collection of large numbers of samples. Thus, to address the data needs required to understand and sustainably manage the American eel population, it is critical that a non-lethal method for obtaining stable isotope data be established for the American eel.

Liver, and muscle tissues, fin clips, and mucus were sampled from American eels to evaluate the viability of non-lethal sampling methods as a replacement for methods that require animal sacrifice. Scale samples were not evaluated due to the potential difficulty of removing scales given the high level of embeddedness of eel scales (Tesch 2003). Fin and mucus samples were evaluated for significant isotopic relationships with liver and muscle tissue. Where

significant relationships were identified among tissue pairs, predictive models were tested with data from eels held in a laboratory to evaluate the transferability of the models to other data sets.

METHODS

To evaluate non-lethal stable isotope sampling methods, 54 American eels were collected from three estuaries: the Parker River Estuary, Massachusetts, the Hampton-Seabrook Estuary, New Hampshire, and the Webhannet Estuary, Maine, USA (Figure 7). Eels were collected from saltmarsh creeks using eel pots and measured for total length to the nearest millimeter (mm). Captured fish were anesthetized, sacrificed, and frozen according to a protocol approved by the Institutional Animal Care and Use Committee of the University of New Hampshire (IACUC permit 070702).

Eels were rinsed with distilled water and mucus samples were collected from the skin surface by scraping with the blunt edge of a scalpel. Each mucus sample was placed on a tin tray for drying. Muscle tissue was collected from the posterior section of each fish between the dorsal fin and the lateral line and livers were dissected while. Fin clips were collected to include primarily membrane portions of the fin. Tissue samples were held in a drying oven at 60^{o}C until they achieved constant weight. Each sample was then dried, ground with a mortar and pestle, weighed to the nearest microgram into a tin capsule, and then compacted into a small cube. Tools and work surfaces were cleaned with 99.5% ethanol and Kimwipes® between processing each sample to prevent cross-contamination. All samples were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes at the University of New Hampshire Stable Isotope Laboratory with a Costech ECS4010 Elemental Analyzer coupled to a Delta Plus XP mass spectrometer (Thermo Finnigan). Stable isotope ratios are reported in delta notation per mil units (‰) as follows:

$$\delta^{X} = [(R_{sample}/R_{standard)}-1)] \times 1000$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Stable isotope ratios were determined using Vienna Pee Dee Belemnite (VPDB) as the reference material for carbon and atmospheric N_2 (air) for nitrogen. Delta^{15}N values are reported on the VPDB scale using International Atomic Energy Agency-N1 (IAEA; 0.4 ‰) and IAEA-N2 (20.3 ‰). Repeated analyses of laboratory standards varied less than 0.15 per mil for both $\delta^{15}N$ and $\delta^{13}C$.

Although mucus is not a tissue, and fin clips are comprised of multiple tissues (e.g., connective tissue, bone, and muscle if clipped close to the body), for the simplicity of referring to all sample types in aggregate, mucus and fin will each be referred to as a tissue throughout. Paired t-tests were used to test the null hypothesis that differences between nonlethal tissues (mucus or fin) and destructive tissues (muscle or liver) were zero. All combinations of nonlethal and destructive tissue results were evaluated for differences for both $\delta^{13}C$ and $\delta^{15}N$. When differences were significant at $p<0.05$, "tissue pair models" were developed with simple linear regression. Regression data are displayed with 95% confidence intervals to examine tissue pair relationships against a line with a slope of 1.

To evaluate the reliability of model predictions, linear regression models were applied to $\delta^{15}N$ and $\delta^{13}C$ data for fin and mucus samples from 9 eels held in the laboratory (hereinafter referred to as "raised eels"). These eels were held on a consistent diet of manufactured eel feed for over a year so presumably have stable isotope values in equilibrium across all tissues measured. Muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values predicted with tissue pair models were tested against observed muscle and liver values for significant differences using paired t-tests.

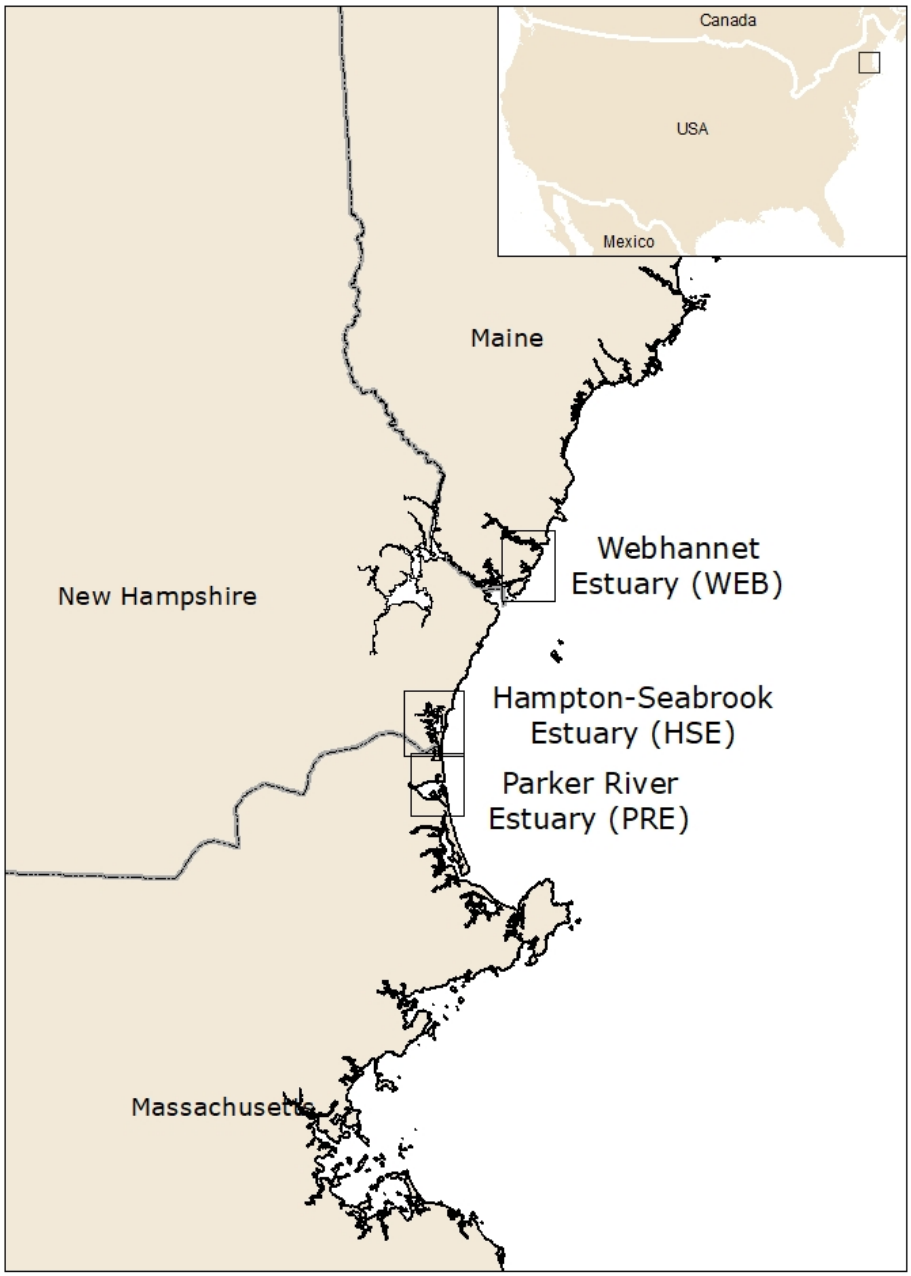

Figure 7. Sampling site locations

Table 9. Sample size, mean carbon and nitrogen isotope values and carbon:nitrogen for each eel tissue type sampled.

	Tissue	N	δ^{15}N ‰ ± SE	δ^{13}C ‰ ± SE	C:N ± SE
Non-lethal					
	fin	54	10.17 ± 0.19	-17.13 ± 0.32	4.16 ± 0.06
	mucus	53	9.80 ± 0.17	-16.76 ± 0.30	3.68 ± 0.02
Lethal					
	liver	54	10.30 ± 0.17	-17.50 ± 0.32	4.41 ± 0.08
	muscle	50	9.80 ± 0.19	-17.35 ± 0.32	3.73 ± 0.11

Given that eels were collected from three estuaries, linear regressions of isotope relationships were tested for an influence of sampling site with site as a blocking factor. To test for an effect of eel size on tissue pair relationships, eel size was regressed against the difference in isotope values (δ^{13}C or δ^{15}N) between each tissue pair combination. Residuals of statistical model outputs were examined for normality and homogeneity of variances; all data met the assumptions of the general linear model.

RESULTS

Tissues from 54 eels with a mean size of 26.4 ± 10.2 cm were analyzed for δ^{15}N and δ^{13}C to evaluate the potential for mucus or fin to serve as non-lethal alternatives to muscle or liver. When compared to the standard of muscle tissue, mucus δ^{15}N values were quite similar while fin tissue was only somewhat enriched in ^{15}N (Table 9). When compared to liver, both mucus and fin were slightly depleted in ^{15}N. Mucus and fin were more enriched in ^{13}C than both muscle and liver tissue. No statistical difference was detected between mucus and muscle samples for δ^{15}N (Table 10). All remaining tissue pairs tested were significantly different for both δ^{15}N and δ^{13}C

(Table 10) so the null hypothesis that the mean differences between the tissue pairs was zero was rejected for all but the $\delta^{15}N$ mucus and muscle comparison.

Table 10. Paired t-test results for each non-lethal (fin or mucus) and lethal (muscle or liver) tissue pair combination for $\delta^{15}N$ and $\delta^{13}C$. Asterisks (*) indicate significant results at an alpha of $p<0.05$.

$\delta^{15}N$

Tissues	Mean difference (±SE)	df	Prob>|t|	t ratio
fin x liver	0.14 ± 0.07	53	0.05	2.01
mucus x liver	-0.49 ± 0.06	52	0.0001	-8.51
fin x muscle	-0.38 ± 0.05	49	0.0001	-7.11
mucus x muscle	-0.02 ± 0.05	48	0.74	-0.33

$\delta^{13}C$

Tissues	Mean difference (±SE)	df	Prob>|t|	t ratio
fin x liver	-0.37 ± 0.11	53	0.001	-3.43
mucus x liver	-0.66 ± 0.09	52	0.0001	-7.74
fin x muscle	-0.37 ± 0.11	49	0.002	-3.28
mucus x muscle	0.70 ± 0.11	48	0.0001	6.53

The relationship between tissue pairs was determined with simple linear regression. Regression models identified significant relationships ($p<0.05$) for all tissue pair combinations analyzed (Figures 8 and 9), with models explaining between 88% and 93% of the variance between tissues. All models included at least a portion of the 1:1 line within the 95% confidence interval of the best fit line.

To evaluate the reliability of the 1:1 relationship observed between mucus and muscle $\delta^{15}N$ data for field collected eels, mucus and muscle $\delta^{15}N$ data from raised eels were evaluated with a paired t-test and were found to be significantly different (Table 11). The regression models developed for the remaining tissue pairs were validated with raised eels to evaluate the reliability of the models for predicting muscle and liver isotope values from nonlethal surrogates

(fin and mucus). A significant difference was detected between fin and liver for $\delta^{15}N$ model, but not for the remaining tissue pairs (Table 11;

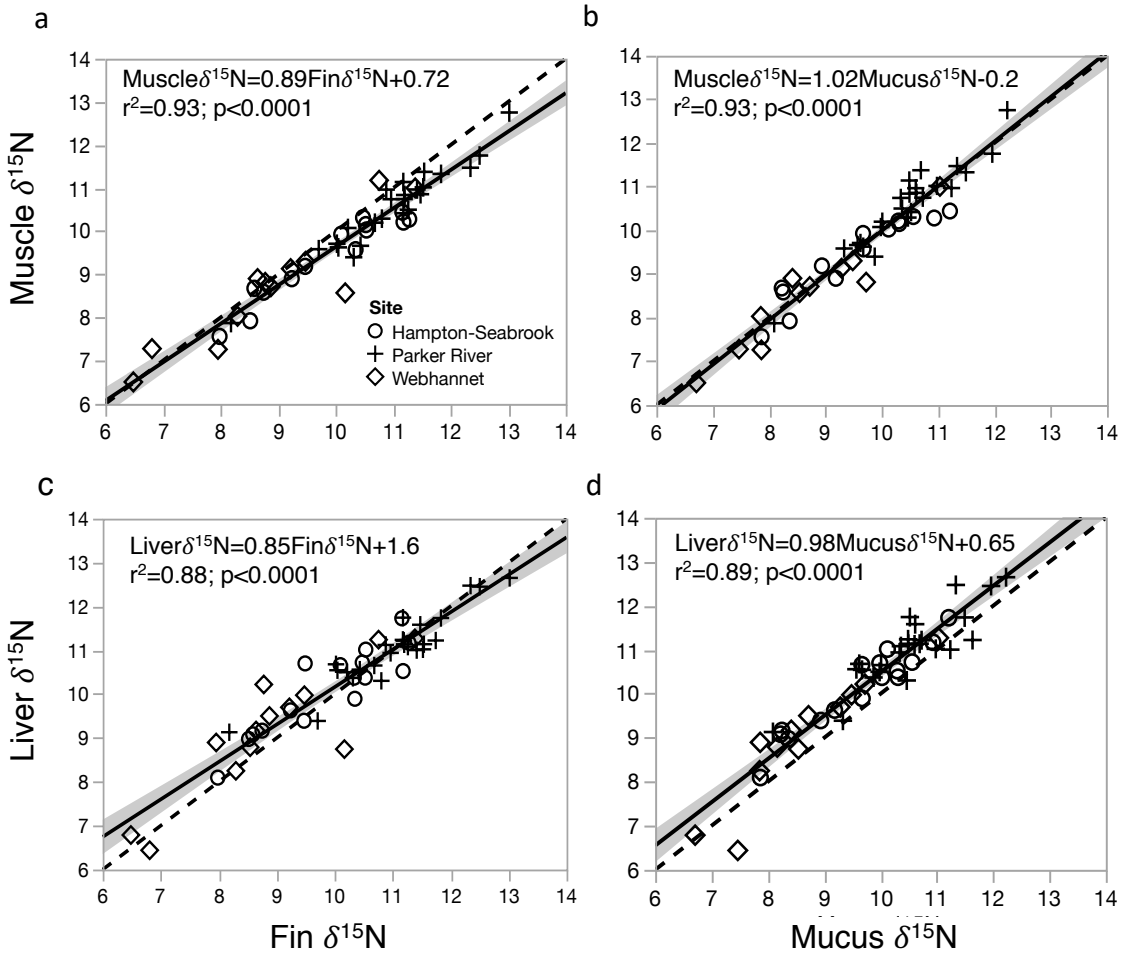

Figure 8. $\delta^{15}N$ values of nonlethal tissues (mucus and fin) regressed against lethal tissues (muscle and liver) for a. $\delta^{15}N$ fin x muscle, b. $\delta^{15}N$ mucus x muscle, c. $\delta^{15}N$ fin x liver, and d. $\delta^{15}N$ mucus x liver. The solid line indicates the best fit line, shading represents the 95% confidence interval of the best fit line, and the dashed line is the 1:1 line for reference.

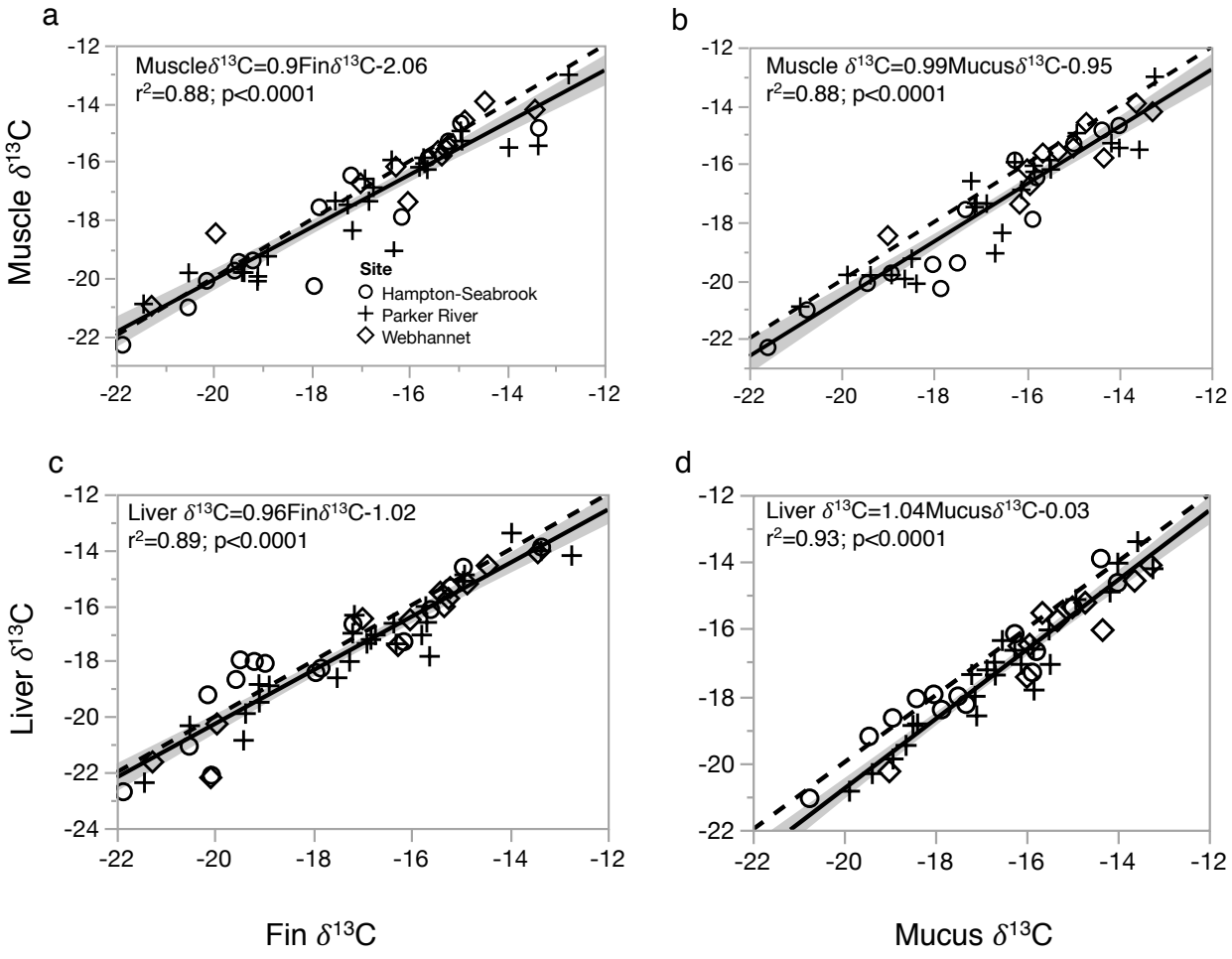

Figure 9. δ^{13}C values of nonlethal tissues (mucus and fin) regressed against destructive tissues (muscle and liver) for a. δ^{13}C fin x muscle, b. δ^{13}C mucus x muscle, c. δ^{13}C fin x liver, and d. δ^{13}C mucus x liver. The solid line indicates the best fit line, shading represents the 95% confidence interval of the best fit line, and the dashed line is the 1:1 line for reference.

