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Tracking diet and movement of Atlantic bluefin tuna (Thunnus thynnus) using carbon and nitrogen stable isotopes

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TRACKING DIET AND MOVEMENT OF ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) USING CARBON AND NITROGEN STABLE ISOTOPES

BY

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ABSTRACT

TRACKING DIET AND MOVEMENT OF ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) USING CARBON AND NITROGEN STABLE ISOTOPES

by

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Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) are important top predators in pelagic ecosystems. Understanding their diet and movements is necessary for proper management, but existing methods are costly and logistically challenging. Naturally occurring stable isotopes have been used to study diet and movement in many aquatic ecosystems and offer a novel approach for studying these large pelagic fishes.

Lipids, which have lower carbon isotope values than protein, can affect isotope analyses of bulk tissue. Both chemical and mathematical corrections were used to address lipid effects on ABFT and their prey. All chemical extraction methods altered nitrogen isotope values. Mathematical corrections closely replicated chemically extracted carbon values while preserving nitrogen isotope and C:N values and were applied to all ABFT samples.

Combined stomach content and stable isotope analyses were performed on ABFT in coastal forage grounds, revealing a diet based on aggregations of small fish and crustaceans. The largest prey components of adult ABFT were Atlantic herring (*Clupea harengus*) and sand lance (*Ammodytes* spp.) in the Gulf of Maine and Atlantic menhaden

(*Brevoortia tyrannus*) and swimming crabs (*Portunus* spp.) in the Mid-Atlantic Bight. Juvenile bluefin tuna fed at a lower trophic level than adult conspecifics due to higher dietary proportions of crustaceans and smaller fishes. Diet composition of ABFT in the western Atlantic was similar to historical observations while juveniles in the Bay of Biscay fed on higher proportions of crustacean prey relative to past observations.

Isotope gradients detected between shelf and open ocean forage grounds were used to infer movements of adult ABFT into Gulf of Mexico spawning grounds and to estimate residency in Gulf of Maine forage grounds in relation to results from tagging experiments. Residency and arrival times varied among individuals in the Gulf of Maine, with late season arrivals having reduced body condition similar to early season fish. Most fish in the Gulf of Mexico had previously fed on shelf forage grounds, demonstrating connectivity between productive coastal waters and this western Atlantic spawning area. Results provide important trophic and migratory information for use in ecosystem-based management strategies and validations for future isotope studies of large pelagic fishes.

INTRODUCTION

Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) and other tuna species are important predators in pelagic food webs, capable of influencing ecosystem functions through top down controls (Hinke et al. 2004; Overholtz 2006). ABFT are commercially important (Mather et al. 1995), and while the extent of fishery removals is still being debated (Fromentin and Powers 2005; Safina and Klinger 2008), recent stock assessments estimate spawning stock biomass to be < 40 % of historical levels (ICCAT 2009). Any reductions in abundance of top predators has the potential to alter food web dynamics and trophic structure in marine systems (Paine 1966; Scheffer et al. 2005).

Prey distribution, abundance, type, and quality can also affect top predator communities (Bertrand et al. 2002; Bearzi et al. 2006). Commercial harvest of mid- and low trophic level species (Pauly et al. 1998) could alter available prey bases. ABFT have wide thermal tolerances (Carey and Teal 1969; Carey and Lawson 1973). Adult feeding migrations would not likely be affected by small temperature shifts induced by climate change, although spawning (Schaefer 1998; Schaefer 2001; Young et al. 2003) and larval survival (Miyashita et al. 2000; Tanaka et al. 2007) are highly temperature dependent, with both generally restricted to 24°C isotherms (Schaefer 2001). Climate shifts should affect lower trophic level prey species (Roessig et al. 2004; Perry et al. 2005; Poulard and Blanchard 2005), and as a result alter ABFT distribution. Shifts towards less energetically favorable prey bases, through reduced density or food quality, could also

impact body condition and reproductive potential (Rosen and Trites 2000; Litzow et al. 2002; Golet et al. 2007).

Understanding diet and movement is important for proper management of ABFT, but also costly and logistically challenging. Diet is commonly determined through stomach content analysis (SCA) while movement is assessed through tracking. Naturally occurring stable isotopes have been used to study diet and movement in many aquatic ecosystems (Fry and Sherr 1984; Hobson 1999), and stable isotope analysis (SIA) offers a novel approach to these fundamental ecological questions that could complement existing monitoring techniques for large pelagic fishes. Stable isotope values in consumer tissues are transferred from diet items providing a chemical tracer for food web analysis (Peterson and Fry 1987). Spatial differences in isotope values incorporated in consumer tissues can then further act as chemical tags to track movement (Hobson 1999; Hobson 2007b).

Stable isotope analysis (SIA) is emerging as an important tool for identifying animal diet, trophic position, and movement (Peterson and Fry 1987; Hobson 1999), including highly migratory and cryptic marine species (Best and Schell 1996; Reich et al. 2007). Isotopes are atoms of a common element that share the same number of protons and electrons, but differ in number of neutrons (Thomson et al. 1921). Differences in neutron number create different atomic masses, which can be measured with an isotope ratio mass spectrometer. Stable isotopes, unlike radioactive isotopes, have combinations of protons and neutrons that are resistant to decay over time. Heavy stable isotopes are rare and comprise a small percentage of total natural abundance for a given element, but are useful as tracers of ecological processes. For carbon and nitrogen, the heavy stable

isotopes ^{13}C and ^{15}N comprise about 1.11 and 0.37 %, respectively, of total percent abundance, while lighter isotopes make up the remainder (Sulzman 2007). Stable isotope values are reported as parts per thousand differences from standard reference material in delta notation, according to the equation:

$$\delta = [(R_{sample}/R_{standard})-1] \times 10^3,$$

where R is the ratio of heavy and light isotopes in a sample (e.g., $^{15}N/^{14}N$ and $^{13}C/^{12}C$) (Peterson and Fry 1987). Reference materials are Pee Dee Belemnite and atmospheric nitrogen.

Stable isotopes of different elements transfer through food webs with fairly consistent trophic level alterations, providing a natural tracer of food web linkages (Peterson and Fry 1987). This isotopic separation between consumer tissue and diet is known as a discrimination factor (Martínez del Rio and Wolf 2004). Stable isotope values reflect an integrated diet over a time scale determined by the metabolic activity of the tissue and organism analyzed (Peterson and Fry 1987). In this way, SIA provides a broader representation of diet and trophic position than standard SCA, which gives a detailed, but time-limited dietary snapshot. SCA is not always a viable method as stomach samples and the taxonomic expertise required to identifiy contents are often unavailable. A combination of stable isotope and stomach content analyses provides two temporal scales of dietary information. Multiple tissue SIA (e.g., slow and fast turnover tissues) may further inform analyses when isotope data are integrated over different time scales.

Carbon ($^{13}C/^{12}C$) stable isotope ratio discrimination across trophic levels is typically minimal (0 - 1 ‰), and can be used to infer sources of primary production

(DeNiro and Epstein 1978; Fry and Sherr 1984). In marine systems, $\delta^{13}C$ varies among benthic, pelagic, and inshore and offshore regions (Hobson et al. 1994; France 1995) and across latitudinal gradients (Rau et al. 1989). Consequently, spatial variations in $\delta^{13}C$ can be used to determine animal movements (Hobson 1999).

Nitrogen ($^{15}N/^{14}N$) stable isotope ratios become enriched in ^{15}N with trophic transfers, presumably due to preferential excretion of light isotopes (^{14}N) in nitrogenous waste and incorporation of heavier isotopes (^{15}N) in consumer tissues (Steele and Daniel 1978). Average trophic discrimination for nitrogen is usually more pronounced than carbon discrimination, providing a more robust measure of trophic position, with trophic enrichment typically ranging from 2 to 4 ‰ (DeNiro and Epstein 1981; Minagawa and Wada 1984; Gannes et al. 1998; Post 2002). Trophic position (TP) can be calculated based on $\delta^{15}N$ according to the equation,

$$TP = \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}}) / \Delta n,$$

where $\delta^{15}N_{\text{base}}$ is the $\delta^{15}N$ of a lower trophic level organism used for comparison, λ is the TP of this baseline organism, and Δn is the discrimination factor for the secondary consumer (Post 2002). Similar to carbon, nitrogen stable isotopes also vary across oceanographic regions and can be used to trace animal movements (Hobson 1999).

Stable isotopes and other chemical tracers have been used to study ABFT physiology (Radtke et al. 1987), stock structure (Secor and Zdanowicz 1998; Rooker et al. 2008a; Rooker et al. 2008b; Dickhut et al. 2009), and trophic ecology (Estrada et al. 2005; Sara and Sara 2007). Early analyses of otolith carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope values demonstrated that $\delta^{18}O$ values were affected by tuna brain temperatures and thus could be used to reconstruct tuna physiological life histories

(Radtke et al. 1987). However, the isotopic estimates of tuna physiology could be confounded by geographic shifts linked to ambient sea water temperature (Campana 1999; Rooker et al. 2008a). These geographic baseline shifts in otolith $\delta^{13}C$ and $\delta^{18}O$ were later used to identify natal origins of Atlantic bluefin tuna based on isotopic differences between Gulf of Mexico and Mediterranean Sea spawning grounds (Rooker et al. 2008a; Rooker et al. 2008b). Geographic variability in otolith isotope values proved to have more resolving power than previous efforts to use trace elements to delineate stock structure (Secor and Zdanowicz 1998; Rooker et al. 2001). Unique chemical signatures in metabolically-active soft tissues have also been used to infer bluefin tuna stock structure and trophic ecology (Estrada et al. 2005; Sara and Sara 2007; Dickhut et al. 2009). Observed gradients in organochlorine ratios between fishes occupying western Atlantic shelf and Mediterranean Sea forage grounds have been used to track recent movements of bluefin tuna across these regions (Dickhut et al. 2009). Known increases in $\delta^{15}N$ values in consumer tissues relative to diet have been used to estimate bluefin tuna trophic position in forage grounds in the NW Atlantic and Mediterranean Sea (Estrada et al. 2005; Sara and Sara 2007).

Chemical tracers clearly show potential to inform diverse aspects of tuna ecology, but all approaches also have potential pitfalls. Estimates of movement and stock structure are challenged by the highly migratory lifestyle of bluefin tuna. Failure to validate spatial signatures of a given tracer for all geographic regions occupied by bluefin tuna can bias results. For analyses using otoliths, values could be biased by physiological differences across ages and regions (Radtke et al. 1987). For soft tissues, which experience metabolic turnover, chemical signatures of past locations could be lost as

tissues equilibrate towards the values reflected in local sampling locations. Understanding physiological aspects surrounding the incorporation of these chemical tags in tuna is critical to proper interpretation in ecological applications (Gannes et al. 1997; Campana 1999).

To apply stable isotope techniques to ABFT ecological studies, several isotope dynamics must first be determined: 1) the timescale of information incorporated in consumer tissues; 2) isotope differences between consumer tissues and diet items; and 3) variability in isotope values among major tissue fractions (e.g., lipids and proteins) (Gannes et al. 1997). Once these are accounted for, stable isotopes can complement stomach content data by providing dietary information over longer time scales.

If the turnover rate of a given tissue or compound used for isotope analysis is unknown, results could be confounded by temporal shifts in diet or habitat. These problems are particularly relevant to large, long-lived pelagic fishes like ABFT, which could occupy multiple habitat types across a broad geographic range during the timescale incorporated in a tissue isotope value.

Discrimination factors are assumed to be ~ 0.4 ‰ for $\delta^{13}C$ and 3.4 ‰ for $\delta^{15}N$ (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987), but vary between species and tissue types (Macko et al. 1982; Pinnegar and Polunin 1999). Incorrect discrimination factors could cause errors in estimates of trophic position (Post 2002) and diet (Phillips and Gregg 2003).

Lipids have lower carbon isotope values than protein (DeNiro and Epstein 1977) and can affect isotope analyses of bulk tissue. For many large pelagic fish tissues (e.g., bluefin tuna muscle), some form of lipid correction is needed to prevent bias of bulk

carbon isotope values (Logan et al. 2008). Many chemical extraction methods that correct tissue $\delta^{13}C$ values can also incidentally alter $\delta^{15}N$ values in other fish species, which could then create error in nitrogen isotope data (Pinnegar and Polunin 1999; Sotiropoulos et al. 2004; Sweeting et al. 2006). Mathematical corrections exist, but their applicability to a diverse range of species and tissue types has been called into question (Kiljunen et al. 2006; Post et al. 2007). To effectively implement stable isotope approaches for ABFT, these fundamental aspects of isotope dynamics need to be better understood.

Atlantic bluefin tuna are highly migratory, have high energetic demands, and are heavily exploited by both commercial and recreational fisheries (Mather et al. 1995). Understanding their diet preferences is important given their high metabolic demands and potentially changing influence as a top predator with fluctuations in population abundance. Observed declines in somatic condition for this species (Golet et al. 2007; Neilson et al. 2007) further warrant detailed dietary analyses as diet shifts are a plausible explanation for such declines.

Stable isotopes can provide a complement to conventional and electronic tagging approaches (Cunjak et al. 2005) to also reveal connectivity among regional forage grounds. While isotope values cannot provide the level of detail captured by electronic tags, the lower costs and logistic challenges associated with SIA relative to electronic tagging could allow this technique to provide increased sample sizes. For regions where tagging is not possible, isotope values can be used to estimate past movements. Recent declines in ABFT abundance in U.S. NW Atlantic shelf waters have limited tagging operations in these regions while observed declines in condition for this assemblage

(Golet et al. 2007) have demonstrated a need for understanding residency and migration schedules for these forage grounds. While ABFT are known to spawn in the Gulf of Mexico (Baglin 1982; Mather et al. 1995), only a small percentage of tagged fish have actually been observed occupying this region (e.g., Lutcavage et al. 1999; Block et al. 2005; Wilson et al. 2005; Galuardi et al. submitted). Understanding connectivity between spawning grounds and regional forage grounds throughout the north Atlantic is important for understanding stock structure and linkages. Stable isotope techniques could help to resolve these movement questions.

Overall, my research objective was to help develop these evolving techniques and apply them to ecological studies of ABFT. Validation approaches will help to reduce uncertainty and ambiguity in future studies while applied components will help provide benchmarks for comparison with past and future studies. Jointly, these components will improve understanding of the trophic ecology of ABFT in the north Atlantic.

CHAPTER 1

METHODS DEVELOPMENT AND VALIDATION

Lipid Corrections in Carbon and Nitrogen Stable Isotope Analyses

Chemical Approaches

Introduction

Carbon and nitrogen stable isotopes are chemical tracers used to study food web dynamics (Peterson and Fry 1987; Kelly 2000) and recently, they have been used to identify previously unknown whereabouts and foraging patterns of highly mobile marine species (Krahn et al. 2007; Reich et al. 2007). Carbon stable isotope ratios ($^{13}C/^{12}C$; $\delta^{13}C$) tend to remain constant through trophic transfers (< 1 ‰ increase in $\delta^{13}C$) and provide information on food web primary production sources (Fry and Sherr 1984). Nitrogen stable isotope ratios ($^{15}N/^{14}N$; $\delta^{15}N$) tend to increase with trophic transfers (~ 3.4 ‰) and provide a more robust measure of trophic position (DeNiro and Epstein 1981; Post 2002). Small changes in $\delta^{13}C$ values from trophic transfers can be obscured by changes in biochemical composition by factors such as lipid content. Fish lipid $\delta^{13}C$ values are ~ 7 ‰ lower than protein (Sweeting et al. 2006), and high or variable lipid content can alter or obscure dietary $\delta^{13}C$ dynamics.

To remove potential bias, biological samples are treated by chemical lipid extraction prior to stable isotope analysis or corrected after using arithmetic normalization techniques (Kiljunen et al. 2006; Sweeting et al. 2006; Post et al. 2007).

Common lipid extractions include soxhlet extractions with chloroform (Godley et al. 1998; Thompson et al. 1999; Hodum and Hobson 2000; Forero et al. 2002) or ether (Lawson and Hobson 2000), exposure to chloroform-methanol mixtures (Hebert et al. 1999; Das et al. 2000; Takai et al. 2000; MacNeill et al. 2005), and hexane (Fry et al. 2003). Extractions using different solvents and techniques prior to SIA can produce significantly different $\delta^{13}C$ values for freshwater and marine fishes and invertebrates (Schlechtriem et al. 2003; Søreide et al. 2006). This variability makes it difficult to compare results from studies using different extraction methods. Differences in the amount of lipid removed among extraction methods (De Boer 1988; Smedes 1999; Iverson et al. 2001) likely causes variability in $\delta^{13}C$ values among methods, with more negative $\delta^{13}C$ values produced by less exhaustive methods relative to methods with higher lipid yields.

Chemical extraction methods also alter $\delta^{15}N$ for fish tissues (Pinnegar and Polunin 1999; Sotiropoulos et al. 2004; Murry et al. 2006; Sweeting et al. 2006). Since both $\delta^{13}C$ and $\delta^{15}N$ can be determined from a single sample with continuous flow isotope ratio mass spectrometry (CFIRMS), duplicate samples must be analyzed to derive accurate $\delta^{13}C$ (lipid-free tissue) and $\delta^{15}N$ (bulk tissue) values. The specific source of $\delta^{15}N$ alteration has not been determined, but removal of proteins attached to structural lipids by polar solvents has been proposed as a possible mechanism (Sotiropoulos et al. 2004; Sweeting et al. 2006; Bodin et al. 2007). Non-polar solvent extractions could provide reliable $\delta^{13}C$ and $\delta^{15}N$ values from a single sample (Sotiropoulos et al. 2004).

I applied several chemical extraction approaches to tissue samples from Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) and associated prey species to better understand

how these methods affect sample $\delta^{13}C$ and $\delta^{15}N$. To assess the impact of lipid content on $\delta^{13}C$ values and to test for possible $\delta^{15}N$ effects, samples were analyzed in duplicate (bulk tissue and lipid extracted with chloroform-methanol). To compare isotope values using chloroform-methanol extractions with a range of non-polar solvents (chloroform, hexane, and diethyl ether), aliquots of ABFT liver and white muscle and whole Atlantic herring (*Clupea harengus*) were analyzed without pre-treatment and following four different extraction methods. Sample C:N ratio (a proxy for lipid content in animal tissues) (Schmidt et al. 2003; Bodin et al. 2007; Post et al. 2007) was used to assess lipid removal for these different methods.

To test for possible impacts of nitrogenous waste removal on $\delta^{15}N$ values, samples of dogfish (*Squalus acanthias*) white muscle (n = 5) were analyzed in triplicate. Dogfish and other elasmobranchs retain urea in their tissues for osmoregulation (Smith 1929; Smith 1936), and provide a good model for urea effects on $\delta^{15}N$ values. Samples were analyzed as bulk tissue, following chloroform-methanol lipid extraction, and following urea extraction.

Methods

Sample Collection and Preparation

Samples of 168 marine fishes and invertebrates representing six families were analyzed for $\delta^{13}C$ and $\delta^{15}N$ with and without chloroform-methanol lipid extraction treatment (Table 1). Additional aliquots of ABFT liver and muscle and whole Atlantic herring were also analyzed following extraction with three different non-polar solvents. All samples were stored frozen before analysis. Comparisons of $\delta^{13}C$ and $\delta^{15}N$ between

lipid-extracted and bulk tissue samples were based on aliquots of dried, homogenized samples subjected to identical frozen storage conditions.

Liver and muscle samples were collected from ABFT and dogfish, thawed, lightly rinsed with deionized water, transferred to glass scintillation vials and dried at 60°C for at least 48 h while the remaining fishes and all invertebrates were dried whole (Table 1). Larger whole Atlantic herring, Atlantic mackerel (*Scomber scombrus*), silver hake (*Merluccius bilinearis*), sand lance (*Ammodytes* spp.), bluefish (*Pomatomus saltatrix*), and shortfin squid (*Illex illecebrosus*) were lightly rinsed with deionized water, then finely minced and dried in aluminum weigh boats at 60°C for at least 48 h until they reached a constant weight over three hours. Dried samples were then homogenized with a Wig-L-Bug® ball and capsule amalgamator (Crescent Industries, Auburn, Maine, U.S.A), Mixer/Mill® (SPEX SamplePrep, LLC Metuchen, New Jersey, U.S.A) and stainless steel grinding vials or a mortar and pestle, depending on tissue volume.

Two aliquots were removed from each homogenized sample; one aliquot was immediately prepared for SIA (see below), while the second underwent lipid extraction using a modification of the Bligh and Dyer (1959) method. Samples were dried at 60°C for 24 h to remove remaining solvent. Euphausiid samples also underwent acid washing after lipid extraction and drying to remove exoskeletal carbonates. Acid washing consisted of addition of 1 N HCl until bubbling ceased (Jacob et al. 2005), and the samples were re-dried at 60°C for 24 h.

Table 1. Marine fish and invertebrate species and tissue types (L = liver, M = muscle, W = whole body) analyzed for $\delta^{13}C$ and $\delta^{15}N$ as bulk tissue and following lipid extraction using chloroform-methanol.

Taxa	n	Tissue	Bulk C:N Range	$\delta^{13}C$ p-value	$\delta^{15}N$ p-value
Fishes					
Atlantic bluefin tuna (*Thunnus thynnus*)	82	M	3.1-6.5	$<0.001^{+*}$	$<0.001^{+}$
Atlantic bluefin tuna (*Thunnus thynnus*)	44	L	3.9-12.0	$<0.001^{+}$	0.242
Atlantic herring (*Clupea harengus*)	29	W	3.9-12.5	$<0.001^{+}$	1.000
Atlantic mackerel (*Scomber scombrus*)	8	W	3.5-9.2	$<0.001^{+}$	0.206
Silver hake (*Merluccius bilinearis*)	25	W	3.1-4.3	$<0.001^{+}$	1.000
Sand lance (*Ammodytes* spp.)	10	W	4.4-6.1	$<0.001^{+}$	$<0.001^{+}$
Bluefish (*Pomatomus saltatrix*)	8	W	3.8-6.3	$<0.001^{+}$	1.000
Spiny dogfish (*Squalus acanthias*)	5	M	4.4 – 6.1	$<0.001^{+}$	0.0267^{+}
Spiny dogfish (*Squalus acanthias*)	5	L	27.5 – 41.7	$<0.001^{+}$	0.9676
Invertebrates					
Krill (Euphausiidae)	10	W	3.7-4.3	$<0.001^{+}$	0.008^{+}
Shortfin squid (*Illex illecebrosus*)	13	W	3.8-4.5	$<0.001^{+}$	1.000

* P – values are Holm-adjusted for multiple comparisons and indicate significant increases ($^{+}$) or decreases ($^{-}$) in $\delta^{13}C$ or $\delta^{15}N$ following lipid extraction with an overall α of 0.05.

Chemical Extractions

Aliquots of all samples were extracted using a modification of the Bligh and Dyer (1959) method. Dried homogenized samples were immersed in a 2:1 ratio of chloroform-methanol with a solvent volume ~ 3-5 times > sample volume. Samples were then mixed for 30 s, left undisturbed for approximately 30 min, and centrifuged for 10 min at 3400

RPMs, and the supernatant containing solvent and lipids was then removed. This process was repeated at least three times or more until the supernatant was clear and colorless following centrifugation. Samples were re-dried at 60°C for 24 h to remove any remaining solvent.

Individual homogenized ABFT liver (n = 10), white muscle (n = 5), and whole Atlantic herring (n = 11) were each also extracted using a Goldfisch® apparatus (Labconco Corporation, Kansas City, Missouri, U.S.A) (a soxhlet-type refluxing instrument) with chloroform, diethyl ether, and hexane. Dried powdered aliquots of each sample were repeatedly flushed with a single solvent for 6-8 h. Samples were then allowed to dry in a fume hood for > 16 h, and transferred to a drying oven to be re-dried at 60°C for 24 h to remove any remaining solvent.

Individual homogenized samples of dogfish white muscle (n = 5) were also extracted with deionized water (Mathew et al. 2002). The deionized water extractions were performed on wet tissue samples, and samples were immersed in ~ 3:1 ratio v/v of deionized water to dogfish tissue for five min. The supernatant was then decanted, and the process was repeated two more times. Samples were then dried at 60°C and were prepared for stable isotope analysis following the same standard methods used for all samples.

Stable Isotope Analysis (SIA)

Aliquots of bulk tissue (non-treated) and lipid-extracted tissue of each sample ranging from 0.6 to 1.2 mg were packed into 4 x 6 mm tin cups. Samples were then analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen through CFIRMS using a DELTA^{plus} isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory of

Northern Arizona University or the University of New Hampshire Stable Isotope Laboratory. All C:N values are reported as uncorrected percent weight calculations. Measurements of commercially available reference materials across all runs were both accurate and precise with mean ± SD of -25.9 ± 0.1 ‰ for $\delta^{13}C$ and 2.0 ± 0.2 ‰ for $\delta^{15}N$ for NIST 1547 (peach leaves, n = 117) and -25.9 ± 0.1 for $\delta^{13}C$ and 1.0 ± 0.2 for $\delta^{15}N$ for acetanilide (n = 7). Replicate analyses of samples produced SD of 0.1 ‰ for $\delta^{13}C$ and 0.1 ‰ for $\delta^{15}N$ (n = 45).

All isotope data are reported in δ notation according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] * 1000$ where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Values are reported relative to international standards of Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on the VPDB and AIR scales with IAEA CH6 (-10.4 ‰), CH7 (-31.8 ‰), N1 (0.4 ‰), and N2 (20.3 ‰).

Statistical Methods

Differences in mean changes in C and N isotopes ($\delta^{13}C'$ - $\delta^{13}C$ and $\delta^{15}N'$ - $\delta^{15}N$) between lipid-extracted (denoted as $\delta^{13}C'$ and $\delta^{15}N'$) and bulk tissue $\delta^{13}C$ and $\delta^{15}N$ samples were tested for all samples. Levene's homogeneity of variance test was applied to each pairwise comparison. If no significant differences were detected between sample variances, differences were then determined using paired t-tests ($\alpha = 0.05$) and a subsequent Holm test to reduce the probability of committing type I errors as a result of multiple comparisons. If significant differences were detected between sample variances, subsequent comparisons were made using Welch's t-test.