Table 11. Results for validation of paired tissue models for predicting $\delta^{15}N$ and $\delta^{13}C$ values of lethal tissues (muscle or liver) from non-lethal tissues (mucus or fin). The first three models for $\delta^{15}N$ were tested by comparing predicted versus actual data for raised eels with paired t-tests. Because the paired tissue model for mucus x muscle $\delta^{15}N$ was a 1:1 relationship, t-test results for mucus x muscle $\delta^{15}N$ represent the comparison of actual mucus and muscle data for raised eels. Asterisks (*) indicate significant results at an alpha of $p<0.05$.

$\delta^{15}N$

Tissues	Mean difference (±SE)	df	Prob>|t|	t ratio
fin x liver	0.49 ±0.15	7	0.0127*	3.32
mucus x liver	-0.01±0.14	7	0.9244	-0.10
fin x muscle	-0.22±0.15	8	0.1788	-1.47
mucus x muscle	0.41 ±0.11	8	0.0084*	3.47272

$\delta^{13}C$

Tissues	Mean difference (±SE)	df	Prob>|t|	t ratio
fin x liver	0.76 ±0.51	7	0.1758	1.51
mucus x liver	0.18 ±0.27	7	0.5391	0.65
fin x muscle	0.35 ±0.24	8	0.1771	1.48
mucus x muscle	0.11 ±0.18	8	0.5743	0.59

i.e., mucus x liver and fin x muscle). No significant differences were found between predicted and observed values for all tissue pair combinations for $\delta^{13}C$ (Table 11) suggesting that these models are robust for predicting eel muscle and liver values from fin and mucus.

Eel body length explained little variation in tissue pair relationships. The difference between fin and muscle tissue $\delta^{15}N$ values had the most variation explained by size of all the tissue pair combinations ($r^2 = 0.22$; $p<0.0006$). When size was added as a factor in the regression model for fin and muscle $\delta^{15}N$, it added only 1% to the simple explanatory model ($r^2_{\text{simple linear regression (SLR)}} = 0.93$ versus $r^2_{\text{SLR with size}} = 0.94$). Because eel size did not explain a large amount of the variability in isotope values between tissues, length was not included as a factor in regression

models. Out of four comparisons each for $\delta^{15}N$ and $\delta^{13}C$, a site effect was significant for two out of the eight models (Table 12). When site was added as a factor in the regression model for each of the two models with significant site effects, it added only 1% to each simple explanatory model (r^2_{SLR} = 0.93 versus $r^2_{SLR\ with\ site}$= 0.94 for $\delta^{15}N$ mucus x muscle, and r^2_{SLR} = 0.93 versus $r^2_{SLR\ with\ site}$= 0.94 for $\delta^{13}C$ mucus x liver). Therefore, site was not considered an important variable in this dataset and was omitted from the models.

Table 12. Results of tests for an effect of sampling site on tissue pair relationships for $\delta^{15}N$ and $\delta^{13}C$. Asterisks (*) indicate significant results at an alpha of $p<0.05$.

	$\delta^{15}N$		$\delta^{13}C$	
	F	p	F	p
Fin x liver				
Site	0.1474	0.8633	1.8726	0.1644
Fin	209.5703	<.0001*	432.0122	<.0001*
Fin x muscle				
Site	0.9242	0.4041	2.3464	0.1071
Fin	396.7804	<.0001*	338.2231	<.0001*
Mucus x liver				
Site	1.4497	0.2445	4.0776	0.0230*
Mucus	242.7527	<.0001*	722.1815	<.0001*
Mucus x muscle				
Site	3.6578	0.0337*	1.2888	0.2856
Mucus	395.4553	<.0001*	303.1995	<.0001*

DISCUSSION

Analysis of carbon and nitrogen isotopes of four American eel tissues suggests that the nonlethal tissues of fin and mucus are suitable predictors for muscle and liver and by extension,

useful indicators of diet and habitat. Significant relationships were identified for all tissue pairs for both $\delta^{13}C$ and $\delta^{15}N$ in the American eel.

Turnover rate

When evaluating the suitability of nonlethal proxies, the turnover rate of each tissue must be considered. Turnover rate identifies the amount of time it takes for a tissue's isotope values to reflect the diet. Based on the isotope values presented here, both mucus and fin can serve as nonlethal surrogates for muscle and liver sampling for eels; however, if the turnover rates for eel tissues vary greatly it may limit the potential uses of mucus and fin as non-lethal proxies. Mucus as a surrogate for eel muscle and liver tissue $\delta^{15}N$ shows great promise due to statistically significant relationships, the copious amounts of available sample material, and the potential for repeated sampling of mucus with minimal damage to the eel. However, in a laboratory experiment evaluating the isotopic turnover of eel tissues, mucus exhibited the quickest ^{15}N turnover with a half-life of 67 days while muscle exhibited the slowest ^{15}N turnover with a half-life of 191 days (Eberhardt, in preparation). Eel fin and liver tissue ^{15}N turned over at similar rates with 90- and 97-day half-lives, respectively (Eberhardt, in preparation). Because eel muscle has a much slower ^{15}N turnover rate than the other tissues, fin and mucus will represent more recent dietary sources than muscle.

Eels exploit many habitats and can forage on a variety of prey resources over both short and long temporal scales (Wenner and Musick 1975; Jessop et al. 2002; Harrod et al. 2005). The agreement between mucus and muscle $\delta^{15}N$ values in the current dataset may be due to the fact that the individual eels had been feeding on diets of similar isotopic composition over a period of 3-9 months or longer (Eberhardt, unpublished data). If eels were to undergo a shift in diet, fin or

mucus $\delta^{15}N$ values could no longer reflect muscle $\delta^{15}N$; however, they would continue to reflect liver $\delta^{15}N$ isotope values given the similar turnover rates of the three tissues. As such, based on turnover rates alone, fin and mucus emerge as promising proxies for liver $\delta^{15}N$ values as nonlethal surrogates. Data for turnover of ^{13}C in eel tissues could not be found at the time of this experiment but would help guide the appropriate use of nonlethal surrogates for eel carbon isotope analysis.

Fin tissue

Fin tissue predictive models were significant for both muscle and liver for $\delta^{15}N$ and $\delta^{13}C$. The similar turnover rates of ^{15}N between fin and liver tissue in eels render fin a suitable non-lethal proxy for liver tissue with regard to the temporal scale of the diet that the tissue reflects. However, while the $\delta^{15}N$ fin-liver model developed from field-collected data was significant, data from raised eels did not fit the model indicating the need for more data to test the transferability of the $\delta^{15}N$ fin-liver model. The predictive models for all other tissue pairs for $\delta^{15}N$ and $\delta^{13}C$ measured in this study were robust when tested with data from raised eels. Fin tissue has emerged as a suitable non-lethal surrogate for muscle in this and other evaluations of other fish species (Jardine et al. 2005; Sanderson et al. 2009; Tronquart et al. 2012). Most studies have evaluated fin as a surrogate for muscle rather than liver tissue and, as in this study, measured slopes slightly above or below 1, necessitating predictive models that account for the offset between fin and muscle tissue isotope values (Willis et al. 2013). Few studies have evaluated fin tissue as a surrogate for both muscle and liver $\delta^{13}C$ and $\delta^{15}N$ and the results vary, with stronger correlations between fin and liver identified for yellow perch, *Perca flavescent,s*

(McCloskey et al. 2018) and fin and muscle for seahorses, *Hippocampus guttulatus* (Valladares and Planas 2012).

Nutrient routing may have played a role in the lack of agreement between the predictive model and test data for $\delta^{15}N$ for fin and liver. Nutrient routing occurs as the macronutrients (i.e., lipids, carbohydrates, proteins) that constitute an organism's diet are assimilated into tissues at varying rates and efficiencies (Schwarcz 1991) and as a result, consumer tissue isotope values represent the nutrient components of the diet rather than the bulk diet (Gannes et al. 1997). The diets of the field and raised eels may have varied in biochemical composition, resulting in differential routing of dietary components to tissues and variation in tissue pair relationships for $\delta^{15}N$. More information is needed to understand the effect of nutrient routing on the utility of non-lethal proxies as the relationships between tissue isotope values calculated for one diet may not hold for a diet with a differing biochemical composition.

Mucus

Mucus emerged as a statistically significant surrogate for both muscle and liver $\delta^{15}N$, but the use of each requires important considerations. The slower turnover rate of eel muscle $\delta^{15}N$ relative to the other tissues measured can limit the utility of mucus as a nonlethal proxy for muscle $\delta^{15}N$. Given that the disparity between eel muscle ^{15}N turnover and mucus ^{15}N turnover was many months, the goals of an experiment will determine whether eel mucus should be used as a surrogate or a compliment to eel muscle samples. For instance, in field-based studies where the goal is to evaluate eel resource use at differing time intervals or movement amongst habitats sampling both mucus and muscle will be beneficial to capture isotope data representing different temporal scales. However, where repeat sampling of the same organism can occur over time,

such as in laboratory experiments or field-based mark-recapture experiments, then sampling only mucus can be effective.

Mucus can also serve as an appropriate proxy for liver given the similar turnover times. The significant validation of the $\delta^{15}N$ mucus-liver model suggests confidence in the transferability of this model to other datasets focused on the yellow life stage of the eel. Mucus has a more homogenized makeup than fin tissue (Shephard 1994; Willis et al. 2013) and likely allows for more frequent sampling with minimal impact to the fish than repeat sampling of fin tissue, although data explicitly testing this are lacking. Therefore, mucus presents a robust option for nonlethal collection of eel $\delta^{15}N$ data.

Influence of eel size

Previous studies evaluating non-lethal methods of stable isotope analysis have identified size-based effects on tissue relationships likely due to variations in habitat use, food sources, and mechanisms of nutrient assimilation with size (Overman and Parrish 2001; Kelly et al 2006). In general, eel body length did not influence isotope values in the present study given that only one out of eight tissue pair models had a significant size effect. Whereas many other species of fish exhibit rapid growth at juvenile life stages and slow growth at adult life stages, the subadult yellow life stage of the American eel is represented by a wide range of sizes and variable growth rates (Helfman and Clark. 1986; Morrison and Secor 2003; Jessop et al. 2004; Lamson et al. 2006). Eel size and growth rate are hypothesized to vary by life stage, habitat, population density, and sex ratio (Krueger and Oliveira 1999; Oliveira 1999). Eels analyzed in the present study were all in the yellow life stage and were captured in the same habitat type (saltmarsh creeks). Little evidence for a site effect was found in this dataset. This may be due to the fact that all sites were comprised of the same habitat types at the same latitude ($\pm 0.3^{o}$) and as a result,

size-based effects, or the correlated factors that influence eel size, are uniform in the present study. Given the many factors that can potentially influence eel size and growth rates, it is recommended that future eel isotope studies evaluate each of the factors that can affect eel size and growth (i.e., latitude, habitat, population density), rather than eel size itself for potential influences on tissue isotope relationships.

In summary, our results provide strong evidence for the use of fin and mucus as nonlethal alternatives to carbon and nitrogen stable isotope analysis of eel muscle and liver tissue. All models were significant suggesting both eel fin and mucus have the potential to serve as nonlethal surrogates for both $\delta^{15}N$ and $\delta^{13}C$. Use of the models developed here for predicting yellow eel $\delta^{15}N$ and $\delta^{13}C$ muscle and liver values from fin and mucus is encouraged with the caution that the relative turnover rates of each tissue must be considered. Models for predicting muscle $\delta^{15}N$ from both mucus and fin were significant; however, the longer turnover time of eel muscle must be accounted for when designing an experiment and interpreting data. Mucus, in particular, can have great utility as a source of isotope data given the potential for repeat sampling due to noninvasive methods and abundant source material. The differing turnover times also create an opportunity to evaluate mucus as a complement to muscle to evaluate dietary shifts over time. If using mucus as a surrogate for muscle, the design of the project must align with and account for the different turnover times between the two tissues. The significant relationship and similar turnover rates of mucus and liver $\delta^{15}N$ create great potential for the use of mucus as a proxy for liver $\delta^{15}N$. Based on our results and similar turnover times, we encourage the use of yellow eel mucus as a surrogate for liver $\delta^{15}N$. Turnover data for ^{13}C in eel tissues are needed to

better evaluate the predictive models developed for predicting $\delta^{13}C$ of eel muscle and liver tissues.

Fin is also a promising surrogate for estimating liver $\delta^{15}N$ due to similar turnover rates; however, when evaluated with test data this model was not validated. More data is needed to evaluate the relationship between fin and liver $\delta^{15}N$ in yellow eels before this model can be used with confidence. Differential routing of food sources to each tissue may play a role in the lack of agreement between predictive models and test data for $\delta^{15}N$ fin-liver and $\delta^{15}N$ mucus-muscle models. The use of nonlethal surrogates will be improved with greater understanding of the effects of nutrient routing on tissue isotope values and turnover rates.

The data presented here contribute to a growing body of literature exploring species-specific relationships of isotope values among tissues, with a focus on identifying the potential for nonlethal sampling methods (e.g., Kelly et al. 2006; Willis et al. 2013). American eels are a population in decline (Castonguay et al. 1994; Haro et al. 2000). By developing predicative models that allow for nonlethal sampling of yellow eels for stable isotope analysis, our data provide an important non-lethal approach for investigating the research priorities outlined by the ASMFC as important for sustaining the American eel population (ASMFC 2000). In particular, the use of the predictive models developed here can help inform our understanding of eel habitat use, movement, and trophic relationships. Given the broad geographic area occupied by American eels, the development of tools to collect data on eel diet and resource use without having to sacrifice the animal will allow for the large-scale data collection efforts needed to inform the management of American eels.

CHAPTER IV

Small scale movements of yellow phase American eels, *Anguilla rostrata,* within saltmarsh creeks

ABSTRACT

Estuarine habitats such as salt marshes are emerging as important for American eels, yet little is known about eel use of these systems. Instream structures such as culverts and self-regulating tide gates are often found where roadways intersect salt marshes creeks. Instream structures have been found to restrict or delay eel movements in freshwater but data are lacking in estuarine systems. A telemetry study was conducted to examine eel movements in two saltmarsh creeks, one of which contained an undersized culvert with a self-regulating tide gate (SRT). Eels moved predominantly under dark conditions and at low tide, presumably moving onto the vegetated marsh at high tide. Eel movement data suggest eels released upstream of the SRT travelled shorter distances than eels in the reference creek, accessing less habitat. The SRT is not a total barrier to eel movement; however, the SRT may delay movement of eels to downstream areas, whether due to the presence of subtidal habitat upstream, altered environmental conditions obscuring cues for movement, or the intermittent periods of a physical barrier. The low sample size precluded conclusions on eel movement; however, trends in the data provide compelling information for future research.

INTRODUCTION

American eels (*Anguilla rostrata*) exhibit a complex life history that includes migrations across a variety of habitat types (Tesch 2003). In addition to two long distance migrations across the Atlantic Ocean to the Sargasso Sea, eels commonly make interhabitat migrations after moving inshore (Jessop et al. 2008). However, little is known about American eel use of specific inshore habitats (ASMFC 2006) and most research on eel habitat use stems from freshwater systems (Glova et al. 1998; Haro et al. 2000.) Estuarine systems including salt marshes have emerged as important habitat for eels (Harrod et al. 2005). In fact, eels have been found to complete their lifecycle in marine or estuarine habitats without ever entering freshwater (Tsukamoto et al. 1998; Lamson et al. 2006) resulting in redefining their life history from obligate to facultative catadromy (Tsukamoto and Arai 2001). Faster eel growth rates have been observed in marine and brackish water than freshwater (e.g., Oliveira 1999; Morrison et al. 2003; Jessop et al. 2006) suggesting that marine and estuarine systems are important for supporting eel production.

Migratory barriers occur where infrastructure such as dams, berms, and culverts are built across waterways to create transportation corridors, regulate water flow, or harness power and are common across freshwater and estuarine portions of eel habitat. In fact, 84% of potential stream habitat for eels from Maine to Connecticut has been obstructed by dams or other migratory barriers (Busch et al. 1998). A review of eel habitat use of unfragmented systems found that 69% of eels moved among habitats (Lamson et al. 2006) while analysis of eels in a Canadian coastal stream obstructed by a dam found that eels did not move between freshwater and estuarine habitats (Cairns et al. 2004). Furthermore, dams have been found to reduce and delay silver eel downstream migration through rivers (Behrmann-Godel and Eckmann 2003;

Besson et al. 2016). Tide gates do not create complete barriers as with dams because the gate is open periodically, yet tide gates have been found to delay silver eel downstream migration potentially increasing energetic demands and the risk of predation (Wright et al. 2015).

In addition to impeding eel movement through systems, structures such as dams, gates, and culverts can fundamentally change habitat conditions by impounding water, degrading water quality, modifying water flow, and altering prey availability (Williams and Wolman 1984; Mor et al. 2018). Eel movements have been associated with specific environmental conditions such as light (Hedger et al. 2010), water level and flow direction (Helfman et al. 1983; Dutil et al. 1998), and water temperature (Vollestad 1986; Dutil et al. 1998). It follows that in addition to physically blocking eel migration, instream structures can disrupt eel migration by altering environmental conditions and cues for eel movement (Crook et al. 2014). Furthermore, decreased connectivity between upstream and downstream habitats can result in eels remaining in suboptimal habitats and suffering decreased access to food and refuge resources (Weisberg and Lotrich 1982). Understanding the impacts of instream structures on eel use of freshwater and estuarine corridors is important as American eel stocks have declined in recent decades and are thought to be at or near historically low levels (ASMFC 2012). Understanding the local habitat movements of eels was listed as a priority to inform the effective management of this species (ASMFC 2006).

Given the demonstrated negative impacts of instream structures on eel movement through freshwater corridors, as well as the emerging data on eel reliance on estuarine systems, a need exists for a clearer understanding of eel movement through estuarine systems such as saltmarsh creeks that contain instream barriers. Understanding eel movement and site use patterns in salt marshes is important to inform salt marsh management to support eels. Therefore, the objectives

of the proposed research are to examine small-scale spatial and temporal variation of eel movements and to examine American eel response to salt marshes impacted by an instream barrier. Nine eels were tagged with transmitters and a telemetry study was conducted in the Webhannet estuary, Wells, ME to evaluate the hypotheses that: 1. Eel movements in saltmarsh creeks differ by temperature, tidal stage, diel period, or light intensity; and 2. The presence of an undersized culvert with a tide gate impacts eel movement in saltmarsh creeks.

METHODS

Sampling site and telemetry array

The Webhannet Estuary is a salt water dominated estuary with a small watershed (3549 hectares) located in Wells, ME. The Webhannet Estuary has a semi-diurnal tidal cycle with a mean tidal range of 2.7 meters (m) at the inlet and is characterized by salt marsh, sand and mud flats, and meandering tidal creeks (Dionne et al. 2006). The Drake's Island Road causeway cuts across the northern section of the estuary and includes a bridge over the western tidal creek (herein referred to as the "reference creek") and a culvert with a self-regulating tide gate (SRT) in the eastern creek (herein referred to as the "SRT creek"; Figure 10). The SRT closes at high tides to limit flooding to upstream residences adjacent to the marsh resulting in reduced tidal exchange to upstream areas (Adamowicz and O'Brien 2012). After each flood event the SRT opens once the downstream tide recedes. Neither the reference nor the SRT creek have major freshwater inputs entering from upstream. The upstream area of the reference creek is intertidal and completely drains at low tidal stages while the area upstream of the SRT creek retains water at all tidal stages due to an undersized culvert and the intermittent gate closures (Adamowicz and O'Brien 2012).

In November 2009, 8 Vemco VR2 or VR2W receivers (Vemco Ltd., Nova Scotia) were

installed in the intertidal creeks in the northern section of the Webhannet estuary. Receivers were set within the creek channel of the reference and SRT creeks, and downstream along the confluence of the two creeks to the estuary inlet (Figure 10). A Hobo pendant data logger (model: UA-002-64, ONSET) was installed adjacent to each receiver to collect data on light intensity (lux) and temperature (degrees Celsius) at 6-minute intervals for the length of the project period.

Laboratory experiment

A laboratory experiment was conducted prior to the field study to ensure no adverse effects of tag implantation on eels and to quantify the exposure time needed for anesthetization. Ten eels were held in a tank equipped with a flow-through seawater system at the Jackson Estuarine Laboratory, University of New Hampshire, from 13 October – 3 November 2009. Five eels were tagged with nonfunctioning transmitters of the same weight and size of the Vemco V7-1 and five eels were left without tags to serve as controls.

Eel capture and tag implantation

Eel pots (Gee's, model G-40EP, Filmore, New York) were set in the Webhannet Estuary to capture eels for the telemetry study. All eels captured in the Webhannet Estuary did not have enough mass to support V7 tags so eels were captured from other salt marsh systems in southern Maine. As such, all eels observed in this study were transplanted into the Webhannet estuary. Captured yellows eels were placed in aerated ambient seawater and brought to the Michele Dionne Research Laboratory at the Wells National Estuarine Research Reserve for tag implantation and monitoring.

Vemco acoustic V7-1 transmitters were used to track eel movements. Transmitters were

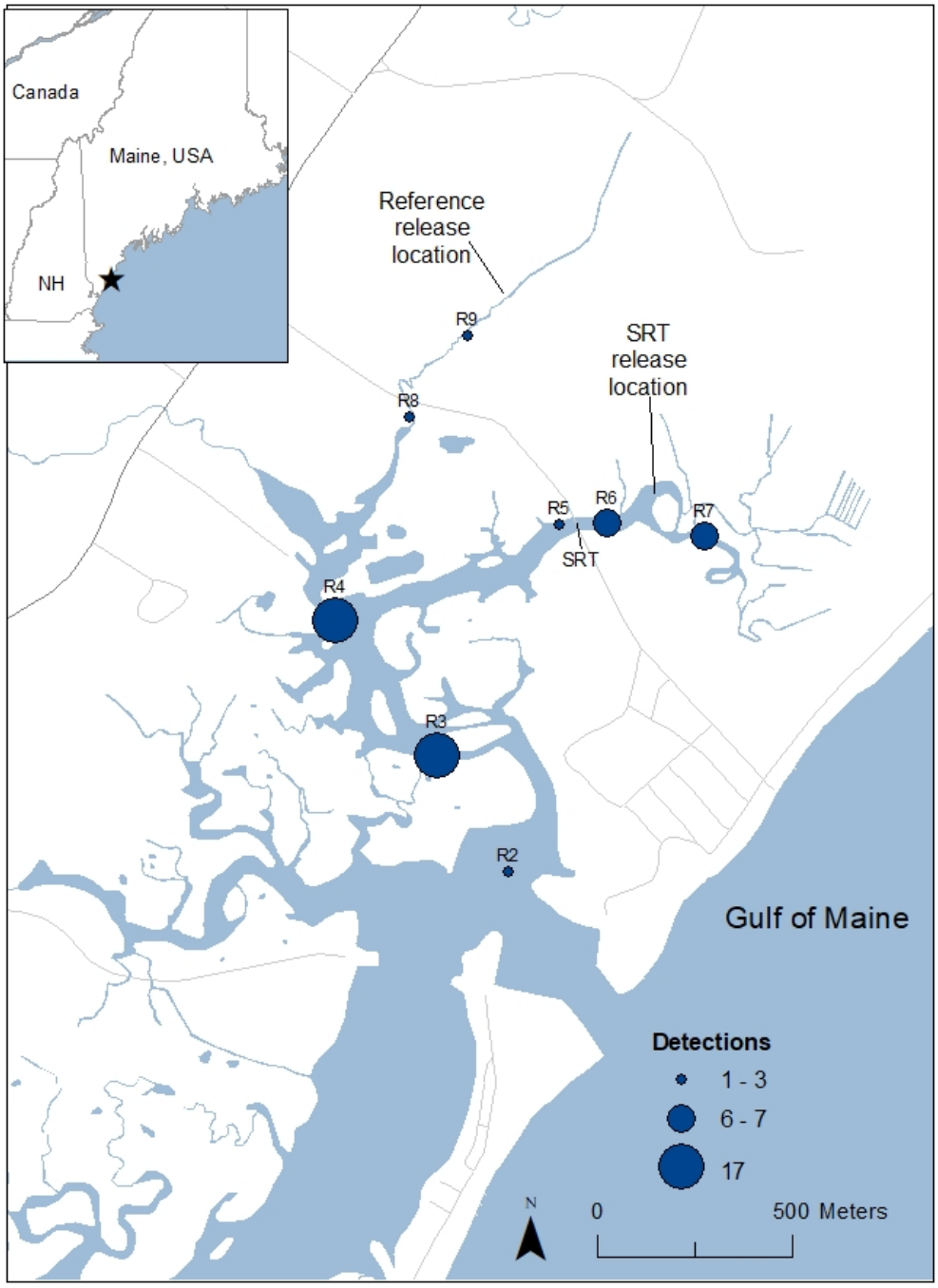

Figure 10. Locations of receivers in the Webhannet Estuary, Wells, Maine. The size of the receiver marker is scaled relative to the number of detections of eel movement at that receiver from 1 to 17.

20 millimeters (mm) long, 7 mm in diameter, weighed 0.7 grams (g) in water, and operated on a frequency of 69 kHz. Each transmitter had a unique identification code that was transmitted randomly every 50 to 130 seconds; at this rate transmitters were estimated to have a battery life of 60 days. A range test at receiver R8 detected transmitters at a distance of over 150 m. Given the small size and sinuosity of the creek system, it is assumed that detection limits at most receivers were defined by the morphology of the creek channels.

Tag implementation methods followed a protocol approved by the University of New Hampshire's Institutional Animal Care and Use Committee (IACUC# 070702). Eels were placed one at a time in a bucket of ambient seawater containing 100 mg/L eugenol. When fish reached stage 2 induction (defined as total loss of equilibrium, very slow opercular rate, and no reaction to stimuli), eels were measured for total length (centimeters) and mass (g). A small incision was made and the transmitter was inserted into the anterior portion of the peritoneal cavity. A small piece of tissue was cut from the dorsal or caudal fin and applied to the incision site with adhesive to serve as a biological bandage (Baras and Jeandrain 1998). Eels were placed in an aerated bucket containing seawater for observation. When stage 2 recovery had been reached (defined as recovery of equilibrium, increased opercular rate, response to stimuli, and normal swimming actions), the incision site was checked to ensure proper closure. If the incision site was deemed to be clean and fully closed, the eel was released in one of 2 locations - upstream of receiver R9 in the reference creek (n=5) or upstream of R6 in the SRT creek (n=4; Figure 10).

Data analysis

Mixed model analysis of variance (ANOVA) was used to test temperature and light data for differences across receiver stations. Data were analyzed for one hour before and after high tide to ensure that data loggers were submerged, and data represent temperature and light values

in the water rather than air. Data for station R7 were removed from analyses as the data variability suggest that the light and temperature logger was out of the water for periods of time that could not be predicted with confidence. Each high tide was numbered sequentially and coded as occurring during the day or night. Mean temperature for each tide was analyzed with a main plot that included the station number and day/night as fixed factors, as well as the station by day/night interaction term. The random factor of tide and the tide by day/night interaction served as the repeated measure subplot. Light data were analyzed for the five daytime high tides within the project period. Individual data points were coded sequentially for "time" to allow for direct comparisons. Light data fit a nonlinear model so were log transformed. Individual light data were analyzed with a main plot that included the station number and the tide as fixed factors and the station by tide interaction term. The random factor of time and the time by tide interaction served as the repeated measure subplot. Data did not meet the normal distribution assumption of ANOVA. Although ANOVA is robust to this violation (Blanca et al. 2017), alpha values for mixed models were set at the 0.0001 level to account for the non-normal distributions.

A limited dataset precludes statistical analysis of movement data to explicitly test hypotheses. Eel movement was defined as subsequent detections of an eel at two different receivers. The small sample size did not allow for examining all potential covariables with one statistical model; therefore, Chi Square Goodness of Fit and Fisher's Exact tests were used to examine potential individual effects of tidal stage, light intensity, temperature, and diel effects on eel movement data comparing eels released in the reference versus SRT creeks. Because the number of movements varied by fish, movement data were used as replicates. Light intensity data were coded as light (equal to or greater than 10 lux) or dark (less than 10 lux) for analysis of light effects on movement (Engineering ToolBox 2004). Temperature effects on movement data

were analyzed with a logistic regression with light as a continuous independent variable and release location as the bivariate dependent variable. The total space used and maximum dispersal was calculated for each eel to understand eel space use in salt marsh creeks.

RESULTS

Laboratory experiment

Ten eels were held in a laboratory experiment to evaluate potential tag effects. Tagged eels (n=5) had a mean length of 34.2 ± 1.7 centimeters (cm) and mean mass of 80.0 ± 9.1 g and control eels (n=5) had a mean length of 32.8 ± 1.9 cm and mean mass of 56.8 ± 2.3 g. Mean induction and recovery times were 5.6 ± 0.5 minutes and 5.4 ± 1.4 minutes, respectively. After 3 weeks of observation, no signs of infection were present in any eels and there was no mortality. After 4 weeks, one tagged eel died. This did not result in a significant difference in mortality between tagged and untagged eels (chi square; $\chi 2=_{(1, 10)} 1.11$; $p>0.29$).

Field experiment

Nine eels were implanted with acoustic transmitters for field study in November 2009. Eels had a mean length of 32.1 (±1.1) cm and mass of 46.35 (± 4.9) g (Table 13). Time to induce eels ranged from 4 to 10 minutes and eel recovery ranged from 10 to 15 min. All incisions were confirmed to be clean and fully closed prior to release and fish were released in good condition.

Receivers were deployed from 3 November 2009 to 10 December 2009. During the 38 days of receiver deployment, 25,753 detections were recorded. The number of detections per fish varied widely among the 9 eels (Table 13). Five fish were released in an upstream section of the reference creek (Fish 1, 2, 3, 8, and 9) and four were released upstream of the SRT (Fish 4, 5, 6, and 7). Three eels had zero (Fish 1 and 3) or few detections (Fish 2) at one receiver suggesting movement of the eel to areas outside of the detection range, loss of the transmitter outside of the

detection range, or a malfunctioning transmitter. Fish 7 had a high frequency of detections at one location over time suggesting loss of the transmitter or mortality of the eel. Fish 6 had nine days of data indicating movement in the SRT creek prior to a pattern of detections suggesting mortality or loss of the transmitter data. Therefore, data recorded after day nine for Fish 6 and all data for Fish 7 were removed prior to analysis. The remaining four fish had detection data indicating movement of eels through the creeks. From the five fish for which movement data were recorded (Fish 4, 5, 6, 8, and 9) there were a total of 56 movements for analysis. More detections were recorded at the two stations upstream of the SRT than in the upstream area of the reference creek (Figure 10).

Water quality data

The tidal range as measured by the tide gauge at Wells Harbor (National Oceanic and Atmospheric Administration station ID 8419317) is 3.5 m with water levels varying from -0.24 to 3.32 m during the project period (Figure 11). Mean high tide water temperature, as measured at each receiver by day and night, varied from 8.9 to 10.3^{o}C (Figure 12). The main effect of receiver ($F_{(6, 126)}$=8.52; $p<0.0001$) and the interaction of receiver and day/night ($F_{(6, 113)}$=16.9; $p<0.0001$) were significant, but not the main effect of day/night ($F_{(1, 113)}$=10.4; $p=0.0053$). Post hoc comparisons (Tukey-Kramer HSD) of the interaction term indicated that the mean water temperature did not vary among stations during the day with the exception of R5. The upstream stations R6 and R9 were significantly colder than all other stations at night; R8 was slightly warmer than R6 and R9 and was significantly different from all other sites.

Table 13. Size and movement data for each tagged eel.

Fish ID	Length (cm)	Weight (g)	Release location	Number of detections	Number of Movements	Number of Receivers	Time between release and first movement (days)	Cumulative distance moved (km)	Maximum dispersal (km)	Movement summary
1	31.1	38.2	reference	0	ND	ND	ND	ND	ND	No detections
2	30.8	ND	reference	4	0	1	ND	0.0	0.0	Few detections, no movement
3	ND	ND	reference	0	ND	ND	ND	ND	ND	No detections
4	36.3	62.5	SRT	411	6	4	6.2	1.6	0.8	Moved downstream
5	30.9	47.3	SRT	133	9	5	0.3	2.7	1.3	Moved downstream
6	35.8	54.8	SRT	24038	4	2	7.2	1.1	0.3	Movement through 11/11, shed tag or died
7	26.5	28.7	SRT	377	0	1	38.0	0.0	0.0	Likely died or shed tag
8	34.3	ND	reference	63	9	5	0.2	4.9	1.7	Moved downstream
9	31.4	46.6	reference	108	28	5	0.3	15.4	1.7	Moved downstream

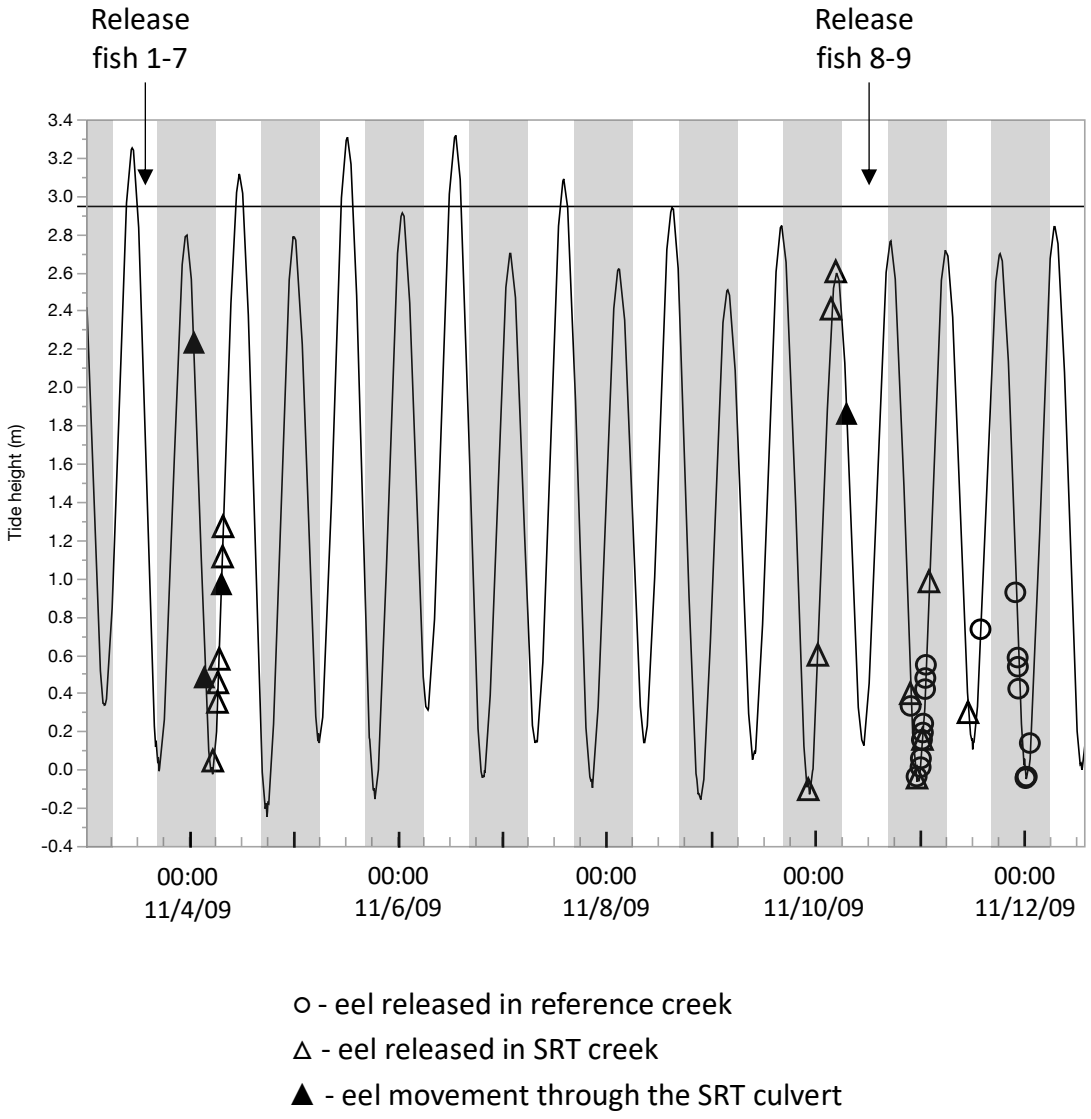

Figure 11. Fish movement and tide data for the duration of the project as measured by the tide gauge at Wells Harbor (National Oceanic and Atmospheric Administration station ID 8419317). The reference line at 2.95 m indicates the mean threshold for SRT closing. Open circles indicate movements of eels released in reference creeks. Triangles are movements of eels released above the SRT with closed triangles indicating movements of eels through the SRT. Shaded areas indicate night while unshaded areas indicate day.

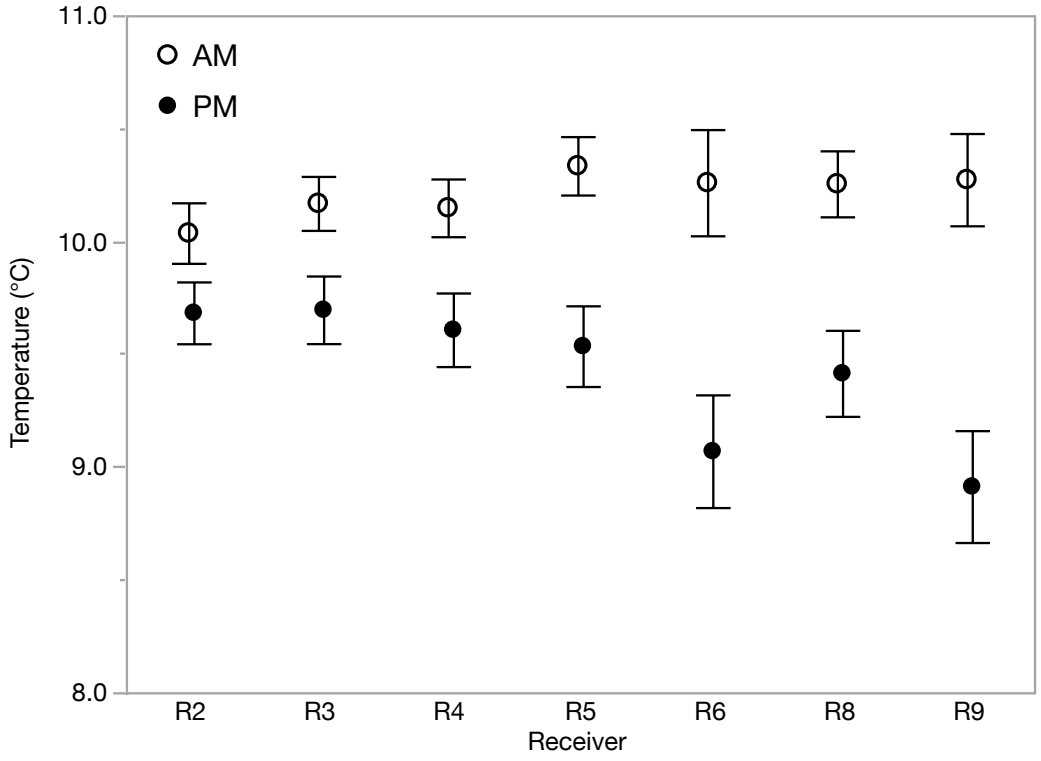

Figure 12. Mean water temperature (°C) one hour before and after high tide recorded at each receiver over the course of the project.