For comparisons among extraction methods, C:N ratios were also statistically compared using paired t-tests with Holm-adjusted p-values ($\alpha = 0.05$). For $\delta^{13}C$ values and C:N ratios, paired comparisons were made within each tissue type among non-treated samples and samples chemically extracted using all four methods. For $\delta^{15}N$ values, paired comparisons were made between non-treated samples and samples pre-treated using each extraction method. Lipid content was compared between tissue types for the non-polar solvent analysis by using $\Delta C:N$ ($C:N_{bulk\ tissue} - C:N_{lipid\text{-}free}$) as a proxy for lipid content to account for differences in baseline $C:N_{lipid\text{-}free}$ between tissue types (Sweeting et al. 2006). Changes in C:N between bulk tissue and samples extracted using chloroform-methanol were used for lipid content comparisons since this method consistently caused the greatest C:N and $\delta^{13}C$ changes. An ANOVA and subsequent pairwise t-tests were used for lipid content comparisons between tissue types. These same statistical tests were performed to compare bulk, urea-extracted, and lipid-extracted dogfish sample $\delta^{15}N$ values. All analyses were performed using the program R (R Development Core Team 2008).

Results

Chloroform-methanol

Carbon stable isotope values were significantly enriched in ^{13}C following lipid extraction for all datasets analyzed (Figure 1; Table 1).

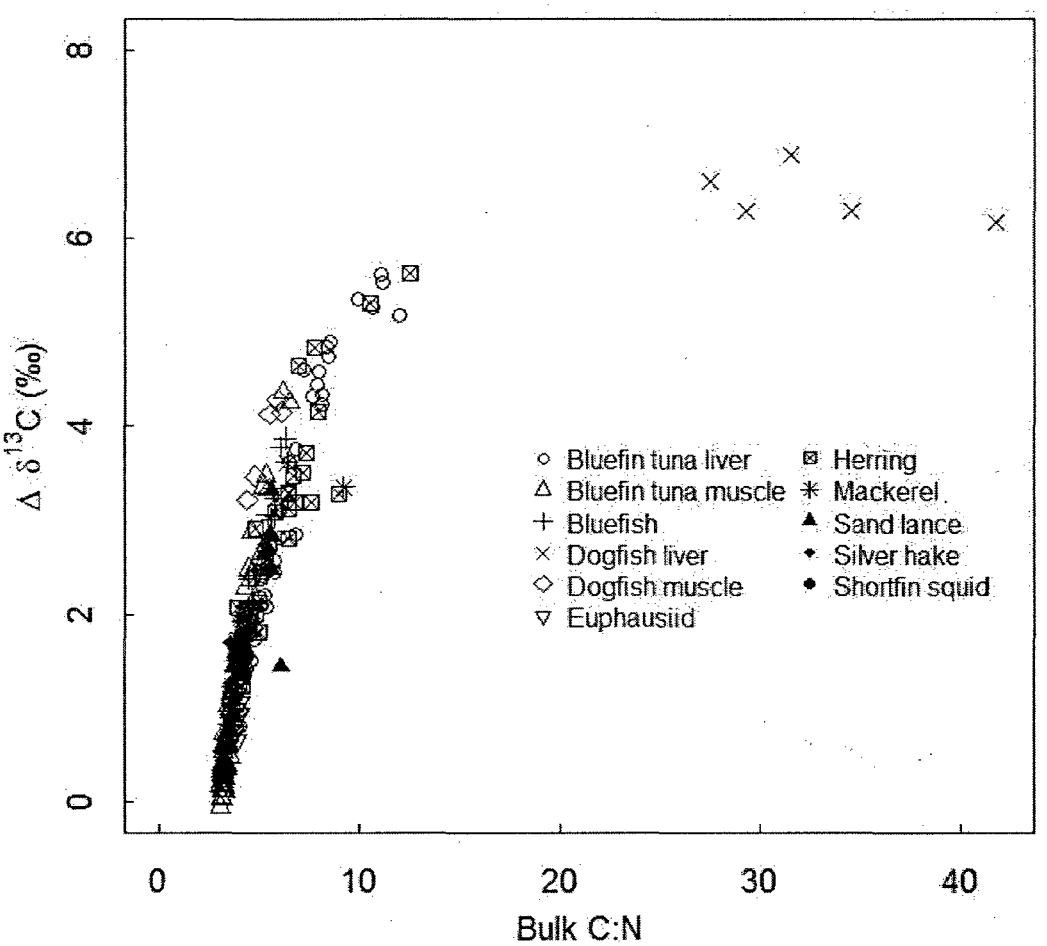

Figure 1. Isotope difference between bulk and lipid-extracted $\delta^{13}C$ values ($\Delta\ \delta^{13}C$) for marine fish and invertebrate samples relative to bulk tissue C:N.

Mean enrichment ranged from 1.0 ± 0.3 for whole euphausiids to 6.4 ± 0.3 for dogfish liver among tissue types analyzed (Table 1). Lipid-extracted C:N ranged from 3.1 (whole bluefish) to 3.6 (ABFT liver).

Among fish tissues, ABFT muscle and whole sand lance were significantly enriched in ^{15}N following extractions (Table 1). No other datasets were significantly altered. Alteration of $\delta^{15}N$ ranged from 0.0 ± 0.6 for whole shortfin squid to 0.7 ± 0.6 for

ABFT muscle (Table 1). Overall, there was a small $\delta^{15}N$ alteration associated with lipid extraction across the complete dataset with a best fit relationship of $\delta^{15}N_{lipid\text{-}free} = 0.948 * \delta^{15}N_{bulk} + 0.907$ (Figure 2; Table 1).

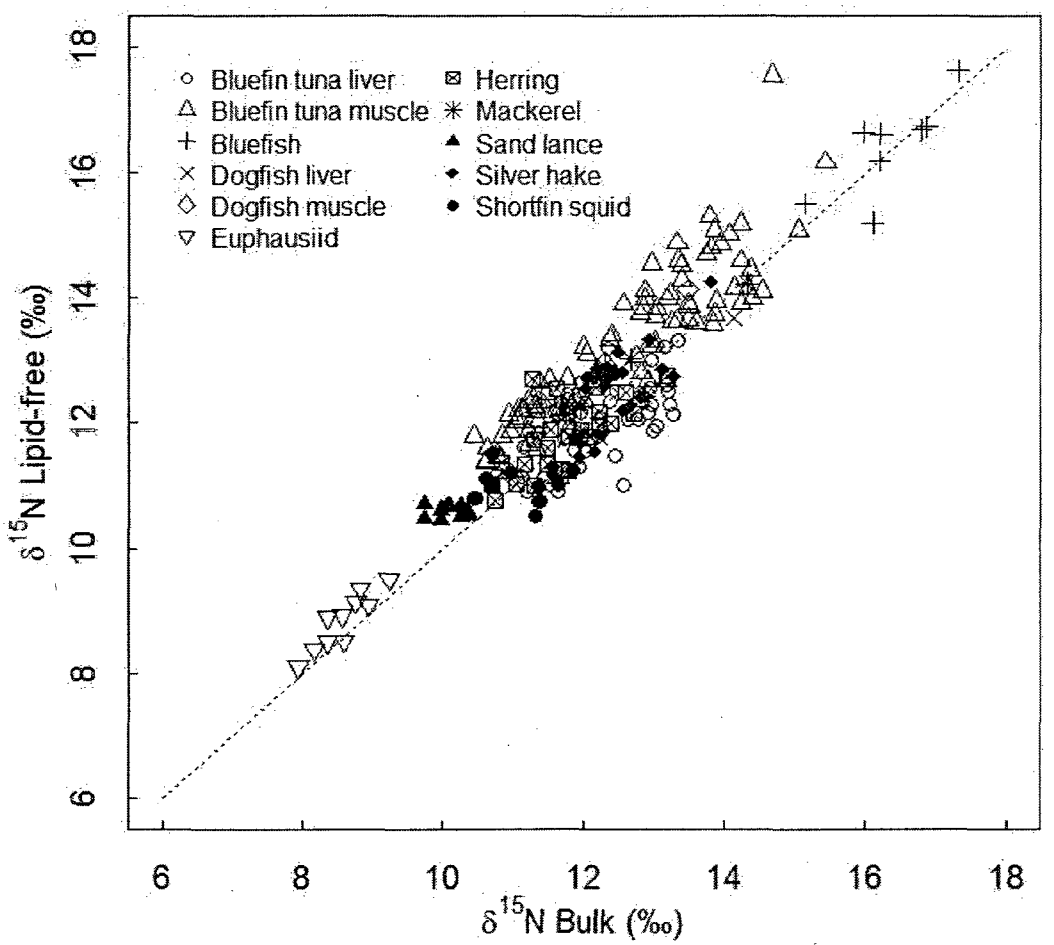

Figure 2. Bulk and lipid-extracted $\delta^{15}N$ values for marine fish and invertebrate samples. A 1:1 line is included to highlight shifts in $\delta^{15}N$ values following lipid removal.

For whole body invertebrates, C:N following extraction ranged from 3.2 – 3.4 (n = 23). Euphausiids were significantly enriched in ^{15}N following extractions while whole shortfin squid were not significantly altered (Table 1).

Solvent Comparisons

Homogeneity of variance

Lipid extractions did not significantly alter sample variance for any paired analyses of sample C:N, $\delta^{13}C$, or $\delta^{15}N$ (Levene's test, $p > 0.05$ using Holm adjustment). Paired t-tests were used for all comparisons between extracted and bulk tissue samples since assumptions of homogeneity of variance were not violated.

C:N ratios

C:N values were consistently lower for chloroform-methanol and chloroform-extracted samples than liver and whole herring samples extracted with diethyl ether or hexane (Figure 3). For bluefin tuna liver (bulk C:N = 6.3 ± 0.7 SE), C:N values were significantly different among all treated and non-treated pairs (Figure 3a). For bluefin tuna white muscle (bulk C:N = 3.3 ± 0.1 SE), only chloroform and diethyl ether samples were significantly different from each other (Figure 3b). In whole Atlantic herring (bulk C:N = 7.7 ± 0.6 SE), all extraction methods produced C:N values significantly lower than bulk tissue (Figure 3c). Chloroform-methanol and chloroform samples were both significantly different from diethyl ether and hexane, but not from each other, and diethyl ether was not significantly different from hexane (Figure 3c).

Differences in C:N between bulk tissue samples and samples extracted with chloroform-methanol were significantly different among the three tissue groups analyzed in this study (ANOVA, $p < 0.001$). Using these differences as proxies for bulk tissue lipid content, white muscle samples had significantly lower lipid content than liver (pairwise t-test, $p < 0.05$) and whole herring (pairwise t-test, $p < 0.001$), and liver had significantly lower lipid content than whole herring (pairwise t-test, $p < 0.05$).

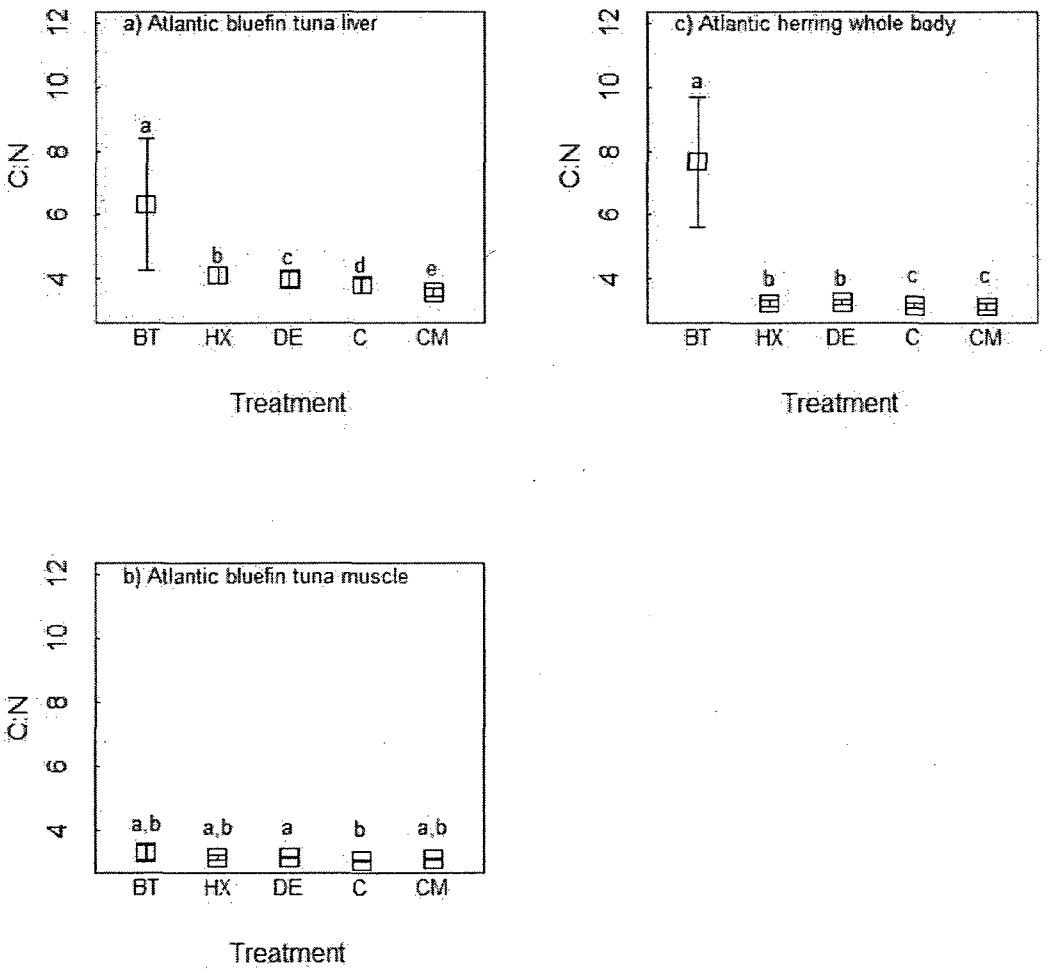

Figure 3. Mean ± SD C:N of a) Atlantic bluefin tuna (*Thunnus thynnus*) liver (n = 10) b) Atlantic bluefin tuna white muscle (n = 5) and c) whole body Atlantic herring (*Clupea harengus*) (n = 11) samples of bulk tissue (BT) and following lipid extraction with hexane (HX), diethyl ether (DE), chloroform (C), or chloroform-methanol (CM). Bars labeled with different letters are significantly different while bars with the same letter are not significantly different from each other.