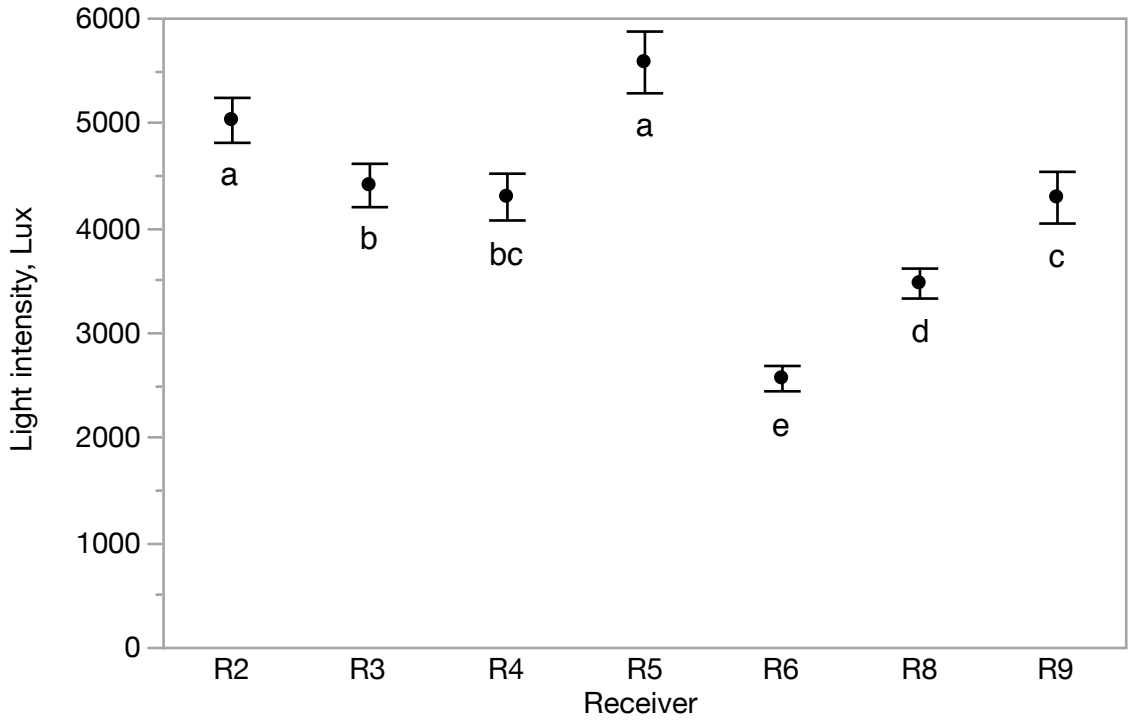

Figure 13. Mean light intensity (lux) recorded at each receiver one hour before and after the day daytime high tides that occurred over the course of the project. Untransformed data are presented. Means that share a letter are not significantly different.

Mean light intensity data for daytime high tides ranged from 2568 lux to 5582 lux across all receivers (Figure 13). The main effects of receiver ($F_{(6, 668)}$=161.9; p<0.0001), tide ($F_{(4, 668)}$=289.6; p<0.0001), and the receiver by tide interaction ($F_{(4, 668)}$=5.36; p<0.0001) were significant. Light intensity downstream of the SRT (R5) and at the entrance to the estuary (R2) recorded significantly higher light intensity than all other stations. The station upstream of the SRT (R6) and one of the upstream locations in the reference creek (R8) had significantly lower light levels from each other and all other stations.

Eel movement and space use

Onset of movement and use of space varied among eels (Table 13). Both eels that were released in the reference creek for which movement was detected moved downstream with the first low tide after release. Fish 8 moved a cumulative distance of 4.9 km with a maximum dispersal of 1.7 km. Fish 9 moved a cumulative distance of 15.4 km with a maximum dispersal also of 1.7 km. Of the three eels released upstream of the SRT for which movement data were detected, one (Fish 5) initiated movement within hours of release while the first movement for the remaining two fish occurred 6-7 days after release. Two of the fish moved through the SRT to downstream regions (Fish 4 and 5). Fish 6 remained site faithful to R6 for 7 days after release prior to making repeated movements between R6 and R7. From then on, the pattern of detections suggest that Fish 6 lost its transmitter or died shortly thereafter. The three fish released in the SRT creek moved a cumulative distance ranging from 1.1 - 1.6 km. with a maximum dispersal ranging from 0.3-0.8 km.

Ninety-three percent of eel movements (52 of 56) occurred at mid tide or below (tidal height of 1.73 m or less as measured at the Wells Harbor tide gauge; Figure 14). Fisher's Exact test showed that the eels released upstream in the SRT creek were more likely to move at high tide than eels released in the reference creek (Fisher's Exact test, $p<0.0106$). The direction of eel movement (i.e., upstream or downstream) did not vary significantly with the direction of the tide (i.e., ebb or flood).

To examine potential diel effects, the proportion of eel movements occurring in day versus night were compared to the proportion of day to night hours during the project period. Fish movement was not equally distributed, and significantly more eel movement occurred at night ($X^2_{(1, n=56)} = 17.44$, $p < .0001$). Diel effects were further explored with light intensity data.

Eighty-six percent of eel movements occurred at a light intensity of 0 lux including 1 of the 9 movements that occurred during the day. A Chi Square was used to examine the proportion of fish movements under light and dark conditions. Eels preferentially moved around the salt marsh under dark conditions ($X^2_{(1, n=56)} = 15.43$, $p < .0001$); however, the eels released upstream of the SRT were more likely to move under light conditions than those released in the reference creek (Fisher's exact test; $p < 0.0001$).

DISCUSSION

Nine eels were tagged with acoustic transmitters to evaluate the impact of environmental factors and the presence of an instream structure on eel movement in intertidal saltmarsh creeks. Eels were translocated from a nearby salt marsh; due to potential translocation effects on fish behavior (LaMothe et al. 2000) as well as small sample sizes, we cannot make conclusions based on the present limited dataset. Despite these limitations, patterns emerged regarding eel movement and space use that may guide future studies of eel movement in salt marshes, particularly those impacted by instream structures.

A general pattern exists in the literature of decreased eel activity with declining temperatures, although the reported temperature threshold at which eel activity decreases is varied (Vollestad 1986; Jellyman 1997; Baras et al. 1998). Walsh et al. (1983) found that eel oxygen consumption rate decreases below 10^{o}C as eels move toward a state of metabolic torpor. Therefore, eels in the present study may have exhibited reduced instream movements due to the relatively low water temperatures (range of 9.4-10.9 ^{o}C) for the October-December project.

Light intensity/diel effects

Eels in the present study moved preferentially under low light conditions and often at night. Strong evidence exists in the literature for eels being negatively phototaxic across life stages (Vollestad 1986; Helfman et al. 1983; Jellyman and Sykes 2003). For instance, eels in the glass eel life stage have been found to move vertically in the water column to avoid light (Bardonnet et al 2005). Furthermore, the tendency of eels to avoid light is relied upon to guide yellow eels through hydroelectric structures to minimize eel injury and mortality (Elvidge et al 2018). The nocturnal movements of eels may be due to the fact that eel foraging relies on olfaction rather than vision (Barbin 2011; Hedger et al 2010) as well as the reduced risk of predation at low light levels (Jellyman and Tsukamoto 2005).

Low light levels had a pronounced effect on eel movement in the present study with 86% of eel movements occurring in darkness of 10 lux or less. The 14% of eel movements under light conditions were all in the SRT creek and all movements but one were associated with movement through the SRT, presumably as eels sought opportunities to pass through the structure (Figure 13). The mean light levels recorded immediately upstream and downstream of the SRT represent the lowest and highest mean light levels recorded in this study, respectively, where an eel encounters a large increase in light as it moves downstream through the SRT (Figure 13). Altered light conditions in the SRT creek as well as reduced opportunities to move through the SRT may have resulted in eel movement under suboptimal conditions, possibly rendering eels more vulnerable to predation (Jellyman and Tsukamoto 2005).

Tidal effects

Evidence exists for eels in tidal systems to be more active at high tide (Dutil et al. 1988); however, eel movements in the present study were recorded under low tide conditions (93% of movements). Helfman et al. (1983) frequently captured eels at the mouths of small tributary creeks at low tide and speculated that these eels were poised to move onto the vegetated marsh when flooded. Although not explicitly tested, we suggest that the preponderance of low tide movements in the present study may be due to the fact that at higher tidal stages the vegetated marsh was flooded and eels moved onto the vegetated marsh and out of detection range. The high marsh provides abundant foraging and refuge opportunities but is limiting to most fish species due to the intermittent flooding, the shallow water depths, high density of vegetation and the risk of stranding (Kneib 1997). Eels may be well suited to exploit the resources of the vegetated marsh given that the undulatory swimming motion and narrow elongate body shape of eels affords them the ability to maneuver through the dense vegetation at any tidal stage. Furthermore, the use of primarily olfaction rather than visual cues to locate prey diminishes the negative effects of dense vegetation on foraging efforts, and the ability to breathe and move out of water minimizes the risk of stranding (Tesch 2003).

High tide movements, although uncommon, were more likely to occur with eels that were released in the SRT creek than the reference creek. Furthermore, all recorded high tide movements were associated with eels moving from upstream to downstream through the SRT or were the movements upstream of the SRT just prior to moving through it. The muted tidal influence and altered environmental conditions upstream of the SRT creek may have reduced tidal cues for movement or alternatively, higher tidal stages (as measured downstream) may present suitable conditions for eel passage through the SRT.

Eel movement and space use

Eel use of estuarine creeks, often reported as home range, is variable in the literature ranging from 0.1 to 5 km (Helfman et al 1983; Ford and Mercer 1986; Dutil et al. 1988; Crook et al 2014; Walker et al. 2014). If the maximum dispersal distance measured is used as a proxy for the home range of each eel (Walker et al. 2014), then the home ranges measured in the present study fall within the range measured in the literature. Although a small sample size precluded statistical analysis, the trend in the data was that the fish that were released above the SRT (Fish 4, 5, and 6) travelled shorter cumulative distances and had shorter maximum dispersal than those released in the reference creek.

In addition, the first movement after release was detected within 7-9 hours for two fish in the reference system and within 6-7 days after release for 2 of the 3 eels released upstream of the SRT. Although the data are insufficient to support statistical analysis, they suggest that the SRT may cause delays in eel movement downstream. Similarly, an SRT in a river in England did not prevent European silver eels from migrating downstream but did delay their movement (Wright et al 2015). High velocity water associated with the SRT may act as a physical or behavioral impediment to fish movement (Enders et al. 2009; Eberhardt et al. 2011). In addition, the high velocity water moving through the undersized culvert associated with the SRT scours out the area immediately upstream of the SRT (Eberhardt et al. 2011) creating a deep pool, conditions similar to downstream subtidal habitats.

Avenues for future research

The lack of replication and short time scale of active eel tracks precludes conclusions on eel use of these systems; however, trends in the data are interesting and may provide the foundations for future research. During the day when light intensity was high, eels were largely

inactive. At night during low tide eels were active in the marsh creeks given the high proportion of low tide movements. It is unclear what eels in the present study were doing during night high tides, but we hypothesize that eels were on the vegetated marsh feeding or seeking refuge. Future research should examine the use and value of the vegetated marsh and associated pools as foraging and refuge resources for eels.

It is clear that the SRT influences instream conditions such as light intensity, water temperature, and tidal height, and in turn, it is possible that the SRT is influencing eel use of the system. The SRT is not a total barrier to eel movement; however, the SRT may delay movement of eels to downstream areas, whether due to the presence of subtidal habitat upstream or the intermittent periods of a physical barrier. Migratory delays of silver eels due to hydropower dams in freshwater systems are well-documented (Behrmann-Godel and Eckmann 2003; Besson et al. 2016); however, the impacts of intertidal barriers such as undersized culverts and SRTs on eel movement and access to resources are unknown. The SRTs may have a negative effect on eel home range by retaining eels upstream and decreasing access to downstream areas. Furthermore, the productivity of the impounded upstream habitat for eels may be compromised due to lower quality trophic resources found there (Eberhardt et al. 2015). We suggest that future studies should evaluate the impacts of instream structures on the timing and extent of eel movements in intertidal systems.

This study provides a rudimentary understanding of eel movement in salt marsh habitats, but more importantly, provides a foundation for further study of eel movement and the possible effects of tidal barriers such as undersized culverts and SRTs on eel use of these habitats. Based on the preliminary data presented here, we recommend future evaluation of eel use of the vegetated salt marsh for foraging and refuge, and the potential impacts of temporary barriers on

upstream environmental conditions, the timing of eel movements, and the spatial extent of resources they access. Determining the factors that govern eel movement and access to resources in saltmarsh systems, as well as factors that may inhibit movement and access, will inform the management of these important landscapes to support conservation of both saltmarsh habitats and the eel population.

CONCLUSION

Summary

Laboratory and field experiments were employed to increase our understanding of American eel use of New England salt marshes. A laboratory experiment determined trophic discrimination factors and turnover rates for nitrogen and carbon isotopes ($\delta^{15}N$ and $\delta^{13}C$) of eel tissues (Chapter I). The data derived from Chapter I informed the interpretation of isotope data from eels collected in salt marshes of three different estuaries. Field data were collected from three salt marshes in the Gulf of Maine and analyzed to compare eel food sources and trophic position in tidally restricted and unrestricted salt marshes (Chapter II). Stable isotope data collected in both the laboratory and the field were analyzed to develop a nonlethal method of sampling eels for stable isotope analysis. Eel mucus and fin tissue $\delta^{15}N$ and $\delta^{13}C$ values were compared to muscle and liver tissue to explore the potential for mucus and fin to serve as nonlethal surrogates (Chapter III). To improve our understanding of eel movements within salt marsh creeks, and the potential impacts of a self-regulating tide gate on eel movement, a telemetry study was conducted in the Webhannet Estuary, ME (Chapter IV). Below are summaries of the key findings of each of the four chapters, followed by a synthesis of the findings to address the primary goals of the project, recommendations for future research, and recommendations to natural resource managers for the conservation of eels.

In Chapter I, eels were held in the laboratory and subject to a diet switch to determine the rates at which eel mucus, fin, muscle, and liver tissue assimilate the carbon and nitrogen isotope values of the diet (turnover rate) and the level of discrimination in each tissue relative to the diet (trophic discrimination factors (TDFs)). TDFs for both $\delta^{15}N$ and $\delta^{13}C$ were found to vary from

those commonly used in the literature suggesting that species and tissue specific TDFs are needed for stable isotope analysis of eels. Nitrogen turnover varied among tissues with mucus the fastest (half-life = 67 days) followed by fin (90 days), liver (97 days), and muscle tissue (191 days). Carbon turnover was slower for all tissues and exceeded the length of the experiment so turnover rates could not be calculated.

In Chapter II, eels were captured in hydrologically restricted and unrestricted creeks in three New England estuaries (Webhannet Estuary, ME, Hampton-Seabrook Estuary, NH, and Parker River Estuary, MA). Potential eel food sources as well as eel muscle and liver tissue were analyzed for $\delta^{15}N$ and $\delta^{13}C$ to determine if salt marsh derived production serves as an important energetic resource for eels and if trophic support is altered in tidally restricted creeks relative to unrestricted systems. Mixing models of isotope data indicated strong evidence of saltmarsh primary producers contributing to the basal diet of eels as well as consumption of marsh resident secondary consumers. Eel gut contents contained abundant saltmarsh secondary consumers, confirming the isotope data finding of reliance on salt marshes for trophic support, and that eels serve as top predators in salt marshes. Given that saltmarsh dietary sources were identified as important at different times scales, eels can be considered saltmarsh residents. Greater eel trophic position measured upstream of reference creeks indicates that tidal restrictions may result in an altered food web in the tidally restricted marsh. Although the experiment was not designed to explicitly test for the degree of restriction, the eels in creeks with more severe tidal restrictions (i.e., Webhannet and Hampton Seabrook estuaries) relied on more local food sources and fed at a lower trophic level.

Chapter III evaluated $\delta^{15}N$ and $\delta^{13}C$ of eel tissues to determine if eel fin or mucus samples can serve as non-lethal substitutes for liver or muscle tissue. All tissue-pair models were

significant suggesting that eel fin and mucus can serve as nonlethal surrogates for muscle and liver $\delta^{15}N$ and $\delta^{13}C$ values. Drawing on the turnover data from Chapter I, the best use of nonlethal methods will account for the relative turnover rates of each tissue. The significant relationship and similar turnover rates of mucus and liver $\delta^{15}N$ create great potential for the use of mucus as a proxy for liver $\delta^{15}N$.

Chapter IV examined eel movements in two saltmarsh creeks, one of which contained an undersized culvert with a self-regulating tide gate. Of the nine fish tagged, usable data was obtained for only five eels. The low sample size precluded the use of comprehensive statistical models on movement data; however, trends in the data provide compelling information for future research. Eel movement data suggest eels released upstream of the SRT travelled shorter distances than eels in the reference creek. Furthermore, the downstream movements of eels released upstream of the SRT delayed movements to downstream areas of the marsh relative to eels in the reference creek.

How data address the primary project goals

The first goal of this project was to improve understanding of the value of salt marsh to the life history of the American eel with respect to trophic interactions and movement. Based simply on the frequent capture of eels throughout the project in each of the three estuaries, we can first conclude that American eels are commonly found in New England salt marshes affirming earlier work (Dionne et al. 1999; Eberhardt et al. 2011). The data from Chapter II provide strong evidence of salt marsh trophic support of eels, including saltmarsh primary production as part of the basal diet as well as direct eel predation on salt marsh consumers. Because stable isotopes are indicators of the diet assimilated by the organism rather than just

what is consumed, the data provide compelling evidence that salt marshes provide important foraging resources to sustain eels. Furthermore, indicators of assimilation of saltmarsh food sources at differing time scales suggest that salt marshes serve as important habitats for eels over long time periods and eels can remain resident in these systems. Evidence in the literature points to eel capture in estuarine systems (Dionne et al. 1999; Eberhardt et al. 2011), and faster growth in brackish and marine waters (Oliveira 1999; Jessop et al. 2004; Morrison and Secor 2003), but little data existed regarding eel use of saltmarsh habitats as a food source (but see Wenner and Musik 1975). The data presented here provide new information on the saltmarsh trophic resources that are assimilated into eel tissues, indicating salt marshes can serve as critical habitats in the life history of eels.

The eel movements observed in Chapter IV also provide information on eel use of salt marshes. Eel movements occurred predominantly at night and at low tide. Evidence in the literature strongly supports the finding of movements under dark conditions (Jellyman and Sykes 2003; Bardonnet et al. 2005; Elvidge et al. 2018). However, another study in a tidal system found eel movements to occur more at high tide than low tide, although the system was a large tributary of the Gulf of St. Lawrence (Quebec, Canada) rather than a small estuary dominated by salt marsh (Dutil et al. 1988). The lack of high tide detections in this project may be due to the fact that eels moved onto the vegetated marsh with the flooding tide where they could not be detected. This hypothesis is supported by the capture of eels in marsh pools in Chapter IV. New England marsh pools and the vegetated marsh are an important foraging resource for mummichogs, *Fundulus heteroclitus*, likely due to the high abundance of grass shrimp, *Palaemonetes pugio* and the benthic microalgae on the sediment (MacKenzie and Dionne 2008). It is also possible that eels use high marsh pools as a winter refuge, as has been observed with the

resident mummichog (Smith and Able 1994). The high marsh provides abundant foraging and refuge opportunities but is limiting to most species due to the intermittent flooding, shallow water depths, high density of vegetation, and risk of stranding (Kneib 1997). American eels may be uniquely adapted to take advantage of the resources of the vegetated marsh and pools. An assessment of fish use of vegetated tidal freshwater marshes found that American eels were the 12th most abundant species using the vegetated marsh out of the 24 species captured (McIvor and Odum 1988). The sinusoidal swimming form of eels is well adapted for moving among structured components such as vegetation and may be well suited for the pursuit of prey in a habitat with a high degree of structure such as the vegetated marsh. Eels may also be efficient predators on the vegetated marsh. They may be less affected by visual obstruction of high stem density since they use olfaction as the primary method of prey detection (Barbin 2011). Therefore, creeks, vegetated marsh, and pools may serve as important foraging resources for eels in salt marshes.