Among the solvents analyzed, chloroform and chloroform-methanol extractions produced the highest $\delta^{13}C$ values (Figure 4). For bluefin tuna liver, all extraction methods produced significantly greater $\delta^{13}C$ values relative to bulk tissue samples, while values for all solvent types were also significantly different from each other (Figure 4a).

For bluefin tuna muscle, no solvents significantly altered $\delta^{13}C$ relative to bulk tissue samples, nor were there significant differences among solvent types (Figure 4b). For whole Atlantic herring, all extraction methods produced significantly greater $\delta^{13}C$ values relative to bulk tissue samples (Figure 4c). Chloroform-methanol samples were also significantly greater than diethyl ether and hexane samples, with no significant difference from chloroform samples. Chloroform was not significantly different from diethyl ether or hexane, and diethyl ether was not significantly different from hexane (Figure 4c).

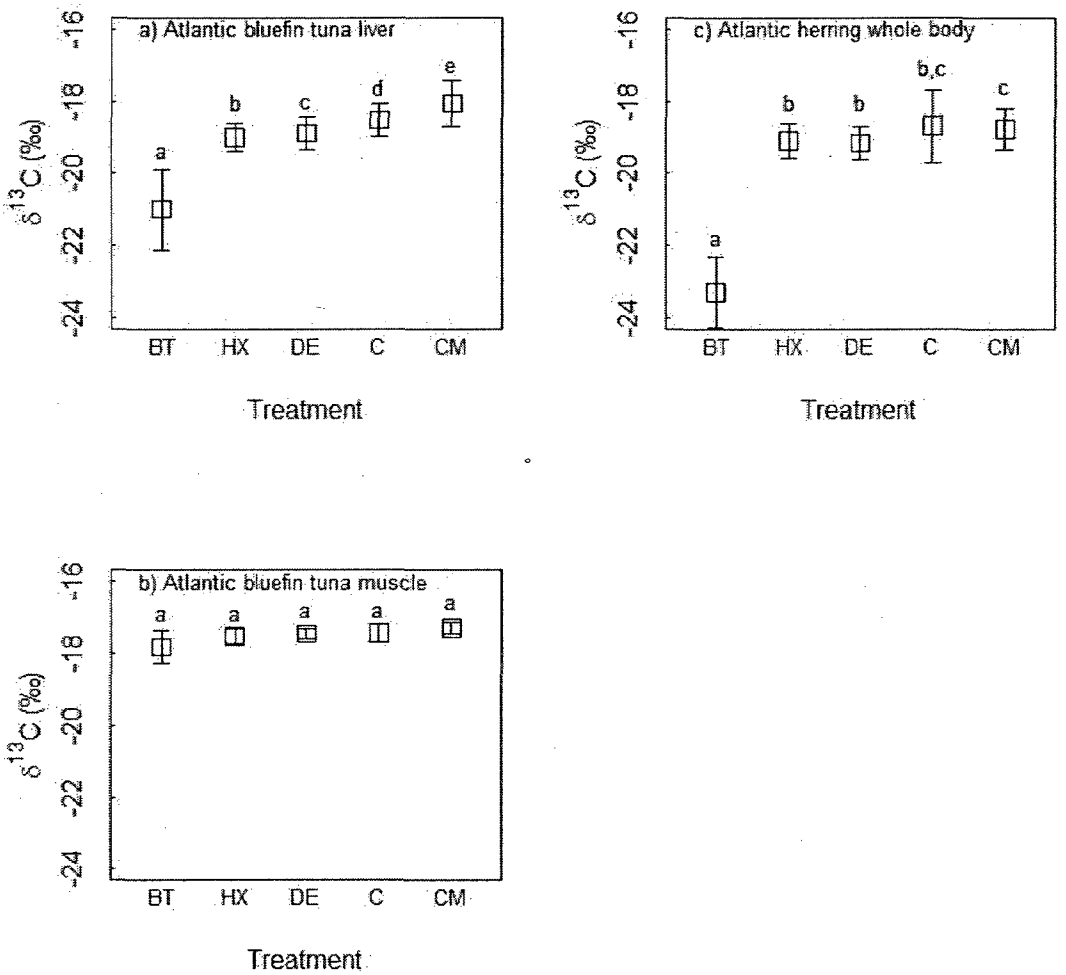

Figure 4. Mean ± SD $\delta^{13}C$ of a) Atlantic bluefin tuna (*Thunnus thynnus*) liver (n = 10) b) Atlantic bluefin tuna white muscle (n = 5) and c) whole body Atlantic herring (*Clupea harengus*) (n = 11) samples of bulk tissue (BT) and following lipid extraction with hexane (HX), diethyl ether (DE), chloroform (C), or chloroform-methanol (CM). Bars labeled with different letters are significantly different while bars with the same letter are not significantly different from each other.

Extraction effects on $\delta^{15}N$ values varied by tissue and solvent type (Figure 5). For example, in ABFT liver, chloroform and hexane significantly decreased $\delta^{15}N$ values relative to non-treated samples while chloroform-methanol and diethyl ether had no significant effect (Figure 5a). In ABFT muscle, all solvent types significantly altered

bulk tissue $\delta^{15}N$ (Figure 5b), while significant differences were not caused by any solvent in whole Atlantic herring (Figure 5c).

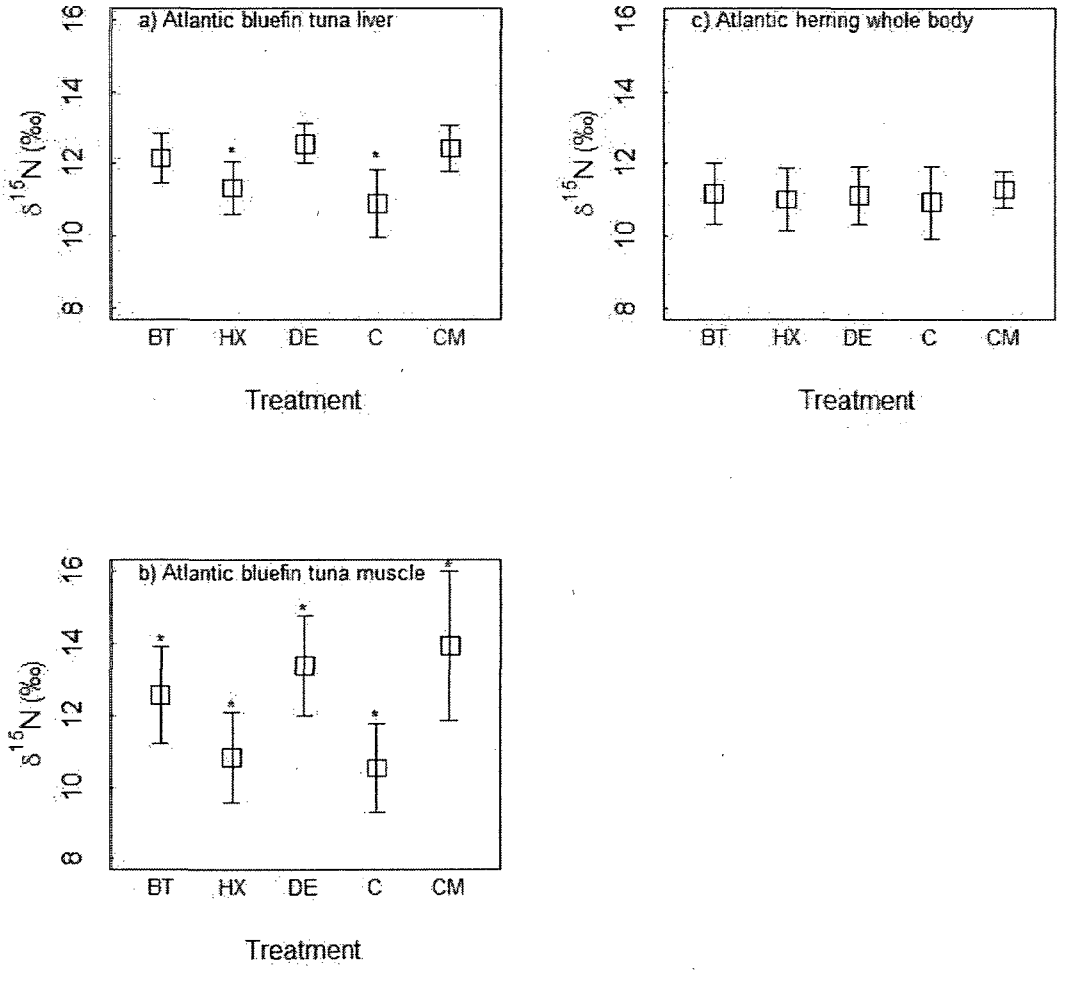

Figure 5. Mean ± SD $\delta^{15}N$ of a) Atlantic bluefin tuna (*Thunnus thynnus*) liver (n = 10) b) Atlantic bluefin tuna white muscle (n = 5) and c) whole body Atlantic herring (*Clupea harengus*) (n = 11) samples of bulk tissue (BT) and following lipid extraction with hexane (HX), diethyl ether (DE), chloroform (C), or chloroform-methanol (CM). Significant differences from bulk tissue samples are indicated by *.

Dogfish Muscle Solvent Comparisons

Assumptions of homogeneity of variance were not violated for any group of dogfish muscle samples (Levene's test, $p > 0.05$ using Holm adjustment), and muscle $\delta^{15}N$ values were significantly higher for lipid-extracted samples than bulk tissue samples ($p = 0.0267$). Muscle $\delta^{15}N$ values did not significantly differ between bulk tissue and urea-extracted samples ($p = 0.4727$) or between urea- and lipid-extracted samples ($p = 3916$).

Discussion

Lipid extractions caused significant increases in $\delta^{13}C$ for almost all species and tissue types, indicating the need to correct for lipid carbon isotope effects, especially when fine scale $\delta^{13}C$ differences are compared. While lipid extractions caused statistically significant increases in $\delta^{13}C$ relative to bulk tissue samples, observed differences were not always biologically significant, with some tissues showing limited $\delta^{13}C$ change. Fish white muscle, for example, typically contains minimal lipids and may not benefit from lipid correction or extraction (Pinnegar and Polunin 1999). In fish muscle samples, bulk C:N values < 3.4 generally produced $\delta^{13}C$ changes < 0.7 ‰. In contrast, fish liver has high lipid content and should be lipid corrected (Sweeting et al. 2006); ABFT liver samples had C:N > 10 and lipid extraction changed $\delta^{13}C > 5$ ‰. For highly migratory fish species like ABFT, lipid corrections may be necessary for both liver and white muscle, as lipid stores can vary seasonally with migration schedules (Mourente et al. 2001).

Bulk liver and white muscle tissue $\delta^{13}C$ values vary seasonally in ABFT in Gulf of Maine forage grounds, with a range of > 4 ‰ in late season (Figure 6). Lipid

corrected values are instead fairly constant over time, with both tissues having values of ~ -18 ‰ (Figure 6). Failure to account for lipid effects for ABFT in this region would result in false detections of seasonal diet shifts, high individual variability in diet, and low trophic position.