The second goal of this project was to evaluate the functional equivalency of both hydrologically restricted and unrestricted salt marshes in the support of eels. Trophic position data from Chapter II indicated that eels in upstream reference regions are feeding one trophic level higher than eels in upstream restricted regions. Undersized culverts result in a lack of tidal flushing that leads to changes in vegetation as typical saltmarsh plants are replaced by invasive species such as common reed *Phragmites australis* (Roman et al. 1984; Burdick et al. 1997). The colonization of tidally restricted systems by non-native species may lead to a cascade of changes that translate to reduced trophic support for eels including changes to infaunal communities (Fell et al. 1998) and reduced densities of mummichogs (Weis and Weis 2003), a species that was identified as important prey for eels in this study. Furthermore, hydrodynamic changes

associated with restricted tidal flow (i.e., less flooding) impact sedimentation rates and types (e.g., organics, Anisfeld 1999), which in turn alters the benthic community that inhabits a site (Stocks and Grassle et al. 2003). As eels are benthic foragers, such changes can have profound effects on eel diet.

In addition to evidence of decreased trophic support upstream, tidal restrictions may also limit the movements of eels through salt marshes. The cumulative distance and maximum dispersal data measured in Chapter IV suggest that eels released above the SRT may have accessed less marsh habitat than eels in the reference system. This may be due to the muted tides decreasing the frequency and extent of marsh inundation at high tide, or to the SRT and culvert serving as a barrier between eel movement between upstream and downstream. The SRT serves as a complete barrier when the gate closes at higher high tides. However, the inhibitory effects of the SRT on eel movement may occur via mechanisms in addition to gate closures due to the presence of an undersized culvert. First, periods of high water velocity moving through undersized culverts may exceed the burst swimming capacity of eels and limit their movement between upstream and downstream areas. In addition, increased water velocity may create behavioral barriers to entering the culvert (Binder and Stevens 2004). Although windows of opportunity exist for eel passage as evidenced by the eels in Chapter IV that moved through the SRT, the SRT coupled with an undersized culvert may limit the times when eels can pass, resulting in decreased distances traveled in the marsh.

By accelerating flow through the structure, undersized culverts result in decreased fish access to upstream areas (Eberhardt et al. 2011) and due to depressed tidal regimes upstream, decreased access to the vegetated marsh. Because the vegetated marsh is hypothesized to serve as a primary foraging habitat for eels at high tidal stages, undersized culverts may restrict eel

foraging opportunities and decrease growth (Weisberg and Lotrich 1982). Therefore, upstream areas of salt marshes with culverts that restrict tides are not likely to provide the same trophic support as unrestricted marshes, as found in Chapter II. Due to the altered trophic support upstream and decreased access to resources associated with the altered tidal regime, this may lead to lower eel condition in estuaries with structures such as undersized culverts.

Another mechanism by which eel movement may be limited in tidally restricted systems is by the presence of subtidal habitat upstream. High velocity water moving through undersized culverts creates scour pools, and the muted tidal range results in standing water upstream of the culvert at all tidal stages (Adamowicz and O'Brien 2012). The presence of subtidal creek habitat upstream of the culvert and SRT at all tidal stages may limit eel movement between upstream and downstream by providing suitable foraging and refuge resources for eels at all tidal stages. In addition, the reduced tidal influence and altered environmental conditions upstream (i.e., lower light during the day and lower temperatures at night) may alter tidal cues for eel movement (Parker and McCleave 1997; Euston et al. 1998). In summary, undersized culverts may delay or inhibit eel movement to downstream areas in intertidal systems.

Recommendations for future research

An additional outcome of this project was the identification of knowledge gaps in the scientific literature, as well as the development of new hypotheses. Below is a short list of ideas for future research identified through this project.

1. Eels were frequently captured in pannes and pools, and movements in the tidal creeks during the telemetry study were not detected at high tide. The vegetated marsh provides abundant food resources and refuge, and eels are well adapted to navigate and survive the

dynamic conditions of the vegetated marsh. Future research should explore the contribution of vegetated marsh resources to eel productivity.

2. Nonlethal methods of sample collection for stable isotope analysis were developed in Chapter III and present a promising method for the noninvasive collection of large numbers of samples. Interpretation of isotope data is subject to variability due to tissue specific turnover time, discrimination, and nutrient routing. Although additional factors may affect these sources of variability, the macronutrient composition of prey is likely an important factor. To establish nonlethal stable isotope collection methods developed here, future studies should identify turnover rates and trophic discrimination factors for eel tissues across diets of varying macronutrient compositions. Furthermore, compound specific isotope analysis (CSIA), that is, the analysis of the incorporation of specific amino acids or fatty acids from diet to tissue, presents a promising method to identify dietary sources with less potential variability than bulk tissue analysis. Bulk tissue or CSIA projects should be planned for multiple months to account for the slow growth rate of yellow eels so that turnover estimates for both $\delta^{15}N$ and $\delta^{13}C$ can be calculated. If resources are limited, projects should focus on developing these data for mucus given that source material is very abundant in eels, mucus allows for repeated sampling of the same individual over time, mucus has a fast turnover rate, and it is a low impact method that presents minimal risk to the eel, an important consideration for species at risk such as eels.
3. Given that intermittent barriers in salt marshes such as undersized culverts and SRTs result in altered trophic support and may inhibit movement of eels, future research should confirm if these instream structures alter environmental cues or create barriers to eel

movement. In particular, the impact of these intermittent barriers on glass eel, elver, and silver eel life stages should be evaluated.

4. Based on the data presented here, eels relied heavily on salt marsh derived production over time. Eels are panmictic and all migrate to the Sargasso Sea, a nutrient poor area of the open ocean, to spawn. Future research should examine the contribution of salt marsh production to Sargasso Sea food webs.

Management Implications

American eels can live in tidal systems throughout their residence in inshore habitats (Tsuakamoto and Arai 2001; Tsukamoto et al. 2002). Estuarine habitats may support enhanced eel productivity relative to freshwater systems (Tsukamoto and Arai 2001) due to the favorable growth conditions (Oliveira 1999, Jessop et al. 2004; Morrison and Secor 2003) and trophic support (this study) found there. Given the clear importance of saltmarsh primary and secondary production to diets of eels inhabiting salt marshes, habitat functional loss through filling, dredging, and tidal restrictions has resulted in a loss of trophic resources for eels. In New England, 37% of salt marshes have been lost over the last 200 years and 20% of the remaining acreage is tidally restricted (Bromberg and Bertness 2005). The cumulative impact of marsh loss may be a contributing factor in the decline of eel populations. As a result, conservation of saltmarsh habitat and maintenance of saltmarsh ecological functions is important for eels.

Removal of instream barriers, including undersized culverts will allow for uninterrupted movement of eels and other nekton. Where full restoration of the tidal prism is not feasible, such as with the SRT in the Webhannet Estuary, saltmarsh trophic resources may not be functionally equivalent to reference systems, but as much tidal flushing as is possible should be restored.

Further, options to provide upstream and downstream passage for eels and other nekton should be explored to allow for unrestricted movement through the system. The increasing frequency of storms and rising seas as a result of climate change (Kossin et al. 2017) results in more water moving through these systems. When developing projects to restore tidal hydrology to salt marshes, projects should design structures to accommodate future scenarios of larger volumes of water in the system. In addition, vegetated buffers should be maintained around salt marshes to allow for saltmarsh migration with rising seas to support the persistence of marsh habitat and American eels. Vegetated buffers upland of salt marshes, as opposed to hardened shorelines, will also maintain present habitat quality to support high densities of eels (Itakura et al 2015) and their prey (Balouskus and Targett 2012). Conservation and restoration of salt marsh as habitat and management of marshes to maintain ecological integrity will provide critical trophic support and access to resources for the American eel population.

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APPENDICES

Appendix A

Bulk carbon and nitrogen isotope measurements of muscle, liver, fin, and mucus samples from American eels held in the laboratory (Chapters 1 and 3).

Identifier	Days since diet switch	Tissue	Initial mass (g)	Final mass (g)	Length (cm)	$\delta^{15}N$	N%	$\delta^{13}C$	C%	C/N
0154L	0	liver	90.7	83.0	38.3	12.3	9.6	-22.3	46.0	4.8
0156L	0	liver	107.7	101.0	41.2	12.5	10.3	-20.6	44.8	4.3
0159L	0	liver	121.2	113.6	38.1	12.6	9.5	-21.7	47.1	5.0
0169L	0	liver	74.7	74.7	35.0	12.5	7.8	-22.0	45.2	5.8
0170L	0	liver	92.5	92.5	38.6	12.4	9.3	-23.2	46.9	5.1
0171L	0	liver	129.0	129.0	43.1	12.1	11.0	-22.5	47.6	4.3
0172L	0	liver	64.2	64.2	33.9	13.6	9.5	-21.8	42.1	4.5
0173L	0	liver	86.8	86.8	37.9	12.2	10.1	-21.7	46.5	4.6
0146L	1	liver	59.6	55.5	34.1	12.5	7.8	-23.9	48.6	6.2
0147L	1	liver	59.8	52.7	29.6	12.6	8.4	-22.3	47.0	5.6
0157L	1	liver	73.3	67.9	34.4	15.7	7.9	-22.1	44.6	5.7
0158L	1	liver	128.8	112.9	40.9	12.6	9.6	-22.2	48.1	5.0
0160L	1	liver	130.7	119.0	40.8	12.7	10.6	-21.4	46.2	4.4
0149L	4	liver	66.4	58.5	34.4	13.2	11.0	-21.0	41.6	3.8
0150L	4	liver	83.5	75.5	37.4	32.4	10.8	-21.1	47.1	4.4
0151L	4	liver	98.6	85.3	36.1	12.1	10.0	-22.6	45.8	4.6
0152L	4	liver	95.1	85.1	41.4	13.8	8.7	-21.5	48.4	5.6
0153L	4	liver	127.7	115.9	38.5	11.9	10.6	-21.3	45.7	4.3
0133L	7	liver	79.8	71.8	36.5	12.9	8.2	-22.0	39.7	4.8
0134L	7	liver	98.4	90.2	34.8	12.8	9.5	-21.8	43.4	4.6
0135L	7	liver	60.9	54.8	32.4	23.8	10.2	-23.3	52.1	5.1
0144L	7	liver	96.2	86.9	39.2	12.8	11.4	-20.2	45.1	4.0
0145L	7	liver	65.7	57.8	34.3	31.8	5.8	-23.4	50.1	8.7
0132L	11	liver	82.4	72.8	36.1	23.8	9.7	-22.2	45.1	4.6
0136L	11	liver	51.6	42.7	34.3	13.1	10.4	-20.5	44.1	4.3
0137L	11	liver	82.8	72.7	37.2	12.8	8.7	-21.6	47.0	5.4
0143L	11	liver	137.5	122.7	41.3	12.8	8.2	-22.4	48.6	6.0
0148L	11	liver	66.5	60.2	35.6	148.3	7.5	-19.9	41.3	5.5
0120L	14	liver	79.4	73.6	34.4	58.6	7.5	-22.1	43.8	5.8
0122L	14	liver	112.6	100.9	42.0	14.2	5.4	-23.4	47.2	8.7
0128L	14	liver	59.2	50.8	31.5	13.2	7.5	-23.2	51.7	6.9

0167L	14	liver	121.4	111.3	41.5	13.9	6.2	-23.7	56.9	9.2
0168L	14	liver	122.6	105.8	40.5	13.1	5.9	-23.4	55.4	9.4
0138L	17	liver	111.1	91.8	42.2	13.5	8.0	-21.7	46.6	5.8
0139L	17	liver	101.4	99.0	46.3	13.1	9.1	-22.2	48.1	5.3
0140L	17	liver	89.9	78.0	37.3	43.0	7.5	-22.4	48.5	6.5
0141L	17	liver	51.0	42.1	29.4	26.6	6.5	-22.8	44.4	6.8
0142L	17	liver	72.0	64.6	34.7	147.3	5.7	-22.9	49.6	8.7
0124L	21	liver	80.4	70.3	36.7	22.4	6.3	-22.8	54.8	8.7
0125L	21	liver	73.2	59.2	36.1	12.7	8.3	-22.1	48.1	5.8
0126L	21	liver	121.6	106.6	41.3	21.8	7.4	-22.7	45.1	6.1
0130L	21	liver	80.0	68.6	36.4	21.3	9.5	-22.9	47.1	5.0
0164L	21	liver	63.5	52.8	33.1	13.6	6.5	-22.9	53.8	8.3
0121L	28	liver	105.2	92.7	37.0	12.6	8.8	-21.7	45.0	5.1
0123L	28	liver	113.5	88.7	40.6	14.9	8.0	-22.3	48.6	6.1
0127L	28	liver	96.7	82.1	36.8	53.3	7.4	-22.3	50.6	6.9
0129L	28	liver	125.4	113.2	45.5	13.4	8.8	-21.0	45.0	5.1
0131L	28	liver	81.6	75.1	36.1	158.1	9.3	-20.5	46.7	5.0
0161L	35	liver	110.2	93.0	39.5	279.9	5.3	-23.0	55.3	10.4
0162L	35	liver	78.9	68.2	34.8	184.6	7.3	-22.0	49.2	6.8
0163L	35	liver	115.1	99.0	43.7	248.4	6.0	-22.1	54.2	9.0
0165L	35	liver	74.6	66.1	37.0	76.0	8.8	-21.0	47.4	5.4
0166L	35	liver	80.8	68.5	36.0	224.6	8.9	-20.0	46.0	5.2
0154S	0	mucus	90.7	83.0	38.3	12.1	11.9	-21.1	43.5	3.7
0155S	0	mucus	102.2	102.7	41.0	12.5	12.1	-20.9	42.7	3.5
0156S	0	mucus	107.7	101.0	41.2	11.7	11.9	-19.3	43.6	3.7
0159S	0	mucus	121.2	113.6	38.1	12.1	12.2	-20.3	44.7	3.7
0169S	0	mucus	74.7	74.7	35.0	11.6	11.2	-20.3	43.6	3.9
0170S	0	mucus	92.5	92.5	38.6	11.9	11.6	-23.4	42.1	3.6
0171S	0	mucus	129.0	129.0	43.1	11.8	12.5	-22.2	45.1	3.6
0172S	0	mucus	64.2	64.2	33.9	12.1	12.4	-20.7	44.9	3.6
0173S	0	mucus	86.8	86.8	37.9	11.8	10.6	-20.6	38.3	3.6
0146S	1	mucus	59.6	55.5	34.1	11.9	10.6	-22.2	37.9	3.6
0147S	1	mucus	59.8	52.7	29.6	12.0	11.2	-20.7	39.6	3.5
0157S	1	mucus	73.3	67.9	34.4	13.3	11.4	-20.7	42.5	3.7
0158S	1	mucus	128.8	112.9	40.9	11.9	12.0	-20.8	43.6	3.6
0160S	1	mucus	130.7	119.0	40.8	12.4	10.1	-21.3	49.5	4.9
0149S	4	mucus	66.4	58.5	34.4	12.1	11.7	-20.7	42.4	3.6
0150S	4	mucus	83.5	75.5	37.4	18.9	11.6	-20.0	41.4	3.6
0151S	4	mucus	98.6	85.3	36.1	11.8	11.6	-21.6	42.1	3.6
0152S	4	mucus	95.1	85.1	41.4	12.7	11.2	-19.5	42.0	3.8

0153S	4	mucus	127.7	115.9	38.5	12.0	11.6	-19.8	43.2	3.7
0133S	7	mucus	79.8	71.8	36.5	12.2	11.9	-20.4	44.7	3.8
0134S	7	mucus	98.4	90.2	34.8	12.2	12.3	-20.0	42.7	3.5
0135S	7	mucus	60.9	54.8	32.4	22.0	11.1	-21.8	40.5	3.6
0144S	7	mucus	96.2	86.9	39.2	12.8	12.3	-19.1	43.9	3.6
0145S	7	mucus	65.7	57.8	34.3	22.4	10.2	-20.3	36.9	3.6
0132S	11	mucus	82.4	72.8	36.1	18.9	11.3	-20.9	39.7	3.5
0136S	11	mucus	51.6	42.7	34.3	12.5	12.2	-19.6	44.5	3.6
0137S	11	mucus	82.8	72.7	37.2	12.4	12.2	-19.9	44.3	3.6
0143S	11	mucus	137.5	122.7	41.3	12.8	10.7	-20.3	39.4	3.7
0120S	14	mucus	79.4	73.6	34.4	55.2	12.0	-20.1	41.9	3.5
0122S	14	mucus	112.6	100.9	42.0	13.2	9.4	-20.2	32.1	3.4
0128S	14	mucus	59.2	50.8	31.5	12.5	12.2	-20.3	44.2	3.6
0167S	14	mucus	121.4	111.3	41.5	12.6	11.0	-20.5	41.5	3.8
0168S	14	mucus	122.6	105.8	40.5	12.7	11.8	-19.9	43.5	3.7
0138S	17	mucus	111.1	91.8	42.2	13.0	12.3	-19.5	45.2	3.7
0139S	17	mucus	101.4	99.0	46.3	12.7	12.0	-20.4	45.3	3.8
0140S	17	mucus	89.9	78.0	37.3	39.1	11.5	-20.0	40.8	3.6
0141S	17	mucus	51.0	42.1	29.4	27.0	11.3	-20.4	41.5	3.7
0142S	17	mucus	72.0	64.6	34.7	88.1	11.4	-20.7	43.4	3.8
0124S	21	mucus	80.4	70.3	36.7	18.4	11.4	-19.6	43.4	3.8
0125S	21	mucus	73.2	59.2	36.1	12.4	12.0	-20.0	43.8	3.7
0126S	21	mucus	121.6	106.6	41.3	19.2	11.2	-20.6	39.6	3.6
0130S	21	mucus	80.0	68.6	36.4	18.6	15.3	-21.6	55.0	3.6
0164S	21	mucus	63.5	52.8	33.1	13.0	11.3	-20.3	43.0	3.8
0121S	28	mucus	105.2	92.7	37.0	12.2	11.3	-20.0	40.1	3.6
0123S	28	mucus	80.4	70.3	36.7	13.4	11.6	-20.1	41.7	3.6
0127S	28	mucus	96.7	82.1	36.8	38.6	12.2	-19.8	43.9	3.6
0129S	28	mucus	125.4	113.2	45.5	12.5	12.0	-19.6	42.5	3.6
0131S	28	mucus	81.6	75.1	36.1	110.4	11.9	-19.9	43.1	3.6
0161S	35	mucus	110.2	93.0	39.5	130.8	11.5	-19.9	43.7	3.8
0162S	35	mucus	78.9	68.2	34.8	121.3	10.5	-20.2	39.7	3.8
0163S	35	mucus	115.1	99.0	43.7	127.7	10.7	-19.5	43.2	4.1
0165S	35	mucus	74.6	66.1	37.0	35.6	10.6	-19.9	40.9	3.9
0166S	35	mucus	80.8	68.5	36.0	121.2	11.9	-19.2	44.1	3.7
0154M	0	muscle	90.7	83.0	38.3	11.8	10.6	-23.0	49.8	4.7
0155M	0	muscle	102.2	102.7	41.0	12.4	10.9	-21.7	44.8	4.1
0156M	0	muscle	107.7	101.0	41.2	11.0	11.1	-20.1	48.2	4.3
0159M	0	muscle	121.2	113.6	38.1	11.8	9.0	-22.2	53.8	6.0
0169M	0	muscle	74.7	74.7	35.0	11.4	12.0	-20.5	47.3	4.0

0170M	0	muscle	92.5	92.5	38.6	11.2	11.5	-24.5	48.1	4.2
0171M	0	muscle	129.0	129.0	43.1	10.7	11.6	-23.5	46.2	4.0
0172M	0	muscle	64.2	64.2	33.9	11.6	12.1	-21.7	50.5	4.2
0173M	0	muscle	86.8	86.8	37.9	11.7	11.2	-21.2	46.8	4.2
0146M	1	muscle	59.6	55.5	34.1	10.9	11.2	-24.1	46.6	4.2
0147M	1	muscle	59.8	52.7	29.6	11.9	9.6	-21.9	42.6	4.4
0157M	1	muscle	73.3	67.9	34.4	12.2	9.0	-22.6	49.8	5.6
0158M	1	muscle	128.8	112.9	40.9	11.9	11.0	-21.9	48.8	4.5
0160M	1	muscle	130.7	119.0	40.8	11.6	9.3	-21.6	44.6	4.8
0149M	4	muscle	66.4	58.5	34.4	11.6	10.6	-22.2	46.5	4.4
0150M	4	muscle	83.5	75.5	37.4	11.8	9.5	-21.7	46.5	4.9
0151M	4	muscle	98.6	85.3	36.1	10.2	11.5	-23.5	46.7	4.1
0152M	4	muscle	95.1	85.1	41.4	11.9	12.1	-19.8	46.1	3.8
0153M	4	muscle	127.7	115.9	38.5	11.4	9.7	-21.3	51.4	5.3
0133M	7	muscle	79.8	71.8	36.5	11.5	5.7	-22.3	28.6	5.0
0134M	7	muscle	98.4	90.2	34.8	11.7	6.8	-23.3	53.3	7.8
0135M	7	muscle	60.9	54.8	32.4	10.9	10.1	-24.5	52.9	5.2
0144M	7	muscle	96.2	86.9	39.2	12.3	11.6	-20.1	47.9	4.1
0145M	7	muscle	65.7	57.8	34.3	11.9	11.1	-22.0	41.0	3.7
0132M	11	muscle	82.4	72.8	36.1	12.8	9.2	-22.9	44.7	4.9
0136M	11	muscle	51.6	42.7	34.3	10.9	11.8	-20.0	44.3	3.7
0137M	11	muscle	82.8	72.7	37.2	10.9	10.7	-21.3	44.8	4.2
0143M	11	muscle	137.5	122.7	41.3	11.6	9.3	-22.0	49.8	5.4
0148M	11	muscle	66.5	60.2	35.6	23.9	10.4	-21.1	48.3	4.7
0120M	14	muscle	79.4	73.6	34.4	15.7	10.2	-21.4	43.0	4.2
0122M	14	muscle	112.6	100.9	42.0	12.0	8.9	-22.0	44.3	5.0
0128M	14	muscle	59.2	50.8	31.5	11.9	11.3	-22.1	45.9	4.1
0167M	14	muscle	121.4	111.3	41.5	11.4	10.0	-22.0	47.0	4.7
0168M	14	muscle	122.6	105.8	40.5	11.9	7.8	-22.7	53.2	6.9
0138M	17	muscle	111.1	91.8	42.2	11.1	11.0	-20.0	43.6	4.0
0139M	17	muscle	101.4	99.0	46.3	11.5	12.3	-20.8	45.4	3.7
0140M	17	muscle	89.9	78.0	37.3	12.7	7.5	-22.7	46.7	6.2
0141M	17	muscle	51.0	42.1	29.4	12.5	9.2	-22.2	39.2	4.2
0142M	17	muscle	72.0	64.6	34.7	22.9	8.8	-22.7	43.1	4.9
0124M	21	muscle	80.4	70.3	36.7	11.1	11.1	-20.0	46.2	4.2
0125M	21	muscle	73.2	59.2	36.1	11.8	10.8	-21.6	50.3	4.7
0126M	21	muscle	121.6	106.6	41.3	12.4	10.5	-22.5	48.6	4.6
0130M	21	muscle	80.0	68.6	36.4	11.2	11.6	-23.4	46.9	4.1
0164M	21	muscle	63.5	52.8	33.1	11.8	10.1	-21.8	44.2	4.4
0121M	28	muscle	105.2	92.7	37.0	11.5	9.6	-21.0	43.5	4.5

0123M	28	muscle	113.5	88.7	40.6	11.6	8.3	-21.6	35.2	4.2
0127M	28	muscle	96.7	82.1	36.8	15.2	8.8	-22.2	49.6	5.6
0129M	28	muscle	125.4	113.2	45.5	11.6	11.0	-20.6	45.5	4.1
0131M	28	muscle	81.6	75.1	36.1	27.0	10.4	-21.7	46.4	4.5
0161M	35	muscle	110.2	93.0	39.5	39.9	10.1	-21.4	44.1	4.4
0162M	35	muscle	78.9	68.2	34.8	28.5	9.6	-22.7	49.9	5.2
0163M	35	muscle	115.1	99.0	43.7	31.4	11.7	-20.7	49.5	4.2
0165M	35	muscle	74.6	66.1	37.0	14.6	7.1	-22.3	50.3	7.3
0166M	35	muscle	80.8	68.5	36.0	27.3	10.8	-20.7	48.1	4.5
0154F	0	fin	90.7	83.0	38.3	12.5	10.0	-21.4	37.9	3.8
0155F	0	fin	102.2	102.7	41.0	14.2	9.7	-21.6	39.8	4.1
0156F	0	fin	107.7	101.0	41.2	11.5	7.6	-20.2	45.6	6.0
0159F	0	fin	121.2	113.6	38.1	12.1	6.6	-22.5	54.0	8.2
0169F	0	fin	74.7	74.7	35.0	12.0	9.9	-20.2	40.9	4.1
0170F	0	fin	92.5	92.5	38.6	11.7	8.0	-25.0	43.7	5.5
0171F	0	fin	129.0	129.0	43.1	11.0	7.3	-24.2	47.1	6.5
0172F	0	fin	64.2	64.2	33.9	12.6	8.9	-21.4	42.2	4.8
0173F	0	fin	86.8	86.8	37.9	12.0	9.5	-20.9	43.6	4.6
0146F	1	fin	59.6	55.5	34.1	11.5	7.2	-24.4	36.9	5.4
0147F	1	fin	59.8	52.7	29.6	12.4	8.2	-21.8	43.5	5.3
0157F	1	fin	73.3	67.9	34.4	13.4	10.1	-21.3	39.2	3.9
0158F	1	fin	128.8	112.9	40.9	12.4	7.7	-22.4	49.0	6.4
0160F	1	fin	130.7	119.0	40.8	12.4	10.3	-20.7	45.0	4.4
0149F	4	fin	66.4	58.5	34.4	12.2	9.1	-21.6	37.0	4.1
0150F	4	fin	83.5	75.5	37.4	17.8	8.3	-20.6	39.1	4.7
0151F	4	fin	98.6	85.3	36.1	10.9	9.0	-23.2	49.0	5.4
0152F	4	fin	95.1	85.1	41.4	13.3	10.2	-18.0	38.1	3.7
0153F	4	fin	127.7	115.9	38.5	11.8	9.2	-20.5	43.9	4.8
0133F	7	fin	79.8	71.8	36.5	12.3	7.4	-21.9	42.0	5.7
0134F	7	fin	98.4	90.2	34.8	12.4	6.8	-22.8	49.4	7.3
0135F	7	fin	60.9	54.8	32.4	17.2	7.9	-24.3	44.9	5.7
0144F	7	fin	96.2	86.9	39.2	13.0	9.8	-19.6	39.1	4.0
0145F	7	fin	65.7	57.8	34.3	15.6	7.9	-22.2	42.0	5.3
0136F	11	fin	51.6	42.7	34.3	11.7	9.0	-18.6	34.8	3.9
0137F	11	fin	82.8	72.7	37.2	11.5	7.7	-21.8	47.4	6.2
0143F	11	fin	137.5	122.7	41.3	12.7	10.1	-20.1	41.1	4.1
0148F	11	fin	66.5	60.2	35.6	45.4	5.4	-21.0	28.4	5.3
0120F	14	fin	79.4	73.6	34.4	38.8	8.9	-20.7	40.8	4.6
0122F	14	fin	112.6	100.9	42.0	13.3	6.2	-21.5	36.7	5.9
0128F	14	fin	59.2	50.8	31.5	12.7	8.0	-22.8	45.6	5.7

0167F	14	fin	121.4	111.3	41.5	12.5	7.4	-22.4	47.2	7.8
0168F	14	fin	122.6	105.8	40.5	12.6	9.6	-20.7	44.9	4.7
0138F	17	fin	111.1	91.8	42.2	11.0	8.7	-17.7	37.2	4.3
0139F	17	fin	101.4	99.0	46.3	12.8	8.8	-21.3	45.3	5.2
0140F	17	fin	89.9	78.0	37.3	27.6	11.7	-19.7	41.8	3.6
0141F	17	fin	51.0	42.1	29.4	24.0	8.8	-21.7	44.0	5.0
0142F	17	fin	72.0	64.6	34.7	40.8	6.8	-23.0	43.6	6.4
0124F	21	fin	80.4	70.3	36.7	16.3	8.4	-20.5	46.3	5.5
0125F	21	fin	73.2	59.2	36.1	12.2	9.4	-20.6	44.7	4.8
0126F	21	fin	121.6	106.6	41.3	15.5	7.1	-22.8	50.3	7.1
0130F	21	fin	80.0	68.6	36.4	13.0	7.9	-24.7	48.4	6.1
0164F	21	fin	63.5	52.8	33.1	12.8	8.4	-21.6	45.1	5.6
0121F	28	fin	105.2	92.7	37.0	12.1	8.5	-20.7	41.5	4.9
0123F	28	fin	113.5	88.7	40.6	13.2	8.9	-21.3	48.5	5.4
0127F	28	fin	96.7	82.1	36.8	23.8	8.6	-21.0	43.0	5.0
0129F	28	fin	125.4	113.2	45.5	12.3	7.1	-21.2	45.2	6.4
0131F	28	fin	81.6	75.1	36.1	84.1	9.7	-19.2	35.7	3.7
0161F	35	fin	110.2	93.0	39.5	94.7	9.3	-20.5	41.4	4.5
0162F	35	fin	78.9	68.2	34.8	98.1	7.5	-22.4	45.0	6.0
0163F	35	fin	115.1	99.0	43.7	84.0	9.0	-20.1	43.2	4.8
0165F	35	fin	74.6	66.1	37.0	24.4	7.7	-21.0	43.5	6.1
0166F	35	fin	80.8	68.5	36.0	46.7	8.0	-20.8	44.0	5.5

Appendix B

Institutional Animal Care and Use Approval Letter

University *of* New Hampshire

Research Conduct and Compliance Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

01-Aug-2007

Burdick, David M
Natural Resources
Jackson Lab
Durham, NH 03824

IACUC #: 070702
Project: The Role of the American Eel, Anguilla rostrata, and the Mummichog, Fundulus Heteroclitus, in Saltmarsh Trophic Dynamics: Implications for Intertidal Resource Management
Category: C
Approval Date: 13-Jul-2007

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Roger Wells at 862-2726 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File
Alison Eberhardt

Appendix C

Bulk carbon and nitrogen isotope measurements of muscle, liver, fin, and mucus samples from American eels collected from salt marshes in three New England estuaries: the Webhannet Estuary, ME (WEB), the Hampton-Seabrook Estuary, NH (HSE), and the Parker River Estuary, MA (PRE; Chapters 2 and 3).

Fish ID	Size (mm)	Site	Location	Hydrology	Tissue type	%N	$\delta^{15}N$	%C	$\delta^{13}C$	C/N
1000F	26.3	WEB	upstream	reference	fin	9.4	11.4	42.1	-17.0	4.5
1000L	26.3	WEB	upstream	reference	liver	10.7	11.3	44.8	-16.5	4.2
1000M	26.3	WEB	upstream	reference	muscle	11.6	11.0	41.4	-16.8	3.6
1000S	26.3	WEB	upstream	reference	mucus	10.0	11.0	38.6	-15.9	3.9
1001F	27.2	WEB	upstream	restricted	fin	10.3	8.3	41.5	-13.4	4.0
1001L	27.2	WEB	upstream	restricted	liver	11.6	8.3	48.1	-14.1	4.2
1001M	27.2	WEB	upstream	restricted	muscle	12.6	8.0	45.3	-14.2	3.6
1001S	27.2	WEB	upstream	restricted	mucus	10.7	7.9	40.2	-13.3	3.7
1002F	17.9	WEB	upstream	restricted	fin	9.9	9.5	48.0	-15.3	4.9
1002L	17.9	WEB	upstream	restricted	liver	10.2	10.0	52.2	-16.0	5.1
1002M	17.9	WEB	upstream	restricted	muscle	12.1	9.3	51.5	-15.8	4.3
1002S	17.9	WEB	upstream	restricted	mucus	13.9	9.5	50.7	-14.3	3.6
1003F	15.7	WEB	upstream	reference	fin	9.8	10.8	44.8	-21.3	4.6
1003L	15.7	WEB	upstream	reference	liver	10.4	11.2	47.1	-21.6	4.5
1003M	15.7	WEB	upstream	reference	muscle	12.4	11.2	47.5	-21.0	3.8
1003S	15.7	WEB	upstream	reference	mucus	11.5	10.5	43.5	-20.5	3.8
1004F	22.0	WEB	upstream	reference	fin	10.9	8.0	48.3	-14.4	4.4
1004L	22.0	WEB	upstream	reference	liver	8.0	8.9	47.2	-14.6	5.9
1004M	22.0	WEB	upstream	reference	muscle	12.5	7.3	45.7	-13.9	3.7
1004S	22.0	WEB	upstream	reference	mucus	9.0	7.9	34.3	-13.6	3.8
1005F	19.4	WEB	downstream	restricted	fin	11.0	6.5	43.4	-15.4	3.9
1005L	19.4	WEB	downstream	restricted	liver	11.8	6.8	50.0	-15.5	4.3
1005M	19.4	WEB	downstream	restricted	muscle	13.6	6.5	45.9	-15.6	3.4
1005S	19.4	WEB	downstream	restricted	mucus	12.3	6.7	45.0	-15.6	3.7
1006F	15.9	WEB	downstream	restricted	fin	11.1	6.8	43.0	-15.2	3.9
1006L	15.9	WEB	downstream	restricted	liver	9.7	6.4	44.9	-15.7	4.6
1006M	15.9	WEB	downstream	restricted	muscle	13.4	7.3	44.4	-15.6	3.3
1006S	15.9	WEB	downstream	restricted	mucus	12.1	7.5	43.2	-15.3	3.6
1007F	17	WEB	downstream	reference	fin	10.3	8.8	41.9	-16.3	4.1

1007L	17	WEB	downstream	reference	liver	9.2	10.2	42.1	-17.4	4.6
1007M	17	WEB	downstream	reference	muscle	14.5	8.8	51.8	-16.2	3.6
1007S	17	WEB	downstream	reference	mucus	11.8	9.7	44.5	-16.0	3.8
1008F	16.8	WEB	upstream	restricted	fin	10.5	10.2	42.0	-14.9	4.0
1008L	16.8	WEB	upstream	restricted	liver	11.0	8.7	43.4	-15.2	3.9
1008M	16.8	WEB	upstream	restricted	muscle	8.1	8.6	27.2	-14.6	3.4
1008S	16.8	WEB	upstream	restricted	mucus	11.5	8.5	42.7	-14.7	3.7
1009F	18.8	WEB	upstream	restricted	fin	9.0	9.2	40.1	-20.0	4.5
1009L	18.8	WEB	upstream	restricted	liver	9.6	9.7	42.5	-20.3	4.4
1009M	18.8	WEB	upstream	restricted	muscle	12.2	9.1	43.5	-18.5	3.6
1009S	18.8	WEB	upstream	restricted	mucus	11.7	9.3	42.0	-19.0	3.6
1010F	17.1	WEB	upstream	restricted	fin	10.6	8.9	42.9	-16.0	4.1
1010L	17.1	WEB	upstream	restricted	liver	10.7	9.5	42.3	-16.5	4.0
1010M	17.1	WEB	upstream	restricted	muscle	12.7	8.7	44.0	-17.4	3.5
1010S	17.1	WEB	upstream	restricted	mucus	12.8	8.7	45.8	-16.1	3.6
1011F	18.1	WEB	upstream	restricted	fin	9.7	8.6	40.5	-15.2	4.2
1011L	18.1	WEB	upstream	restricted	liver	10.9	9.2	44.3	-15.4	4.1
1011M	18.1	WEB	upstream	restricted	muscle	12.3	8.9	45.5	-15.5	3.7
1011S	18.1	WEB	upstream	restricted	mucus	11.7	8.4	42.7	-15.0	3.7
1012F	10.3	WEB	downstream	restricted	fin	10.9	8.5	50.3	-20.1	4.6
1012L	10.3	WEB	downstream	restricted	liver	7.8	8.8	55.2	-22.2	7.0
1012S	10.3	WEB	downstream	restricted	mucus	11.2	8.2	45.5	-19.6	4.1
1013F	18.1	HSE	downstream	restricted	fin	11.3	8.0	45.6	-15.2	4.0
1013L	18.1	HSE	downstream	restricted	liver	10.3	8.1	47.5	-15.4	4.6
1013M	18.1	HSE	downstream	restricted	muscle	12.4	7.6	43.7	-15.3	3.5
1013S	18.1	HSE	downstream	restricted	mucus	10.9	7.9	41.0	-15.0	3.8
1014F	53.5	HSE	downstream	restricted	fin	10.4	11.3	39.1	-15.6	3.7
1014L	53.5	HSE	downstream	restricted	liver	11.4	11.2	46.2	-16.2	4.0
1014M	53.5	HSE	downstream	restricted	muscle	12.1	10.3	44.5	-15.9	3.7
1014S	53.5	HSE	downstream	restricted	mucus	11.8	10.9	44.5	-16.3	3.8
1015F	51.6	HSE	downstream	restricted	fin	11.1	11.2	43.1	-13.3	3.9
1015L	51.6	HSE	downstream	restricted	liver	9.6	10.5	41.0	-13.9	4.3
1015M	51.6	HSE	downstream	restricted	muscle	10.5	10.2	39.8	-14.8	3.8
1015S	51.6	HSE	downstream	restricted	mucus	10.2	10.3	41.3	-14.4	4.0
1016F	27.7	HSE	upstream	restricted	fin	7.3	9.5	30.7	-20.1	4.2
1016L	27.7	HSE	upstream	restricted	liver	12.2	9.4	50.3	-19.2	4.1
1016M	27.7	HSE	upstream	restricted	muscle	12.0	9.2	40.7	-20.1	3.4
1016S	27.7	HSE	upstream	restricted	mucus	10.5	8.9	38.5	-19.4	3.7