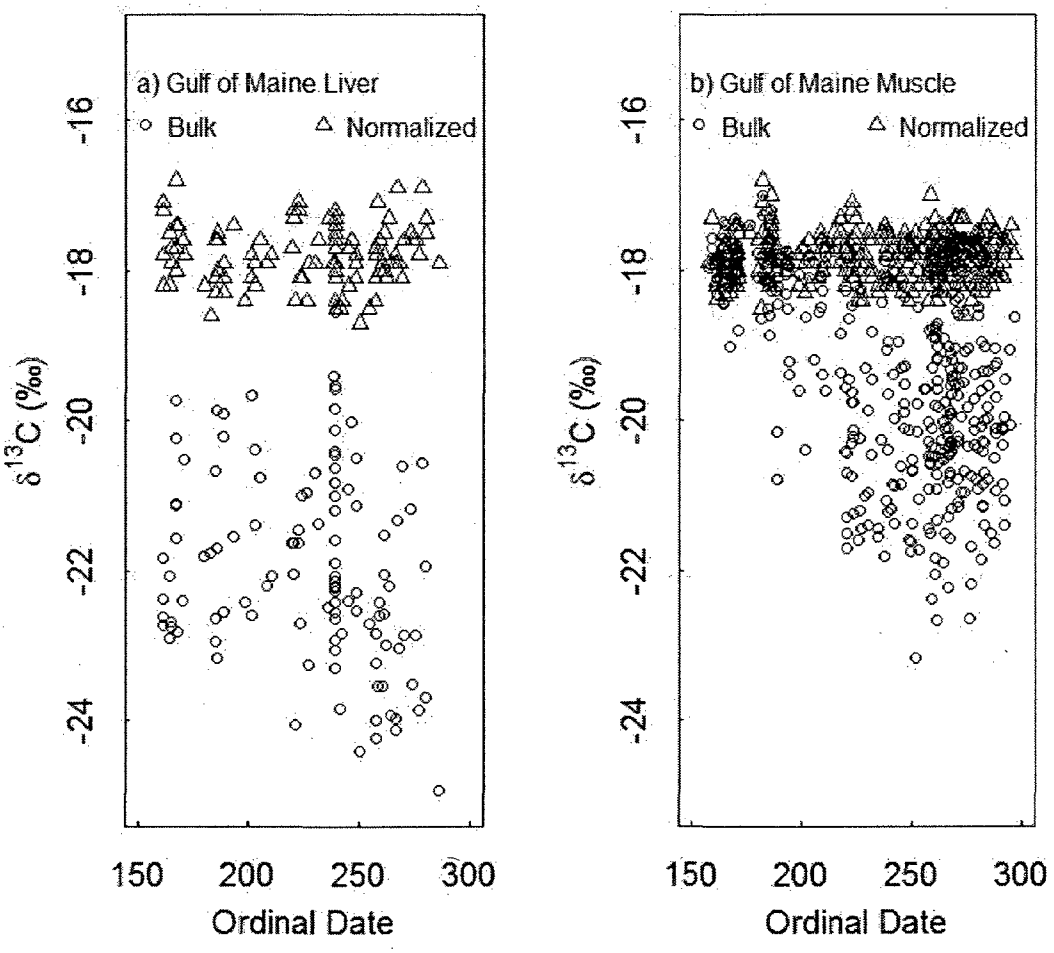

Figure 6. Bulk and lipid-corrected $\delta^{13}C$ values of a) liver and b) white muscle from adult Atlantic bluefin tuna (*Thunnus thynnus*) in Gulf of Maine forage grounds.

Since lipid content of whole body fish and invertebrate samples are highly variable, bulk C:N provided a less clear predictor of change in $\delta^{13}C$ from lipid removal. Lipid-free C:N varied by species and certain tissues showed a moderate $\delta^{13}C$ response to extraction despite high $C:N_{bulk}$. For example, euphausiids have bulk C:N ~ 4, but lipid extraction increased $\delta^{13}C$ by ~ 1 ‰.

Given the broad range of ecological studies that use carbon isotopes, the biological significance of $\delta^{13}C$ changes will vary by study and the ecological questions being posed. With estimation of marine vs. terrestrial (Bearhop et al. 1999) or C-3 vs. C-4 or CAM primary producer contributions to diet (Peterson et al. 1985; Wolf et al. 2002), $\delta^{13}C$ end members may be sufficiently distinct, and lipid correction will not alter isotope data interpretation. Where end-members are less isotopically distinct (e.g., Abend and Smith 1997), proper lipid correction may be critical in determining food sources for consumers. Decisions regarding lipid correction will ultimately be decided by the scientific questions being addressed in a given study.

Overall, each tissue dataset had greater than a per mil mean increase in $\delta^{13}C$ following extraction, deviations that would alter interpretations of results in many food web studies. The implication is that an analysis of bulk tissue C:N values and correction of $\delta^{13}C$ through chemical extractions or modeling approaches may be necessary. Alternatively, transitioning from bulk $\delta^{13}C$ and $\delta^{15}N$ analyses to compound-specific analyses (Hammer et al. 1998; Fantle et al. 1999) could remove variability due to isotopic heterogeneity.

Lipid extractions with chloroform-methanol altered sample $\delta^{15}N$ to varying degrees (e.g., sample $\delta^{15}N$ range relative to nontreated samples of -2.4 – +2.9 ‰).

Increases in $\delta^{15}N$ occurred for ABFT white muscle and fish and invertebrate whole body samples. Fish liver $\delta^{15}N$ values were not significantly altered by chloroform-methanol extractions, and the total dataset had minimal $\delta^{15}N$ alteration. Previous studies for individual species have found significant increases in $\delta^{15}N$ associated with lipid extraction, including tissues such as fish white muscle, whole bodies, and liver (Pinnegar and Polunin 1999; Sotiropoulos et al. 2004; Murry et al. 2006). These conflicting results support caution in subjecting all samples to lipid extraction prior to $\delta^{15}N$ analysis and the need for additional studies to determine the specific mechanism for $\delta^{15}N$ alteration.

For chemical lipid extractions applied to fish tissues for carbon and nitrogen SIA, non-polar solvents also caused $\delta^{15}N$ alteration previously observed in chloroform-methanol extractions (Pinnegar and Polunin 1999; Sotiropoulos et al. 2004; Sweeting et al. 2006). This latter method actually has less impact on $\delta^{15}N$ than chloroform and hexane, significantly altering only one (white muscle) of the three tissue types examined. Chloroform-methanol extractions are also more effective than the single nonpolar solvents chloroform, hexane, or diethyl ether, as chloroform-methanol extractions produced the lowest C:N ratios and highest $\delta^{13}C$ values.

Using C:N as a proxy for sample lipid content, more significant differences were found among methods for higher lipid content liver tissue and whole herring relative to low lipid white muscle, suggesting that choice of extraction method is more critical for higher lipid content samples. Similarly, no significant differences in lipid estimates were detected among methods for low lipid (~ 1 %) fish muscle (Honeycutt et al. 1995), but differences were detected between methods for higher lipid content (> 2 %) marine fishes and invertebrates (Iverson et al. 2001). Among all methods, differences in $\delta^{13}C$ were

greatest for liver tissue, and showed about a 1 ‰ separation among extraction methods, a range typically identified as a full trophic position shift (DeNiro and Epstein 1978; Fry and Sherr 1984; Peterson and Fry 1987). Chloroform-methanol extractions also produced higher percent lipid estimates for bluefish livers relative to semipolar and nonpolar solvent systems (Randall et al. 1998). This variability among methods is also reflected in the range of mean C:N values (3.6 - 4.1) for lipid extracted liver samples. Whole Atlantic herring had a separation across methods of about 0.5 ‰ and a narrower mean C:N range (3.1 - 3.3).

As expected, low lipid white muscle samples had minimal variation in $\delta^{13}C$ while higher lipid content liver and whole herring had significant differences between non-treated and chemically extracted samples. Lipid-extracted white muscle from this study (mean $C:N_{lipid\text{-}extracted} = 3.1$) and from other marine and freshwater fish species (mean $C:N_{lipid\text{-}extracted} = 3.1$ (Kiljunen et al. 2006) and 3.2 (Logan et al. 2008)) are only slightly lower than bulk C:N values observed in this study. Variation in $\delta^{13}C$ values among all methods was about 0.2 ‰, similar to analytical precision. Consequently, these results suggest that lipid extractions may not be necessary for low lipid tissues such as fish white muscle. However, larger sample sizes may yield a broader range of bulk C:N values and call for lipid correction of outlier samples (see Figure 6). All extraction methods produced $\delta^{13}C$ values more than 2 ‰ above bulk tissue for liver and 4 ‰ for whole herring, values which could be interpreted as indicative of multiple trophic level transfers (DeNiro and Epstein 1978; Fry and Sherr 1984; Peterson and Fry 1987). These larger variations between extracted and bulk tissue samples indicate that lipid correction should

be used in these tissue types when source end member $\delta^{13}C$ values are within the range of variability caused by lipid content.

Significant differences in $\delta^{13}C$ between samples analyzed with chloroform-methanol and single nonpolar solvent extractions were not surprising, because these solvents do not remove complex or "polar" lipids, but $\delta^{13}C$ values did not vary between methods strictly in relation to total lipid content. Given that liver samples had lower lipid contents than herring samples, and greater changes in $\delta^{13}C$ were observed for liver samples than herring, tissue-specific differences in attributes such as relative proportions of polar and nonpolar lipids are also likely to affect extraction efficiency. Polar lipids comprised approximately 15 % of total lipids in liver tissues from hatchery reared lake trout (*Salvelinus namaycush*), while carcass total lipids contained less than 1 % polar lipids (Hoffman et al. 1999). Percent lipid removed should increase for nonpolar solvents as the percentage of polar lipids decreases.

Nonpolar solvents also removed N-containing compounds despite there being reduced lipid removal relative to chloroform-methanol. While observed $\delta^{15}N$ alteration (1-2 ‰) was less than typical trophic level discrimination factors (DeNiro and Epstein 1981; Post 2002), changes in $\delta^{15}N$ were similar to many measured fish diet-tissue discrimination factors (Herzka and Holt 2000; Logan et al. 2006), and could create errors in isotope mixing models (Phillips and Gregg 2001; McCutchan Jr et al. 2003). Alteration of $\delta^{15}N$ occurred with weak single nonpolar solvents. This suggests that while removal of amino acids associated with polar structural lipids may account for $\delta^{15}N$ differences in certain instances, other mechanisms must also contribute to $\delta^{15}N$ alteration. Shifts in $\delta^{15}N$ following extractions were greatest for the low lipid content white muscle

sample dataset, similar to negative relationships observed between bulk tissue lipid content and extraction-induced $\delta^{15}N$ changes within fish white muscle (Ingram et al. 2007) and liver (Sweeting et al. 2006) datasets. These results indicate that $\delta^{15}N$ changes cannot simply be normalized based on total lipid content. Since $\delta^{15}N$ changes are greatest when little lipid is removed, the $\delta^{15}N$ value of the extract component must vary widely from the remaining pool to create a detectable shift in the extracted sample.

Removal of nitrogenous waste products by lipid extraction procedures may also alter $\delta^{15}N$ values (Bearhop et al. 2000). If observed $\delta^{15}N$ increase following chloroform-methanol and diethyl ether extractions is mainly due to removal of nitrogenous waste, bulk $\delta^{15}N$ values would be less accurate than lipid extracted $\delta^{15}N$. Significant increases in $\delta^{15}N$ values were only observed for white muscle samples extracted with chloroform-methanol and diethyl ether. Observed decreases in $\delta^{15}N$ values following chloroform and hexane extractions of bluefin tuna liver and white muscle suggest that other nitrogenous compounds are being removed, since nitrogenous waste has lower $\delta^{15}N$ values relative to consumer tissues (Steele and Daniel 1978; Peterson and Fry 1987). Given that $\delta^{15}N$ values of dogfish white muscle were significantly higher in samples lipid extracted with chloroform-methanol than bulk tissue samples, but urea-extracted sample $\delta^{15}N$ values did not differ from bulk values, observed differences between lipid-extracted and bulk samples are not caused solely by removal of nitrogenous waste. Relative to bulk tissue values, individual amino acid $\delta^{15}N$ values vary widely (Hare and Estep 1983; McClelland and Montoya 2002; Montoya et al. 2002; Schmidt et al. 2004), and preferential removal of different amino acids by different extraction methods could produce significant $\delta^{15}N$ increases (e.g., threonine removal) and decreases (e.g., glutamic acid removal) for a given

tissue type as observed in this study. Following these results, alteration of protein nitrogen is a more likely explanation of extraction-induced $\delta^{15}N$ alteration than nitrogenous waste removal.

In summary, extraction methods produced different $\delta^{13}C$ values, and all methods altered $\delta^{15}N$ for some subsets of tested fish tissues. For these reasons, 2:1 chloroform-methanol extractions appear to offer the most exhaustive extraction approach, but these methods should follow initial paired analysis of bulk tissue and treated samples to test whether extractions will induce $\delta^{15}N$ alteration. Future studies should directly test for mechanisms of $\delta^{15}N$ alteration following chemical extractions and further examine modeling alternatives to better resolve this central problem of lipid correction for ecological studies using carbon and nitrogen SIA.

Mathematical Approaches

Introduction

Several different mathematical normalization equations have been applied in stable isotope studies of food webs (Kelly 2000), but a standard protocol for lipid correction does not exist. Most mathematical corrections use elemental carbon to nitrogen ratios (C:N) of bulk tissue as a proxy for lipid content in estimating $\delta^{13}C$ of lipid-free tissue (McConnaughey and McRoy 1979; Fry 2002; Kiljunen et al. 2006; Sweeting et al. 2006; Post et al. 2007). Through the use of an elemental analyzer, solid biological samples are combusted at high temperature and converted into CO_2 and N_2 gases (Jardine and Cunjak 2005), a process that also allows for quantification of sample % C and % N. Since lipids are composed mainly of carbon and most lipid classes contain

no nitrogen, increases in C:N ratios closely track increases in lipid content in animal tissues (Schmidt et al. 2003; Bodin et al. 2007; Post et al. 2007; Logan et al. 2008).

Lipids are a major component of energy flow in food webs, and valuable ecological information is lost when they are extracted (Arts et al. 2001). Since C:N ratios increase linearly with lipid content, analysis of bulk tissue samples can provide information on body condition (Estrada et al. 2005) in addition to diet and movement. Given observed effects of chemical extractions on $\delta^{15}N$ values and the importance of lipid data in many ecological studies, alternative mathematical approaches to lipid correction for $\delta^{13}C$ were explored.

Methods

To determine the relationship between bulk tissue C:N and % lipid for marine fishes, a quantitative modification of the Folch et al. (1957) method was performed on Atlantic bluefin tuna white muscle samples. Frozen samples were thawed and homogenized. Two aliquots of the homogenate, each weighing ~ 1.5 g, were weighed (± 0.0001 g) and immersed in 30 ml of 2:1 chloroform-methanol for ~ 16 hours. Contents were then poured over Whatman filter paper into glass centrifuge tubes. Deionized water with 0.88 % NaCL (7 ml) was added, and the centrifuge tube was capped and inverted to mix the solution. Each tube was then centrifuged (~ 1,000 RPMs) for 20 minutes, and the upper aqueous phase was then removed. The remaining solvent phase was then pipetted over $NaSO_4$ into a pre-weighed glass centrifuge tube. The tube was then immersed in a water bath (~ 24°C), and the surface was agitated with a constant stream of N_2 gas until all solvent was removed. The remaining lipid fraction was then weighed ± 0.0001 g and % lipid was calculated using the formula

$$\% \, lipid = \frac{lipid \; weight \; (g)}{wet \; weight \; (g)} * 100$$

Statistical Models and Analyses

The accuracy of various mathematical correction approaches was assessed by comparing model fits and estimations of lipid-free $\delta^{13}C$ using paired $\delta^{13}C$ data from the chloroform-methanol fish and invertebrate dataset (Table 1) and additional paired data (Table 2) using the same extraction method derived from the literature (Sweeting et al. 2006; Logan et al. 2008).

To compare lipid extraction results with those expected by applying several models previously described in the literature, log-likelihood values (assuming normally distributed errors) were calculated for models described by McConnaughey and McRoy (1979), Kiljunen et al. (2006), Fry (2002), and Sweeting et al. (2006). The Akaike Information Criterion (AIC_c) value was also calculated for each model. AIC_c values were determined according to the equation

$$AIC_c = -2 * log - likelihood + 2k + \frac{2k(k+1)}{n-k-1}$$

where k equals the number of parameters and lower AIC_c values correspond to improved model fits. AIC_c values are also presented as AIC differences (Δ_i) according to the equation

$$\Delta_i = AIC_{ci} - minAIC_c,$$

where AIC_{ci} corresponds to the AIC_c value for model *i* and min AIC_c is the model with the lowest AIC_c value among tested models (Burnham and Anderson 1998). AIC_c differences were calculated between individual models. Models with Δ_i of about 0-2 have substantial support as best model fits, Δ_i of 4-7 indicates considerably less support,

and $\Delta_i > 10$ provide essentially no support for a given model (Burnham and Anderson 1998). All tested models use bulk tissue C:N as a predictor of $\delta^{13}C' - \delta^{13}C$.

Table 2. Freshwater (FW) and marine (M) fish and invertebrate species and tissue types (L = liver, M = muscle, W = whole body) used in lipid correction modeling exercises.

Taxa	n	Habitat	Tissue	Bulk C:N Range
Fishes				
American eel (*Anguilla rostrata*)[a]	50	FW	M	3.3-9.8
Brook trout (*Salvelinus fontinalis*)[a]	12	FW	M	3.1-3.6
Bony bream (*Nematalosa erebi*)[a]	36	FW	W	2.9-8.0
Golden perch (*Macquaria ambigua*)[a]	36	FW	W	3.2-6.4
Silver tandan (*Porochilus argenteus*)[a]	36	FW	W	3.3-12.1
Spangled perch (*Leiopotherapon unicolor*)[a]	29	FW	W	2.7-9.3
Atlantic bluefin tuna (*Thunnus thynnus*)	82	M	M	3.1-6.5
Atlantic bluefin tuna (*Thunnus thynnus*)	44	M	L	3.9-12.0
Atlantic herring (*Clupea harengus*)	11	M	W	6.3-12.5
Silver hake (*Merluccius bilinearis*)	12	M	W	3.5-4.1
European sea bass (*Dicentrarchus labrax*)[b]	47	M	L	6.4-21.7
European sea bass (*Dicentrarchus labrax*)[b]	53	M	M	3.3-4.1
Invertebrates				
Mayflies (Heptageniidae)[a]	26	FW	W	5.2-10.7
Stoneflies (Perlidae)[a]	15	FW	W	4.3-5.3
Water pennies (Psephenidae)[a]	12	FW	W	4.3-7.5
Water striders (*Aquarius remigis*)[a]	12	FW	W	4.1-6.0
Krill (Euphausids)	10	M	W	3.7-4.3

* Data for model fitting were partly derived from literature values published in Logan et al. (2008)[a] and Sweeting et al. (2006)[b].

The first tested model form is based on the McConnaughey and McRoy (1979) model (eq. 1),

$$\delta^{13}C' - \delta^{13}C = D(\theta + \frac{3.90}{1 + \frac{287}{L}})$$

(1)

where $$L = \frac{93}{1+(0.246*C:N-0.775)^{-1}}$$

and L and D represent sample lipid content and protein-lipid discrimination, respectively. McConnaughey and McRoy (1979) assumed D and θ (a constant term relating to the x-intercept) are known (D = 6 and θ = -0.207). These values set $C:N_{lipid\text{-}free}$ to equal four, forcing any bulk tissue C:N values less than four to predict $\delta^{13}C'$ - $\delta^{13}C$ as negative values. The Kiljunen et al. (2006) model (eq. 1a) is the same as the original McConnaughey and McRoy (1979) model (eq. 1), but where D and θ are based on a dataset of fish muscle tissue with values of 7.018 and 0.048, respectively. These values set $C:N_{lipid\text{-}free}$ to equal 3.003. The McConnaughey and McRoy (1979) model (eq. 1) was also fit with D and θ based on a dataset of aquatic fishes and invertebrates (Table 2) as eq. 1b. A new generalized model based on eq. 1 was developed that maintains the non-linear relationship of the difference in $\delta^{13}C$ between bulk tissue and lipid-extracted tissue, but aggregates assumed values into three parameters,

$$\delta^{13}C' - \delta^{13}C = \frac{a * C:N + b}{C:N + c}$$

(1c)

The y-asymptote, or D in eq. 1, corresponds to a in eq. 1c. The model estimate $C{:}N_{lipid\text{-}free}$ is represented by $-b/a$ (x-intercept), whereas b/c (y-intercept) is the $\delta^{13}C$ difference corresponding to a C:N value of zero (Table 3).

The second tested model form is based on the Fry (2002) model (eq. 2),

$$\delta^{13}C' - \delta^{13}C = P - \frac{P * F}{C{:}N} \quad \textbf{(2)}$$

where P and F represent protein-lipid discrimination and $C{:}N_{lipid\text{-}free}$, respectively. The Fry (2002) model (eq. 2) assigns a value of six for P and 3.7 for F. The Sweeting et al. (2006) model (eq. 2a) is

$$\delta^{13}C' - \delta^{13}C = \frac{B(C{:}N_{sample} - C{:}N_{protein})}{C{:}N_{sample}} \quad \textbf{(2a)}$$

with protein-lipid discrimination (B) assumed to be 7.08 and $C{:}N_{protein}$ and $C{:}N_{sample}$ corresponding to lipid-extracted and bulk tissue C:N, respectively. If B and $C{:}N_{protein}$ are assigned values equal to P and F, the Sweeting et al. (2006) model (eq. 2a) condenses to equal the Fry (2002) model (eq. 2). The Fry (2002) model (eq. 2) was also fit with P and F based on a dataset of aquatic fishes and invertebrates (Table 2) as eq. 2b.

A new model of the difference in $\delta^{13}C$ between bulk and lipid-extracted tissue and log-transformed C:N,

$$\delta^{13}C' - \delta^{13}C = \beta_0 + \beta_1 \ln(C{:}N) \quad \textbf{(3)}$$

was also explored. The model estimate of $C{:}N_{lipid\text{-}free}$ is represented by $e^{\frac{-\beta_0}{\beta_1}}$.

Normally distributed error terms, $\varepsilon \sim N(0,\sigma^2)$, were assumed and models were fit based on eqs. 1b, 1c, 2b, and 3 to data from different tissue types and species with sample sizes greater than 10 (Table 2). For fish species, three nested models of a given type were fitted. The simplest model assumed all model parameters (D, θ and σ^2 for eq. 1b, a, b, c and σ^2 for eq. 1c, P, F and σ^2 for eq. 2b, and β_0, β_1 and σ^2 for eq. 3) were the same across species and tissue types, the intermediate model assumed that the parameters were tissue-specific and the full model assumed that parameters were both tissue and species-specific. For invertebrate species, two models of a given type (based on eqs. 1b, 1c, 2b, or 3) were fitted where the simpler model again assumed all model parameters were the same across species and the full model assumed parameters were species-specific. Likelihood ratio tests were performed for each model type and species group (fish or invertebrates) to determine the most parsimonious models. Parameters were estimated for all models using least-squares procedures available in R (R Development Core Team 2008).

Results

Bulk tissue C:N maintained a strong linear relationship with % lipid in ABFT muscle (Figure 7; $r^2 = 0.9662$), and a closer asymptotic relationship with the observed change in $\delta^{13}C$ due to extraction in fish tissues (Figure 8) than whole invertebrates (Figure 9). The x-intercepts for the simplest fish tissue models (liver, muscle, and whole body combined) were 3.02 (eq. 1c) and 0.962 (eq. 3), indicating a C:N of 2.6 (calculated as $e^{0.962}$) for eq. 3 for pure protein, or the C:N at which no change in $\delta^{13}C$ occurred due to lipid extraction (Table 3). A best fit equation for quantitative lipid extractions of ABFT muscle produced a $C:N_{lipid\text{-}free}$ of 2.9 (Figure 7).

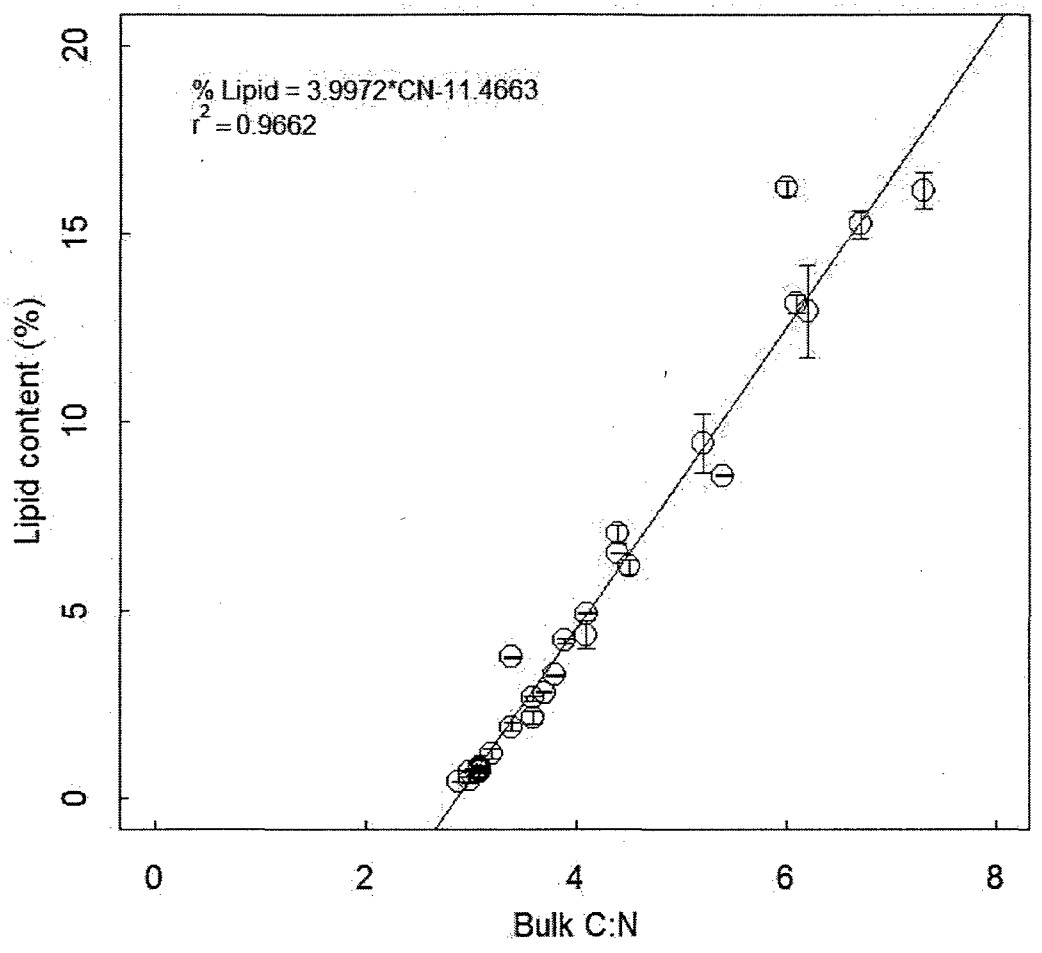

Figure 7. Relationship between bulk C:N and % lipid content ± SD for Atlantic bluefin tuna (*Thunnus thynnus*) white muscle. Lipid content estimates are based on gravimetric estimates on wet tissue using a 2:1 chloroform-methanol extraction (Folch et al. 1957).