1017F	30.7	HSE	upstream	restricted	fin	8.6	10.5	47.4	-19.2	5.5
1017L	30.7	HSE	upstream	restricted	liver	9.7	10.4	39.7	-18.0	4.1
1017M	30.7	HSE	upstream	restricted	muscle	11.5	10.2	50.0	-19.4	4.4
1017S	30.7	HSE	upstream	restricted	mucus	11.9	10.3	44.2	-17.5	3.7
1018F	30.7	HSE	upstream	restricted	fin	8.2	10.3	47.9	-19.5	5.9
1018L	30.7	HSE	upstream	restricted	liver	10.0	9.9	42.0	-18.0	4.2
1018M	30.7	HSE	upstream	restricted	muscle	11.3	9.6	45.1	-19.5	4.0
1018S	30.7	HSE	upstream	restricted	mucus	11.1	9.7	43.1	-18.0	3.9
1019F	20.3	HSE	upstream	restricted	fin	10.9	8.7	41.7	-21.9	3.8
1019L	20.3	HSE	upstream	restricted	liver	10.9	9.2	49.8	-22.7	4.6
1019M	20.3	HSE	upstream	restricted	muscle	13.0	8.6	45.1	-22.3	3.5
1019S	20.3	HSE	upstream	restricted	mucus	12.5	8.3	45.5	-21.6	3.7
1020F	18.3	HSE	upstream	restricted	fin	11.6	8.6	43.5	-20.5	3.8
1020L	18.3	HSE	upstream	restricted	liver	11.8	9.1	50.8	-21.1	4.3
1020M	18.3	HSE	upstream	restricted	muscle	12.3	8.7	41.5	-21.0	3.4
1020S	18.3	HSE	upstream	restricted	mucus	12.9	8.2	45.5	-20.7	3.5
1021F	29.2	HSE	upstream	restricted	fin	9.3	10.3	41.5	-19.0	4.5
1021L	29.2	HSE	upstream	restricted	liver	9.3	10.4	44.2	-18.1	4.8
1021S	29.2	HSE	upstream	restricted	mucus	11.6	10.0	41.4	-18.4	3.6
1022F	30.5	HSE	upstream	restricted	fin	11.9	10.1	45.0	-17.9	3.8
1022L	30.5	HSE	upstream	restricted	liver	10.8	10.7	47.4	-18.4	4.4
1022M	30.5	HSE	upstream	restricted	muscle	9.5	9.9	52.9	-20.3	5.5
1022S	30.5	HSE	upstream	restricted	mucus	10.1	9.7	37.1	-17.9	3.7
1023F	24.5	HSE	downstream	reference	fin	8.4	8.5	35.7	-14.9	4.2
1023L	24.5	HSE	downstream	reference	liver	8.1	9.0	37.0	-14.6	4.6
1023M	24.5	HSE	downstream	reference	muscle	12.7	7.9	42.8	-14.7	3.4
1023S	24.5	HSE	downstream	reference	mucus	11.6	8.4	40.6	-14.0	3.5
1024F	29.2	HSE	downstream	reference	fin	11.2	9.2	43.9	-17.8	3.9
1024L	29.2	HSE	downstream	reference	liver	10.2	9.6	42.0	-18.3	4.1
1024M	29.2	HSE	downstream	reference	muscle	12.4	8.9	39.4	-17.6	3.2
1024S	29.2	HSE	downstream	reference	mucus	11.4	9.2	41.2	-17.3	3.6
1025F	19.9	HSE	upstream	reference	fin	10.8	9.5	41.7	-20.1	3.8
1025L	19.9	HSE	upstream	reference	liver	11.2	10.7	46.1	-22.1	4.1
1025S	19.9	HSE	upstream	reference	mucus	12.0	10.0	42.6	-21.2	3.6
1026F	28.1	HSE	upstream	reference	fin	10.5	10.5	46.2	-19.6	4.4
1026L	28.1	HSE	upstream	reference	liver	9.3	10.7	43.3	-18.7	4.6
1026M	28.1	HSE	upstream	reference	muscle	11.8	10.3	42.3	-19.8	3.6
1026S	28.1	HSE	upstream	reference	mucus	10.0	10.6	35.6	-18.9	3.6

1027F	16.3	HSE	upstream	reference	fin	8.9	10.5	41.5	-17.2	4.7
1027L	16.3	HSE	upstream	reference	liver	11.3	11.0	46.8	-16.7	4.1
1027M	16.3	HSE	upstream	reference	muscle	11.2	10.0	38.5	-16.5	3.4
1027S	16.3	HSE	upstream	reference	mucus	11.3	10.1	39.7	-15.8	3.5
1028F	43.8	HSE	upstream	reference	fin	9.1	11.2	42.4	-16.1	4.6
1028L	43.8	HSE	upstream	reference	liver	8.5	11.7	46.8	-17.3	5.5
1028M	43.8	HSE	upstream	reference	muscle	7.8	10.4	57.2	-17.9	7.3
1028S	43.8	HSE	upstream	reference	mucus	11.2	11.2	44.4	-15.9	4.0
1029F	21.7	PRE	downstream	reference	fin	11.0	9.7	43.5	-16.7	3.9
1029L	21.7	PRE	downstream	reference	liver	11.6	9.4	45.7	-17.1	3.9
1029M	21.7	PRE	downstream	reference	muscle	13.2	9.6	44.1	-16.9	3.3
1029S	21.7	PRE	downstream	reference	mucus	12.7	9.3	44.4	-16.1	3.5
1030F	28.2	PRE	downstream	reference	fin	10.6	10.3	39.7	-15.7	3.7
1030L	28.2	PRE	downstream	reference	liver	10.7	10.4	47.2	-16.6	4.4
1030M	28.2	PRE	downstream	reference	muscle	14.0	9.4	46.1	-16.1	3.3
1030S	28.2	PRE	downstream	reference	mucus	11.6	9.9	41.9	-15.8	3.6
1031F	27.2	PRE	downstream	reference	fin	10.2	8.2	38.1	-12.7	3.7
1031L	27.2	PRE	downstream	reference	liver	10.4	9.1	46.6	-14.2	4.5
1031M	27.2	PRE	downstream	reference	muscle	12.9	7.9	42.6	-13.0	3.3
1031S	27.2	PRE	downstream	reference	mucus	10.4	8.1	38.2	-13.2	3.7
1032F	51.7	PRE	downstream	reference	fin	11.0	11.8	50.5	-14.9	4.6
1032L	51.7	PRE	downstream	reference	liver	8.9	11.7	47.5	-14.9	5.4
1032M	51.7	PRE	downstream	reference	muscle	11.7	11.3	47.8	-15.3	4.1
1032S	51.7	PRE	downstream	reference	mucus	11.0	11.5	42.7	-14.2	3.9
1033F	29.9	PRE	downstream	reference	fin	8.4	10.8	41.3	-14.0	4.9
1033L	29.9	PRE	downstream	reference	liver	10.1	10.3	37.3	-13.4	3.7
1033M	29.9	PRE	downstream	reference	muscle	10.6	10.3	53.8	-15.5	5.1
1033S	29.9	PRE	downstream	reference	mucus	13.2	10.6	56.6	-13.8	4.3
1034F	50.3	PRE	downstream	reference	fin	9.3	11.4	34.7	-13.4	3.7
1034L	50.3	PRE	downstream	reference	liver	9.1	11.0	41.3	-14.0	4.5
1034M	50.3	PRE	downstream	reference	muscle	10.6	11.0	43.2	-15.5	4.1
1034S	50.3	PRE	downstream	reference	mucus	11.1	11.2	41.8	-14.0	3.8
1035F	24.1	PRE	downstream	restricted	fin	9.6	10.4	40.9	-16.4	4.2
1035L	24.1	PRE	downstream	restricted	liver	11.0	10.6	44.1	-16.6	4.0
1035M	24.1	PRE	downstream	restricted	muscle	14.7	9.7	47.9	-15.9	3.3
1035S	24.1	PRE	downstream	restricted	mucus	7.9	9.6	28.6	-16.3	3.6
1036F	22.1	PRE	downstream	restricted	fin	10.7	11.0	42.2	-15.7	3.9
1036L	22.1	PRE	downstream	restricted	liver	11.6	10.9	44.2	-16.0	3.8

1036M	22.1	PRE	downstream	restricted	muscle	14.9	10.7	48.4	-15.9	3.2
1036S	22.1	PRE	downstream	restricted	mucus	11.6	10.3	41.3	-15.5	3.6
1037F	23.6	PRE	downstream	restricted	fin	8.9	11.3	33.3	-15.8	3.7
1037L	23.6	PRE	downstream	restricted	liver	11.7	11.1	50.4	-17.1	4.3
1037M	23.6	PRE	downstream	restricted	muscle	10.4	10.5	33.0	-16.2	3.2
1037S	23.6	PRE	downstream	restricted	mucus	12.3	10.4	43.0	-15.5	3.5
1038F	25.5	PRE	downstream	restricted	fin	10.6	10.7	39.9	-16.9	3.8
1038L	25.5	PRE	downstream	restricted	liver	12.0	10.7	47.4	-17.4	4.0
1038M	25.5	PRE	downstream	restricted	muscle	11.6	10.2	38.4	-16.6	3.3
1038S	25.5	PRE	downstream	restricted	mucus	11.8	10.0	42.7	-17.2	3.6
1039F	46.2	PRE	downstream	restricted	fin	11.0	11.5	44.0	-14.9	4.0
1039L	46.2	PRE	downstream	restricted	liver	9.2	11.0	43.2	-15.1	4.7
1039M	46.2	PRE	downstream	restricted	muscle	13.6	11.0	45.7	-15.0	3.4
1039S	46.2	PRE	downstream	restricted	mucus	11.5	11.0	44.1	-14.9	3.8
1040F	17.4	PRE	upstream	restricted	fin	10.7	10.9	46.1	-20.5	4.3
1040L	17.4	PRE	upstream	restricted	liver	11.3	11.1	43.4	-20.3	3.8
1040M	17.4	PRE	upstream	restricted	muscle	13.0	11.0	43.4	-19.8	3.3
1040S	17.4	PRE	upstream	restricted	mucus	12.2	10.6	42.3	-19.4	3.5
1041F	21.1	PRE	upstream	restricted	fin	10.9	11.2	45.6	-19.4	4.2
1041L	21.1	PRE	upstream	restricted	liver	11.5	11.2	44.2	-19.9	3.8
1041M	21.1	PRE	upstream	restricted	muscle	11.6	11.1	43.4	-19.8	3.7
1041S	21.1	PRE	upstream	restricted	mucus	10.9	10.5	38.5	-18.9	3.5
1042F	19.2	PRE	upstream	restricted	fin	11.0	10.0	41.4	-19.4	3.8
1042L	19.2	PRE	upstream	restricted	liver	10.1	10.5	41.9	-20.9	4.2
1042M	19.2	PRE	upstream	restricted	muscle	12.8	9.6	45.1	-19.8	3.5
1042S	19.2	PRE	upstream	restricted	mucus	12.6	9.7	44.7	-19.9	3.5
1043F	18.3	PRE	upstream	restricted	fin	10.4	10.2	47.1	-21.4	4.5
1043L	18.3	PRE	upstream	restricted	liver	10.5	10.5	48.2	-22.4	4.6
1043M	18.3	PRE	upstream	restricted	muscle	12.4	10.1	41.5	-20.9	3.3
1043S	18.3	PRE	upstream	restricted	mucus	12.6	10.0	44.3	-20.9	3.5
1044F	23.3	PRE	upstream	restricted	fin	12.0	11.2	46.9	-19.1	3.9
1044L	23.3	PRE	upstream	restricted	liver	11.7	11.1	46.5	-19.5	4.0
1044M	23.3	PRE	upstream	restricted	muscle	11.8	10.8	40.1	-20.0	3.4
1044S	23.3	PRE	upstream	restricted	mucus	14.8	10.5	51.0	-18.6	3.5
1045F	26.7	PRE	upstream	restricted	fin	11.8	11.5	47.0	-19.1	4.0
1045L	26.7	PRE	upstream	restricted	liver	11.0	11.1	51.4	-18.8	4.7
1045M	26.7	PRE	upstream	restricted	muscle	13.0	11.4	45.1	-20.1	3.5
1045S	26.7	PRE	upstream	restricted	mucus	12.1	10.7	43.9	-18.4	3.6

1046F	24.1	PRE	upstream	restricted	fin	11.3	11.7	41.9	-17.2	3.7
1046L	24.1	PRE	upstream	restricted	liver	10.9	11.2	42.5	-17.0	3.9
1046S	24.1	PRE	upstream	restricted	mucus	13.3	11.6	46.9	-16.7	3.5
1047F	47.0	PRE	upstream	restricted	fin	10.2	12.5	44.5	-17.2	4.4
1047L	47.0	PRE	upstream	restricted	liver	9.0	12.5	42.9	-16.4	4.8
1047M	47.0	PRE	upstream	restricted	muscle	12.3	11.8	53.9	-18.4	4.4
1047S	47.0	PRE	upstream	restricted	mucus	11.4	12.0	42.2	-16.5	3.7
1048F	22.7	PRE	upstream	reference	fin	12.1	10.0	49.2	-17.5	4.1
1048L	22.7	PRE	upstream	reference	liver	9.6	10.7	41.3	-18.6	4.3
1048M	22.7	PRE	upstream	reference	muscle	12.4	9.7	41.4	-17.4	3.3
1048S	22.7	PRE	upstream	reference	mucus	12.0	9.6	41.9	-17.1	3.5
1049F	21.8	PRE	upstream	reference	fin	11.0	11.2	42.3	-18.9	3.9
1049L	21.8	PRE	upstream	reference	liver	5.1	11.2	20.0	-18.9	3.9
1049M	21.8	PRE	upstream	reference	muscle	14.4	10.7	48.1	-19.3	3.3
1049S	21.8	PRE	upstream	reference	mucus	11.1	10.7	41.2	-18.5	3.7
1050F	27.0	PRE	upstream	reference	fin	11.1	11.5	40.9	-16.8	3.7
1050L	27.0	PRE	upstream	reference	liver	10.8	11.6	40.1	-17.2	3.7
1050M	27.0	PRE	upstream	reference	muscle	16.0	10.9	54.1	-17.4	3.4
1050S	27.0	PRE	upstream	reference	mucus	8.8	10.6	31.1	-16.9	3.5
1051F	26.4	PRE	upstream	reference	fin	11.5	11.2	44.1	-17.3	3.8
1051L	26.4	PRE	upstream	reference	liver	11.1	11.7	47.6	-18.0	4.3
1051M	26.4	PRE	upstream	reference	muscle	15.3	10.4	51.1	-17.5	3.3
1051S	26.4	PRE	upstream	reference	mucus	10.6	10.5	38.3	-17.1	3.6
1052F	30.0	PRE	upstream	reference	fin	12.6	13.0	48.2	-16.3	3.8
1052L	30.0	PRE	upstream	reference	liver	9.9	12.6	50.4	-17.4	5.1
1052M	30.0	PRE	upstream	reference	muscle	9.4	12.8	54.8	-19.1	5.8
1052S	30.0	PRE	upstream	reference	mucus	12.4	12.2	48.3	-16.7	3.9
1053F	34.6	PRE	upstream	reference	fin	11.8	12.3	42.5	-15.6	3.6
1053L	34.6	PRE	upstream	reference	liver	11.7	12.5	52.2	-17.8	4.5
1053M	34.6	PRE	upstream	reference	muscle	13.4	11.5	44.7	-16.3	3.3
1053S	34.6	PRE	upstream	reference	mucus	11.9	11.3	43.6	-15.8	3.7
1000F	26.3	WEB	upstream	reference	fin	9.4	11.4	42.1	-15.6	4.5
1000L	26.3	WEB	upstream	reference	liver	10.7	11.3	44.8	-15.2	4.2
1000M	26.3	WEB	upstream	reference	muscle	11.6	11.0	41.4	-15.9	3.6
1000S	26.3	WEB	upstream	reference	mucus	10.0	11.0	38.6	-14.9	3.9
1001F	27.2	WEB	upstream	restricted	fin	10.3	8.3	41.5	-12.3	4.0
1001L	27.2	WEB	upstream	restricted	liver	11.6	8.3	48.1	-12.9	4.2
1001M	27.2	WEB	upstream	restricted	muscle	12.6	8.0	45.3	-13.4	3.6

1001S	27.2	WEB	upstream	restricted	mucus	10.7	7.9	40.2	-12.3	3.7
1002F	17.9	WEB	upstream	restricted	fin	9.9	9.5	48.0	-13.7	4.9
1002L	17.9	WEB	upstream	restricted	liver	10.2	10.0	52.2	-14.2	5.1
1002M	17.9	WEB	upstream	restricted	muscle	12.1	9.3	51.5	-14.5	4.3
1002S	17.9	WEB	upstream	restricted	mucus	13.9	9.5	50.7	-13.4	3.6
1003F	15.7	WEB	upstream	reference	fin	9.8	10.8	44.8	-19.8	4.6
1003L	15.7	WEB	upstream	reference	liver	10.4	11.2	47.1	-20.2	4.5
1003M	15.7	WEB	upstream	reference	muscle	12.4	11.2	47.5	-20.0	3.8
1003S	15.7	WEB	upstream	reference	mucus	11.5	10.5	43.5	-19.6	3.8
1004F	22.0	WEB	upstream	reference	fin	10.9	8.0	48.3	-13.0	4.4
1004L	22.0	WEB	upstream	reference	liver	8.0	8.9	47.2	-12.3	5.9
1004M	22.0	WEB	upstream	reference	muscle	12.5	7.3	45.7	-13.0	3.7
1004S	22.0	WEB	upstream	reference	mucus	9.0	7.9	34.3	-12.6	3.8
1005F	19.4	WEB	downstream	restricted	fin	11.0	6.5	43.4	-14.3	3.9
1005L	19.4	WEB	downstream	restricted	liver	11.8	6.8	50.0	-14.2	4.3
1005M	19.4	WEB	downstream	restricted	muscle	13.6	6.5	45.9	-14.9	3.4
1005S	19.4	WEB	downstream	restricted	mucus	12.3	6.7	45.0	-14.8	3.7
1006F	15.9	WEB	downstream	restricted	fin	11.1	6.8	43.0	-14.2	3.9
1006L	15.9	WEB	downstream	restricted	liver	9.7	6.4	44.9	-14.2	4.6
1006M	15.9	WEB	downstream	restricted	muscle	13.4	7.3	44.4	-14.9	3.3
1006S	15.9	WEB	downstream	restricted	mucus	12.1	7.5	43.2	-14.5	3.6
1007F	17	WEB	downstream	reference	fin	10.3	8.8	41.9	-15.1	4.1
1007L	17	WEB	downstream	reference	liver	9.2	10.2	42.1	-16.0	4.6
1007M	17	WEB	downstream	reference	muscle	14.5	8.8	51.8	-15.3	3.6
1007S	17	WEB	downstream	reference	mucus	11.8	9.7	44.5	-15.0	3.8
1008F	16.8	WEB	upstream	restricted	fin	10.5	10.2	42.0	-13.7	4.0
1008L	16.8	WEB	upstream	restricted	liver	11.0	8.7	43.4	-14.1	3.9
1008M	16.8	WEB	upstream	restricted	muscle	8.1	8.6	27.2	-13.9	3.4
1008S	16.8	WEB	upstream	restricted	mucus	11.5	8.5	42.7	-13.8	3.7
1009F	18.8	WEB	upstream	restricted	fin	9.0	9.2	40.1	-18.5	4.5
1009L	18.8	WEB	upstream	restricted	liver	9.6	9.7	42.5	-18.9	4.4
1009M	18.8	WEB	upstream	restricted	muscle	12.2	9.1	43.5	-17.6	3.6
1009S	18.8	WEB	upstream	restricted	mucus	11.7	9.3	42.0	-18.1	3.6
1010F	17.1	WEB	upstream	restricted	fin	10.6	8.9	42.9	-14.9	4.1
1010L	17.1	WEB	upstream	restricted	liver	10.7	9.5	42.3	-15.4	4.0
1010M	17.1	WEB	upstream	restricted	muscle	12.7	8.7	44.0	-16.6	3.5
1010S	17.1	WEB	upstream	restricted	mucus	12.8	8.7	45.8	-15.3	3.6
1011F	18.1	WEB	upstream	restricted	fin	9.7	8.6	40.5	-14.0	4.2