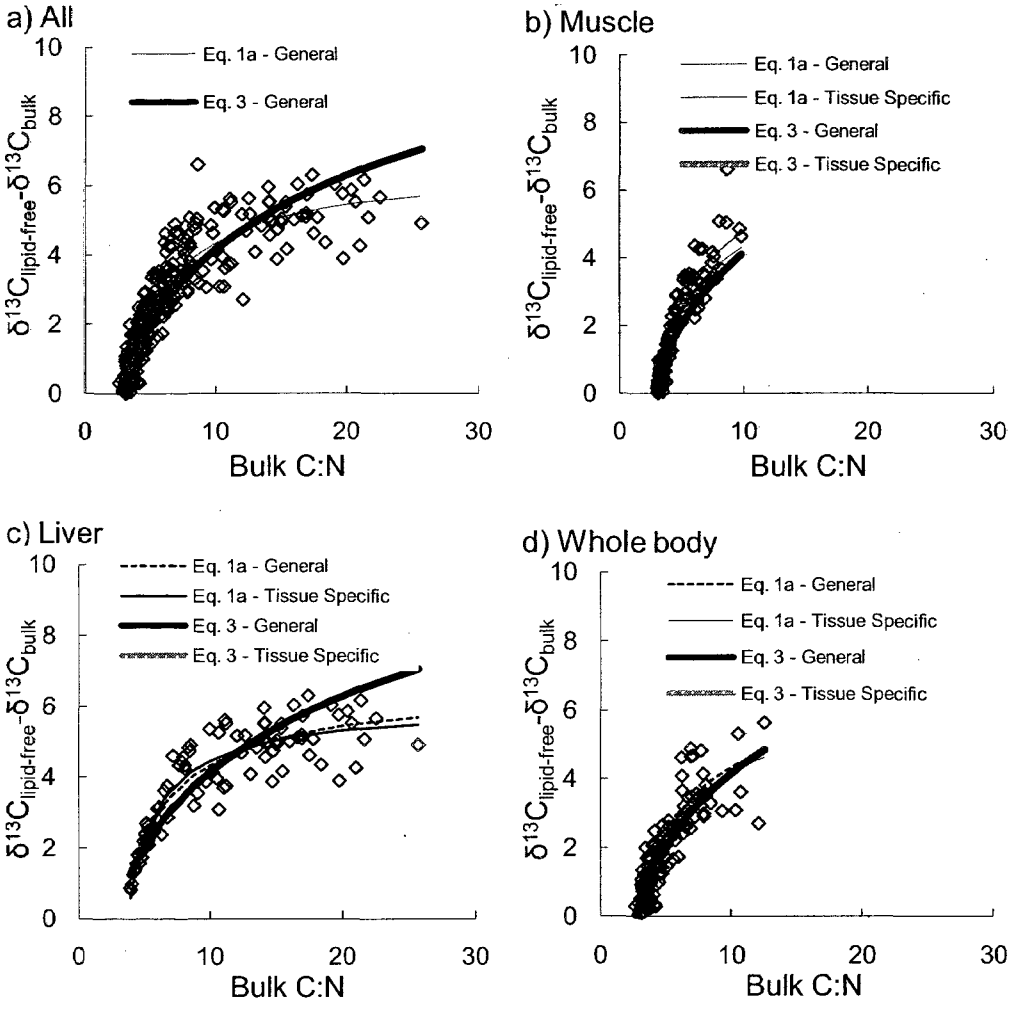

Figure 8 a,b,c,d. Model fits to differences in fish tissue bulk $\delta^{13}C$ and lipid-free $\delta^{13}C$ following lipid extractions with 2:1 chloroform-methanol. Models were fit a) to a general dataset of fish muscle, liver, and whole body paired $\delta^{13}C$ samples, and b-d) to tissue-specific datasets. Equations defined in Methods section for Lipid Corrections in Carbon and Nitrogen Stable Isotope Analyses – Mathematical Approaches.

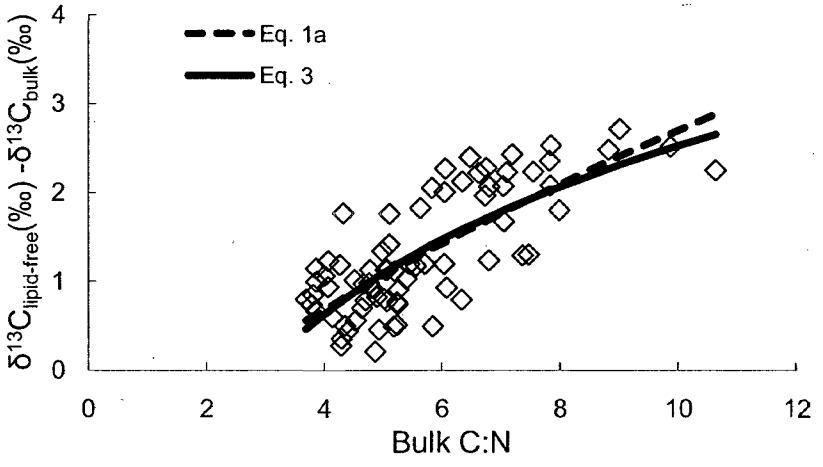

Figure 9. Model fits to differences in whole body invertebrate bulk $\delta^{13}C$ and lipid-free $\delta^{13}C$ following lipid extractions with 2:1 chloroform-methanol. Equations defined in Methods section for Lipid Corrections in Carbon and Nitrogen Stable Isotope Analyses – Mathematical Approaches.

Table 3. Model estimates of $C{:}N_{lipid\text{-}free}$ and protein-lipid discrimination for fish liver, muscle, and whole bodies (Table 2).

Model	Dataset	$C{:}N_{lipid\text{-}free}$	Protein-lipid Discrimination
Eq. 1b $\delta^{13}C' - \delta^{13}C = D(\theta + \frac{3.90}{1+\frac{287}{L}})$	All	3.09	6.34
	Liver	3.33	7.21
	Muscle	3.10	6.83
	Whole	3.01	5.50
Eq. 1c $\delta^{13}C' - \delta^{13}C = \frac{a * C{:}N + b}{C{:}N + c}$	All	3.02	6.63
	Liver	3.68	6.06
	Muscle	3.07	7.42
	Whole	2.87	6.98
Eq. 2b $\delta^{13}C' - \delta^{13}C = P - \frac{P * F}{C{:}N}$	All	3.08	6.23
	Liver	3.31	6.52
	Muscle	3.10	6.70
	Whole	3.00	5.57
Eq. 3 $\delta^{13}C' - \delta^{13}C = \beta_0 + \beta_1 \ln(C{:}N)$	All	2.62	-
	Liver	1.90	-
	Muscle	2.95	-
	Whole	2.73	-

Application of eqs. 1, 1a, 2, and 2a to the aquatic dataset (Table 2) predicted lipid-free $\delta^{13}C$ with varying degrees of accuracy (Table 4). For fish samples, the original McConnaughey and McRoy (1979) model (eq. 1) and Fry (2002) model (eq. 2) consistently underestimated lipid-free $\delta^{13}C$ and had higher AIC_c values relative to modified versions of each equation (Kiljunen et al. (2006) and Sweeting et al. (2006)) when applied to all fish samples, muscle, and whole body samples. For liver samples, the Fry (2002) model had a reduced AIC_c value comparable to values for Kiljunen et al. (2006) and Sweeting et al. (2006). Overall AIC_c values were highest for the original McConnaughey and McRoy (1979) model (eq. 1) and the Fry (2002) model (eq. 2) as both models generally underestimated lipid-free $\delta^{13}C$ while the Kiljunen et al. (2006) model (eq. 1a) and the Sweeting et al. (2006) model (eq. 2a) produced AIC_c values that were lower than those for eqs. 1 and 2 (Table 4). For invertebrate samples, the opposite pattern was observed with original models generating lower AIC_c values (Table 4).

Table 4. Statistical comparisons of literature lipid correction models fit to a dataset of aquatic species. Models were applied to datasets consisting of liver, muscle, and whole body samples from a range of species for fish and whole body samples from a range of species for invertebrates (Table 2).

Model – Fish Data	Dataset	Log Likelihood	AIC_c
Eq. 1	All Data	-490.2	982.3
$\delta^{13}C'-\delta^{13}C = 6\left(-0.207+\frac{3.90}{1+287/L}\right)$	Liver	-102.5	207.0
	Muscle	-214.8	431.7
	Whole Body	-171.9	345.8
Eq. 1a	All Data	-332.5	669.0
$\delta^{13}C'-\delta^{13}C = 7.018\left(0.048+\frac{3.90}{1+287/L}\right)$	Liver	-73.5	159.2
	Muscle	-110.2	232.6
	Whole Body	-129.5	271.1
Eq. 2	All Data	-414.7	835.4
$\delta^{13}C'-\delta^{13}C = 6-\frac{22.2}{C:N}$	Liver	-73.5	153.0
	Muscle	-187.1	380.2
	Whole Body	-149.8	305.7
Eq. 2a	All Data	-295.7	593.5
$\delta^{13}C'-\delta^{13}C = \frac{7\left(C:N_{sample}-C:N_{protein}\right)}{C:N_{sample}}$	Liver	-67.1	136.2
	Muscle	-98.9	199.7
	Whole Body	-109.9	221.9

Model – Invertebrate Data	Dataset	Log Likelihood	AIC_c
Eq. 1	All Data	-50.6	103.2
$\delta^{13}C'-\delta^{13}C = 6\left(-0.207+\frac{3.90}{1+287/L}\right)$			
Eq. 1a	All Data	-88.5	183.3
$\delta^{13}C'-\delta^{13}C = 7.018\left(0.048+\frac{3.90}{1+287/L}\right)$			
Eq. 2	All Data	-63.4	128.8
$\delta^{13}C'-\delta^{13}C = 6-\frac{22.2}{C:N}$			
Eq. 2a	All Data	-64.2	128.5
$\delta^{13}C'-\delta^{13}C = \frac{7\left(C:N_{sample}-C:N_{protein}\right)}{C:N_{sample}}$			

For models fit to the aquatic dataset (Table 2), fits improved significantly with increased model specificity (Table 5). For the group of fish species, likelihood ratio tests of the three models based on eqs. 1b, 1c, 2b, and 3 showed that the tissue-specific model

fit the data significantly better than the simplest model (with parameters constant across all tissue types) and the species-tissue-specific model fit the data significantly better than the tissue-specific model (Table 5). Model estimates of $C:N_{lipid\text{-}free}$ ranged from 1.9 to 3.7 among fish models and tissue types with a mean of 3.0 ± 0.1. Protein-lipid discrimination estimates ranged from 5.5 to 7.4 with a mean of 6.5 ± 0.2 among tissue types for eqs. 1b, 1c, and 2b (Table 3). Eq. 3 did not have a y-asymptote for protein-lipid discrimination estimation (Figure 8), therefore a value for protein-lipid discrimination could not be derived from that equation. The species-specific model also fit invertebrate data significantly better than the simpler model with parameters constant across species for all models (Table 5).

Table 5. Statistical comparisons of nested lipid correction models fit to a dataset of aquatic species. Models were fit to a dataset of liver, muscle, and whole body samples from fish and whole body samples from invertebrates (Table 2).