1011L	18.1	WEB	upstream	restricted	liver	10.9	9.2	44.3	-14.2	4.1
1011M	18.1	WEB	upstream	restricted	muscle	12.3	8.9	45.5	-14.5	3.7
1011S	18.1	WEB	upstream	restricted	mucus	11.7	8.4	42.7	-14.1	3.7
1012F	10.3	WEB	downstream	restricted	fin	10.9	8.5	50.3	-18.6	4.6
1012L	10.3	WEB	downstream	restricted	liver	7.8	8.8	55.2	-19.2	7.0
1012S	10.3	WEB	downstream	restricted	mucus	11.2	8.2	45.5	-18.5	4.1
1013F	18.1	HSE	downstream	restricted	fin	11.3	8.0	45.6	-14.1	4.0
1013L	18.1	HSE	downstream	restricted	liver	10.3	8.1	47.5	-13.9	4.6
1013M	18.1	HSE	downstream	restricted	muscle	12.4	7.6	43.7	-14.5	3.5
1013S	18.1	HSE	downstream	restricted	mucus	10.9	7.9	41.0	-14.0	3.8
1014F	53.5	HSE	downstream	restricted	fin	10.4	11.3	39.1	-14.7	3.7
1014L	53.5	HSE	downstream	restricted	liver	11.4	11.2	46.2	-15.0	4.0
1014M	53.5	HSE	downstream	restricted	muscle	12.1	10.3	44.5	-15.0	3.7
1014S	53.5	HSE	downstream	restricted	mucus	11.8	10.9	44.5	-15.3	3.8
1015F	51.6	HSE	downstream	restricted	fin	11.1	11.2	43.1	-12.3	3.9
1015L	51.6	HSE	downstream	restricted	liver	9.6	10.5	41.0	-12.6	4.3
1015M	51.6	HSE	downstream	restricted	muscle	10.5	10.2	39.8	-13.9	3.8
1015S	51.6	HSE	downstream	restricted	mucus	10.2	10.3	41.3	-13.2	4.0
1016F	27.7	HSE	upstream	restricted	fin	7.3	9.5	30.7	-18.9	4.2
1016L	27.7	HSE	upstream	restricted	liver	12.2	9.4	50.3	-18.0	4.1
1016M	27.7	HSE	upstream	restricted	muscle	12.0	9.2	40.7	-19.4	3.4
1016S	27.7	HSE	upstream	restricted	mucus	10.5	8.9	38.5	-18.6	3.7
1017F	30.7	HSE	upstream	restricted	fin	8.6	10.5	47.4	-17.1	5.5
1017L	30.7	HSE	upstream	restricted	liver	9.7	10.4	39.7	-16.8	4.1
1017M	30.7	HSE	upstream	restricted	muscle	11.5	10.2	50.0	-18.1	4.4
1017S	30.7	HSE	upstream	restricted	mucus	11.9	10.3	44.2	-16.6	3.7
1018F	30.7	HSE	upstream	restricted	fin	8.2	10.3	47.9	-17.2	5.9
1018L	30.7	HSE	upstream	restricted	liver	10.0	9.9	42.0	-16.7	4.2
1018M	30.7	HSE	upstream	restricted	muscle	11.3	9.6	45.1	-18.3	4.0
1018S	30.7	HSE	upstream	restricted	mucus	11.1	9.7	43.1	-17.0	3.9
1019F	20.3	HSE	upstream	restricted	fin	10.9	8.7	41.7	-20.9	3.8
1019L	20.3	HSE	upstream	restricted	liver	10.9	9.2	49.8	-21.2	4.6
1019M	20.3	HSE	upstream	restricted	muscle	13.0	8.6	45.1	-21.5	3.5
1019S	20.3	HSE	upstream	restricted	mucus	12.5	8.3	45.5	-20.7	3.7
1020F	18.3	HSE	upstream	restricted	fin	11.6	8.6	43.5	-19.6	3.8
1020L	18.3	HSE	upstream	restricted	liver	11.8	9.1	50.8	-19.7	4.3
1020M	18.3	HSE	upstream	restricted	muscle	12.3	8.7	41.5	-20.3	3.4
1020S	18.3	HSE	upstream	restricted	mucus	12.9	8.2	45.5	-19.9	3.5

1021F	29.2	HSE	upstream	restricted	fin	9.3	10.3	41.5	-17.6	4.5
1021L	29.2	HSE	upstream	restricted	liver	9.3	10.4	44.2	-16.5	4.8
1021S	29.2	HSE	upstream	restricted	mucus	11.6	10.0	41.4	-17.6	3.6
1022F	30.5	HSE	upstream	restricted	fin	11.9	10.1	45.0	-17.0	3.8
1022L	30.5	HSE	upstream	restricted	liver	10.8	10.7	47.4	-17.1	4.4
1022M	30.5	HSE	upstream	restricted	muscle	9.5	9.9	52.9	-18.2	5.5
1022S	30.5	HSE	upstream	restricted	mucus	10.1	9.7	37.1	-17.0	3.7
1023F	24.5	HSE	downstream	reference	fin	8.4	8.5	35.7	-13.7	4.2
1023L	24.5	HSE	downstream	reference	liver	8.1	9.0	37.0	-13.1	4.6
1023M	24.5	HSE	downstream	reference	muscle	12.7	7.9	42.8	-14.0	3.4
1023S	24.5	HSE	downstream	reference	mucus	11.6	8.4	40.6	-13.2	3.5
1024F	29.2	HSE	downstream	reference	fin	11.2	9.2	43.9	-16.8	3.9
1024L	29.2	HSE	downstream	reference	liver	10.2	9.6	42.0	-17.1	4.1
1024M	29.2	HSE	downstream	reference	muscle	12.4	8.9	39.4	-17.0	3.2
1024S	29.2	HSE	downstream	reference	mucus	11.4	9.2	41.2	-16.5	3.6
1025F	19.9	HSE	upstream	reference	fin	10.8	9.5	41.7	-19.1	3.8
1025L	19.9	HSE	upstream	reference	liver	11.2	10.7	46.1	-20.9	4.1
1025S	19.9	HSE	upstream	reference	mucus	12.0	10.0	42.6	-20.3	3.6
1026F	28.1	HSE	upstream	reference	fin	10.5	10.5	46.2	-18.2	4.4
1026L	28.1	HSE	upstream	reference	liver	9.3	10.7	43.3	-17.1	4.6
1026M	28.1	HSE	upstream	reference	muscle	11.8	10.3	42.3	-18.9	3.6
1026S	28.1	HSE	upstream	reference	mucus	10.0	10.6	35.6	-18.1	3.6
1027F	16.3	HSE	upstream	reference	fin	8.9	10.5	41.5	-15.6	4.7
1027L	16.3	HSE	upstream	reference	liver	11.3	11.0	46.8	-15.5	4.1
1027M	16.3	HSE	upstream	reference	muscle	11.2	10.0	38.5	-15.7	3.4
1027S	16.3	HSE	upstream	reference	mucus	11.3	10.1	39.7	-15.0	3.5
1028F	43.8	HSE	upstream	reference	fin	9.1	11.2	42.4	-14.6	4.6
1028L	43.8	HSE	upstream	reference	liver	8.5	11.7	46.8	-15.2	5.5
1028M	43.8	HSE	upstream	reference	muscle	7.8	10.4	57.2	-14.8	7.3
1028S	43.8	HSE	upstream	reference	mucus	11.2	11.2	44.4	-14.8	4.0
1029F	21.7	PRE	downstream	reference	fin	11.0	9.7	43.5	-15.6	3.9
1029L	21.7	PRE	downstream	reference	liver	11.6	9.4	45.7	-16.0	3.9
1029M	21.7	PRE	downstream	reference	muscle	13.2	9.6	44.1	-16.2	3.3
1029S	21.7	PRE	downstream	reference	mucus	12.7	9.3	44.4	-15.3	3.5
1030F	28.2	PRE	downstream	reference	fin	10.6	10.3	39.7	-14.7	3.7
1030L	28.2	PRE	downstream	reference	liver	10.7	10.4	47.2	-15.2	4.4
1030M	28.2	PRE	downstream	reference	muscle	14.0	9.4	46.1	-15.4	3.3
1030S	28.2	PRE	downstream	reference	mucus	11.6	9.9	41.9	-15.0	3.6

1031F	27.2	PRE	downstream	reference	fin	10.2	8.2	38.1	-11.8	3.7
1031L	27.2	PRE	downstream	reference	liver	10.4	9.1	46.6	-12.8	4.5
1031M	27.2	PRE	downstream	reference	muscle	12.9	7.9	42.6	-12.4	3.3
1031S	27.2	PRE	downstream	reference	mucus	10.4	8.1	38.2	-12.3	3.7
1032F	51.7	PRE	downstream	reference	fin	11.0	11.8	50.5	-13.4	4.6
1032L	51.7	PRE	downstream	reference	liver	8.9	11.7	47.5	-12.9	5.4
1032M	51.7	PRE	downstream	reference	muscle	11.7	11.3	47.8	-14.1	4.1
1032S	51.7	PRE	downstream	reference	mucus	11.0	11.5	42.7	-13.1	3.9
1033F	29.9	PRE	downstream	reference	fin	8.4	10.8	41.3	-12.2	4.9
1033L	29.9	PRE	downstream	reference	liver	10.1	10.3	37.3	-12.5	3.7
1033M	29.9	PRE	downstream	reference	muscle	10.6	10.3	53.8	-13.7	5.1
1033S	29.9	PRE	downstream	reference	mucus	13.2	10.6	56.6	-12.5	4.3
1034F	50.3	PRE	downstream	reference	fin	9.3	11.4	34.7	-12.4	3.7
1034L	50.3	PRE	downstream	reference	liver	9.1	11.0	41.3	-12.6	4.5
1034M	50.3	PRE	downstream	reference	muscle	10.6	11.0	43.2	-14.3	4.1
1034S	50.3	PRE	downstream	reference	mucus	11.1	11.2	41.8	-13.0	3.8
1035F	24.1	PRE	downstream	restricted	fin	9.6	10.4	40.9	-15.1	4.2
1035L	24.1	PRE	downstream	restricted	liver	11.0	10.6	44.1	-15.5	4.0
1035M	24.1	PRE	downstream	restricted	muscle	14.7	9.7	47.9	-15.3	3.3
1035S	24.1	PRE	downstream	restricted	mucus	7.9	9.6	28.6	-15.4	3.6
1036F	22.1	PRE	downstream	restricted	fin	10.7	11.0	42.2	-14.6	3.9
1036L	22.1	PRE	downstream	restricted	liver	11.6	10.9	44.2	-15.1	3.8
1036M	22.1	PRE	downstream	restricted	muscle	14.9	10.7	48.4	-15.3	3.2
1036S	22.1	PRE	downstream	restricted	mucus	11.6	10.3	41.3	-14.7	3.6
1037F	23.6	PRE	downstream	restricted	fin	8.9	11.3	33.3	-14.8	3.7
1037L	23.6	PRE	downstream	restricted	liver	11.7	11.1	50.4	-15.8	4.3
1037M	23.6	PRE	downstream	restricted	muscle	10.4	10.5	33.0	-15.6	3.2
1037S	23.6	PRE	downstream	restricted	mucus	12.3	10.4	43.0	-14.7	3.5
1038F	25.5	PRE	downstream	restricted	fin	10.6	10.7	39.9	-15.9	3.8
1038L	25.5	PRE	downstream	restricted	liver	12.0	10.7	47.4	-16.3	4.0
1038M	25.5	PRE	downstream	restricted	muscle	11.6	10.2	38.4	-15.9	3.3
1038S	25.5	PRE	downstream	restricted	mucus	11.8	10.0	42.7	-16.3	3.6
1039F	46.2	PRE	downstream	restricted	fin	11.0	11.5	44.0	-13.8	4.0
1039L	46.2	PRE	downstream	restricted	liver	9.2	11.0	43.2	-13.6	4.7
1039M	46.2	PRE	downstream	restricted	muscle	13.6	11.0	45.7	-14.3	3.4
1039S	46.2	PRE	downstream	restricted	mucus	11.5	11.0	44.1	-13.9	3.8
1040F	17.4	PRE	upstream	restricted	fin	10.7	10.9	46.1	-19.2	4.3
1040L	17.4	PRE	upstream	restricted	liver	11.3	11.1	43.4	-19.3	3.8

1040M	17.4	PRE	upstream	restricted	muscle	13.0	11.0	43.4	-19.2	3.3
1040S	17.4	PRE	upstream	restricted	mucus	12.2	10.6	42.3	-18.6	3.5
1041F	21.1	PRE	upstream	restricted	fin	10.9	11.2	45.6	-18.1	4.2
1041L	21.1	PRE	upstream	restricted	liver	11.5	11.2	44.2	-18.9	3.8
1041M	21.1	PRE	upstream	restricted	muscle	11.6	11.1	43.4	-18.9	3.7
1041S	21.1	PRE	upstream	restricted	mucus	10.9	10.5	38.5	-18.1	3.5
1042F	19.2	PRE	upstream	restricted	fin	11.0	10.0	41.4	-18.4	3.8
1042L	19.2	PRE	upstream	restricted	liver	10.1	10.5	41.9	-19.6	4.2
1042M	19.2	PRE	upstream	restricted	muscle	12.8	9.6	45.1	-19.0	3.5
1042S	19.2	PRE	upstream	restricted	mucus	12.6	9.7	44.7	-19.1	3.5
1043F	18.3	PRE	upstream	restricted	fin	10.4	10.2	47.1	-20.0	4.5
1043L	18.3	PRE	upstream	restricted	liver	10.5	10.5	48.2	-20.9	4.6
1043M	18.3	PRE	upstream	restricted	muscle	12.4	10.1	41.5	-20.2	3.3
1043S	18.3	PRE	upstream	restricted	mucus	12.6	10.0	44.3	-20.1	3.5
1044F	23.3	PRE	upstream	restricted	fin	12.0	11.2	46.9	-18.0	3.9
1044L	23.3	PRE	upstream	restricted	liver	11.7	11.1	46.5	-18.4	4.0
1044M	23.3	PRE	upstream	restricted	muscle	11.8	10.8	40.1	-19.2	3.4
1044S	23.3	PRE	upstream	restricted	mucus	14.8	10.5	51.0	-17.9	3.5
1045F	26.7	PRE	upstream	restricted	fin	11.8	11.5	47.0	-18.0	4.0
1045L	26.7	PRE	upstream	restricted	liver	11.0	11.1	51.4	-17.3	4.7
1045M	26.7	PRE	upstream	restricted	muscle	13.0	11.4	45.1	-19.4	3.5
1045S	26.7	PRE	upstream	restricted	mucus	12.1	10.7	43.9	-17.5	3.6
1046F	24.1	PRE	upstream	restricted	fin	11.3	11.7	41.9	-16.3	3.7
1046L	24.1	PRE	upstream	restricted	liver	10.9	11.2	42.5	-15.9	3.9
1046S	24.1	PRE	upstream	restricted	mucus	13.3	11.6	46.9	-15.9	3.5
1047F	47.0	PRE	upstream	restricted	fin	10.2	12.5	44.5	-15.8	4.4
1047L	47.0	PRE	upstream	restricted	liver	9.0	12.5	42.9	-14.7	4.8
1047M	47.0	PRE	upstream	restricted	muscle	12.3	11.8	53.9	-17.0	4.4
1047S	47.0	PRE	upstream	restricted	mucus	11.4	12.0	42.2	-15.6	3.7
1048F	22.7	PRE	upstream	reference	fin	12.1	10.0	49.2	-16.4	4.1
1048L	22.7	PRE	upstream	reference	liver	9.6	10.7	41.3	-17.3	4.3
1048M	22.7	PRE	upstream	reference	muscle	12.4	9.7	41.4	-16.7	3.3
1048S	22.7	PRE	upstream	reference	mucus	12.0	9.6	41.9	-16.3	3.5
1049F	21.8	PRE	upstream	reference	fin	11.0	11.2	42.3	-17.9	3.9
1049L	21.8	PRE	upstream	reference	liver	5.1	11.2	20.0	-17.8	3.9
1049M	21.8	PRE	upstream	reference	muscle	14.4	10.7	48.1	-18.6	3.3
1049S	21.8	PRE	upstream	reference	mucus	11.1	10.7	41.2	-17.5	3.7
1050F	27.0	PRE	upstream	reference	fin	11.1	11.5	40.9	-15.9	3.7

1050L	27.0	PRE	upstream	reference	liver	10.8	11.6	40.1	-16.3	3.7
1050M	27.0	PRE	upstream	reference	muscle	16.0	10.9	54.1	-16.7	3.4
1050S	27.0	PRE	upstream	reference	mucus	8.8	10.6	31.1	-16.1	3.5
1051F	26.4	PRE	upstream	reference	fin	11.5	11.2	44.1	-16.3	3.8
1051L	26.4	PRE	upstream	reference	liver	11.1	11.7	47.6	-16.7	4.3
1051M	26.4	PRE	upstream	reference	muscle	15.3	10.4	51.1	-16.8	3.3
1051S	26.4	PRE	upstream	reference	mucus	10.6	10.5	38.3	-16.3	3.6
1052F	30.0	PRE	upstream	reference	fin	12.6	13.0	48.2	-15.3	3.8
1052L	30.0	PRE	upstream	reference	liver	9.9	12.6	50.4	-15.6	5.1
1052M	30.0	PRE	upstream	reference	muscle	9.4	12.8	54.8	-16.8	5.8
1052S	30.0	PRE	upstream	reference	mucus	12.4	12.2	48.3	-15.6	3.9
1053F	34.6	PRE	upstream	reference	fin	11.8	12.3	42.5	-14.8	3.6
1053L	34.6	PRE	upstream	reference	liver	11.7	12.5	52.2	-16.4	4.5
1053M	34.6	PRE	upstream	reference	muscle	13.4	11.5	44.7	-15.6	3.3
1053S	34.6	PRE	upstream	reference	mucus	11.9	11.3	43.6	-14.9	3.7

Appendix D

Bulk carbon and nitrogen isotope measurements of *Geukensia demissus* (ribbed mussel), *Mytilus edulis* (blue mussel), *Melampus, bidentatus* (coffee bean snail), and *Littorina littorea* (common periwinkle) collected from New England salt marshes and used to estimate trophic position (Chapter 2).

Site	**Hydrology**	**Location**	**Species**	**N**	**$\delta^{15}N$**
HSE	reference	downstream	*L. littorea*	1	6.1
		upstream	*M. bidentatus, G. demissa*	2	6.1
	restricted	downstream	*M. edulis, L. littorea*	2	7.8
		upstream	*L. littorea*	1	7.5
PRE	reference	downstream	*G. demissa*	1	7.5
		upstream	*M. bidentatus*	1	7.5
	restricted	downstream	*L. littorea, G. demissa*	2	8.4
		upstream	*G. demissa*	1	7.5
WEB	reference	downstream	*L. littorea*	1	6.5
		upstream	*L. littorea*	1	6.5
	restricted	downstream	*L. littorea*	1	6.7
		upstream	*L. littorea*	1	7.5