Model – Fish Data	Dataset	Log Likelihood	AIC_c	df	χ^2	P-value
Eq. 1b $\delta^{13}C' - \delta^{13}C = D(\theta + \frac{3.90}{1+\frac{287}{L}})$	All	-281.8	569.6	2	-	-
	Tissue-Specific	-262.7	543.7	6	38.2	<0.001
	Tissue-Species Specific	-193.4	465.3	24	132.9	<0.001
Eq. 1c $\delta^{13}C' - \delta^{13}C = \frac{a * C:N + b}{C:N + c}$	All	-279.2	566.5	3	-	-
	Tissue-Specific	-258.4	541.5	9	40.0	<0.001
	Tissue-Species Specific	-184.4	507.1	36	144.4	<0.001
Eq. 2b $\delta^{13}C' - \delta^{13}C = P - \frac{P * F}{C:N}$	All	-281.3	568.6	2	-	-
	Tissue-Specific	-261.9	542.2	6	37.6	<0.001
	Tissue-Species Specific	-192.9	464.3	24	133.5	<0.001
Eq. 3 $\delta^{13}C' - \delta^{13}C = \beta_0 + \beta_1 \ln(C:N)$	All	-310.9	625.9	2	-	-
	Tissue-Specific	-272.9	531.9	6	77.3	<0.001
	Tissue-Species Specific	-192.4	463.3	24	159.6	<0.001
Model – Invertebrate Data						
Eq. 1b $\delta^{13}C' - \delta^{13}C = D(\theta + \frac{3.90}{1+\frac{287}{L}})$	All	-44.4	95.2	2	-	-
	Species-Specific	-25.4	89.0	6	38.0	<0.001
Eq. 1c $\delta^{13}C' - \delta^{13}C = \frac{a * C:N + b}{C:N + c}$	All	-42.2	92.9	3	-	-
	Species-Specific	-21.9	99.3	9	39.1	0.001
Eq. 2b $\delta^{13}C' - \delta^{13}C = P - \frac{P * F}{C:N}$	All	-44.1	94.6	2	-	-
	Species-Specific	-24.3	86.7	8	37.9	<0.001
Eq. 3 $\delta^{13}C' - \delta^{13}C = \beta_0 + \beta_1 \ln(C:N)$	All	-43.5	93.4	2	-	-
	Species-Specific	-33.1	104.4	6	20.8	0.008

Lowest AIC_c values (min AIC_c) were not consistently produced by a single model form among general, tissue, and species-tissue specific models. For fish samples, eq. 1c had the lowest AIC_c value among general and tissue-specific fitted models. For species-tissue-specific models, eq. 2b and 3 had similar lowest AIC_c values. For invertebrate samples, eq. 2b had the lowest AIC_c value among general models and eqs. 1b and 2b had lowest AIC_c values for species specific models (Table 5).

Discussion

Multiple models fit to the dataset of fish and aquatic invertebrate tissues closely followed the dynamics of change in $\delta^{13}C$ between bulk tissue and lipid-extracted samples with increasing bulk tissue C:N. Original models by McConnaughey and McRoy (1979) and Fry (2002) generally underestimated $\delta^{13}C$ for fish and aquatic invertebrate tissue samples, but modifications of these models produced better fits, and are a suitable alternative to lipid extraction for $\delta^{13}C$ corrections. McConnaughey and McRoy (1979) assigned values for $C:N_{lipid\text{-}free}$ and protein-lipid discrimination of 4 and 6 and Fry (2002) similarly used 3.7 and 6. With the exception of eq. 1c for fish liver, these $C:N_{lipid\text{-}free}$ values were greater than values estimated by equations fit to the aquatic dataset (Table 2), which were generally closer to 3. McConnaughey and McRoy (1979) and Fry (2002) protein-lipid discrimination estimates instead were lower than model estimates. By replacing constant terms representing protein-lipid discrimination and lipid-free C:N with parameters fit to the aquatic dataset (Table 2), SSE and AIC_c values decreased and were similar to SSE and AIC_c values for new models (eqs. 1b,c, 2b and 3).

Invertebrate data were derived from homogenized whole organisms, and relationships between C:N and change in $\delta^{13}C$ with lipid removal were less pronounced

than in fish tissues. While many aquatic invertebrate species store considerable amounts of lipid (Lee et al. 1975; Meier et al. 2000; Iverson et al. 2002; Fisk et al. 2003), greater heterogeneity in whole organism invertebrate samples that contain high proportions of chitin, in addition to lipids and protein, may weaken the observed relationship between bulk tissue C:N and $\delta^{13}C$ (Kiljunen et al. 2006). In modeling approaches, analysis of soft tissue components may produce a stronger relationship than whole organism analyses. For whole organism samples, poor model fits that do not demonstrate a clear relationship between C:N and changes in $\delta^{13}C$ (Figure 9) indicate that lipid removal using chloroform-methanol and acid treatment provide better $\delta^{13}C$ estimates of lipid-free tissue.

By using a correction approach rather than extracting lipids prior to analysis, the movement of lipids through food webs can be preserved in ecological studies (Arts et al. 2001). Multiple models fit to the dataset of fish tissues tracked changes in $\delta^{13}C$ between bulk tissue and lipid extracted samples with increasing bulk tissue C:N. The asymptotic relationship between $C:N_{bulk}$ and $\delta^{13}C$ indicates that a linear fit correction (e.g., Post et al. 2007) may not be appropriate over a wide range of $C:N_{bulk}$.

When dealing with new taxa for which necessary $C:N_{bulk}$, $\delta^{13}C_{bulk}$ and $\delta^{13}C_{lipid\text{-}free}$ data do not exist, extracting a subset of samples of the species and tissues of interest to develop the necessary correction equations is recommended. These new correction equations can then be applied to remaining data to account for a large lipid load and biased $\delta^{13}C$. This will reduce sample preparations, eliminate the need to analyze all samples before and after extraction, and decrease $\delta^{13}C$ variability among individuals yielding improved estimates of dietary source proportions in mixing models (Phillips and Gregg 2001). Most analytical laboratories provide % C and % N data with isotope data,

making it easy to evaluate whether lipids bias $\delta^{13}C$ measurements. Values for C:N ratios can be reported based on uncorrected % element data or with correction for differences in atomic weight between elements (i.e. % C / % N * 1.16667). If either method is used consistently, results will not be affected, but applying both methods in a single analysis would reduce accuracy of $\delta^{13}C$ estimates (Sweeting et al. 2006). All C:N values used in conjunction with reported parameter estimates should use uncorrected elemental C:N.

The significant χ^2 differences for all nested models demonstrate that species- and tissue-specific parameters provide the best $\delta^{13}C_{lipid\text{-}free}$ estimates. Model parameters (slope and intercept) relate to protein-lipid $\delta^{13}C$ discrimination and baseline C:N of lipid-free tissue. As model fits improved with specificity, the data used to generate parameters is more important than the specific model selected. Developing equations specific to the tissue and taxa of interest should produce more accurate estimates of diet and habitat.

No single model performed best across nested models but instead model performance was related to parameter specificity. The modification of the McConnaughey and McRoy (1979) model, eq. 1c, had the lowest AIC_c among general and tissue-specific models, but the highest AIC_c among species and tissue-specific models. The opposite pattern was observed for eq. 3. When tissue-specific parameters are used instead of species- and tissue-specific parameters, the modification of the McConnaughey and McRoy (1979) model (eq 1c) is most appropriate. The Fry (2002) equation using parameters fit to the aquatic dataset (eq 2c) had essentially indistinguishable AIC_c values ($\Delta_i \sim 2$) from eq. 3 for the species-tissue specific fit. Since eq. 3 failed to accurately reflect protein-lipid discrimination, the Fry (2002) equation fit

(eq. 2b) is more appropriate for use with species- and tissue-specific parameters (e.g., brook trout muscle).

For fish tissues, datasets could be created through pairwise isotope analysis of a study's bulk tissue and lipid-extracted tissue for each species and tissue type presented. Models (based on tissue C:N) could then be fit to each data subset, and fit parameters could be used to predict lipid-free $\delta^{13}C$ for remaining samples. Alternatively, literature parameter estimates from this study or previous published studies (Kiljunen et al. 2006; Post et al. 2007) could be applied to datasets with similar species and bulk tissue C:N ranges. This approach would be similar to current techniques that estimate trophic position of consumers by using diet-tissue discrimination factors ($\delta^{15}N$ consumer- $\delta^{15}N$ diet) from the literature (Post 2002; McCutchan Jr et al. 2003; Vanderklift and Ponsard 2003), or ideally, with species- or taxon-specific estimates in controlled laboratory rearing experiments (Hobson et al. 2002). Continued application of this approach in published literature will help to build a library of parameter values across a broader range of taxa and tissue types for use in SIA.

CHAPTER 2

TROPHIC ECOLOGY OF ATLANTIC BLUEFIN TUNA

Diet of Young Atlantic Bluefin Tuna in Eastern and Western Atlantic Forage Grounds

Introduction

Coastal waters of the Bay of Biscay (BYB) and Mid-Atlantic Bight (MAB) are productive summer forage grounds (Figure 10) for Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) (Ortiz de Zárate and Cort 1986; Eggleston and Bochenek 1990). Commercial and recreational fisheries target these regional aggregations (Richards 1965; Rodríguez-Marín et al. 2003). Both regions have estuarine outflows, shelf break fronts, eddies, and seasonal upwelling (Koutsikopoulos and LeCann 1996; Mouw and Yoder 2005), conditions that support productive waters and dense aggregations of zooplantivorous fishes. In the MAB, juvenile ABFT have historically fed mainly on schooling sand lance (*Ammodytes* spp.) (Eggleston and Bochenek 1990; Barr 1991) and on anchovies (*Engraulis encrasicholus*) in the BYB (Ortiz de Zárate and Cort 1986). Schools of ABFT arrive from late May to early June and remain through autumn (Mather et al. 1995; Rodríguez-Marín et al. 2003). In addition to north-south migrations along coastal foraging grounds, young (i.e. 1-3 yrs) ABFT also undertake trans-Atlantic migrations, and mix on both sides of the Atlantic (Mather et al. 1995; Rooker et al. 2007). Consequently, individual fish may visit both the MAB and BYB during their first years

of life, although inter-annual mixing rates may be highly variable (Arregui et al. 2006; Rooker et al. 2007).

Atlantic bluefin tuna are "energy speculators" (Brill 1996; Korsmeyer et al. 1996) with high metabolic rates (Korsmeyer and Dewar 2001; Fitzgibbon et al. 2007), regional endothermy (Carey and Teal 1966), and rapid gut evacuation (Butler and Mason 1978). Like other temperate tunas, they undertake long migrations to access productive forage grounds throughout the north Atlantic (Rivas 1955; Mather et al. 1995). These demands require large inputs of high caloric prey (Fitzgibbon et al. 2007). Given that recent changes in prey abundance or environmental conditions may have influenced ABFT distributions and migration patterns (Fromentin 2009), identification of exploited prey bases is important. Recent changes in both ABFT somatic condition (Golet et al. 2007; Neilson et al. 2007) and coastal abundance (Lutcavage et al. unpubl. data) point to possible disturbances in their prey base that further warrant dietary studies, as prey shifts are likely to affect fecundity and stock production (e.g., Benson and Trites 2002) and reduce economic returns in the commercial fisheries.

Atlantic bluefin tuna are opportunistic predators with a wide range of prey (Dragovich 1969). In the NW Atlantic, their diet includes butterfish (*Peprilus triacanthus*), Atlantic mackerel (*Scomber scombrus*), cephalopods, and euphausiids (Chase 2002; Estrada et al. 2005). In the NE Atlantic and Mediterranean Sea, clupeids (Sanz Brau 1990; Orsi Relini et al. 1995; Sinopoli et al. 2004) and anchovies (Aloncle 1964; Ortiz de Zárate and Cort 1986) as well as zooplankton, cephalopods, and other teleosts (Sara and Sara 2007) are major prey items. Little is known about the winter distributions and diet of young individuals in either coastal area (Mather et al. 1995),

although larger fish consume cephalopods and mesopelagic fishes while offshore (Matthews et al. 1977), and menhaden (*Brevoortia tyrannus*) and swimming crabs (*Portunus* spp.) inshore (Butler 2007).

Previous studies primarily used either stomach content analysis (SCA) (Ortiz de Zárate and Cort 1986; Eggleston and Bochenek 1990; Chase 2002; Sinopoli et al. 2004) or stable isotope analysis (SIA) (Estrada et al. 2005; Sara and Sara 2007) as tools to identify young ABFT diet. Traditional SCA techniques can provide detailed information on diet composition, prey size distribution, and consumption rate estimates (Chipps and Garvey 2006), but only a snapshot of an individual's diet. Alternatively, SIA data track diet over longer time scales, depending on compound or tissue turnover rates (Gannes et al. 1998). For tunas, dietary isotope values indicate average assimilated diet over weeks in liver tissue and months in white muscle (Graham et al. submitted).

A combined SCA and SIA approach was used to assess dietary preferences of young ABFT (~ 60 – 150 cm CFL) on forage grounds in the MAB and BYB. Trophic analyses have not been performed for these regions in the past two decades and SCA was the only dietary metric (Ortiz de Zárate and Cort 1986; Eggleston and Bochenek 1990). This study builds on these earlier works to more thoroughly identify diet preferences for this commercially and ecologically important species, to assess possible trophic shifts, and to establish a current baseline for ABFT trophic relationships that can be used in ecosystem studies.

Methods

Study Sites

ABFT were sampled from the Mid-Atlantic Bight (MAB) and Bay of Biscay (BYB). MAB forage grounds are located ~ 30 to 60 km off the eastern shore of Virginia, U.S.A. (Figure 10), and are characterized by sandy substrate and water depths of 30 to 40 m (Bochenek 1989). The BYB is an open oceanic bay partly enclosed by a wide shelf region along the western coast of France and a narrow shelf along the northern coast of Spain (Koutsikopoulos and LeCann 1996). Samples were collected from the southeastern region off the coast of Spain (Figure 10).

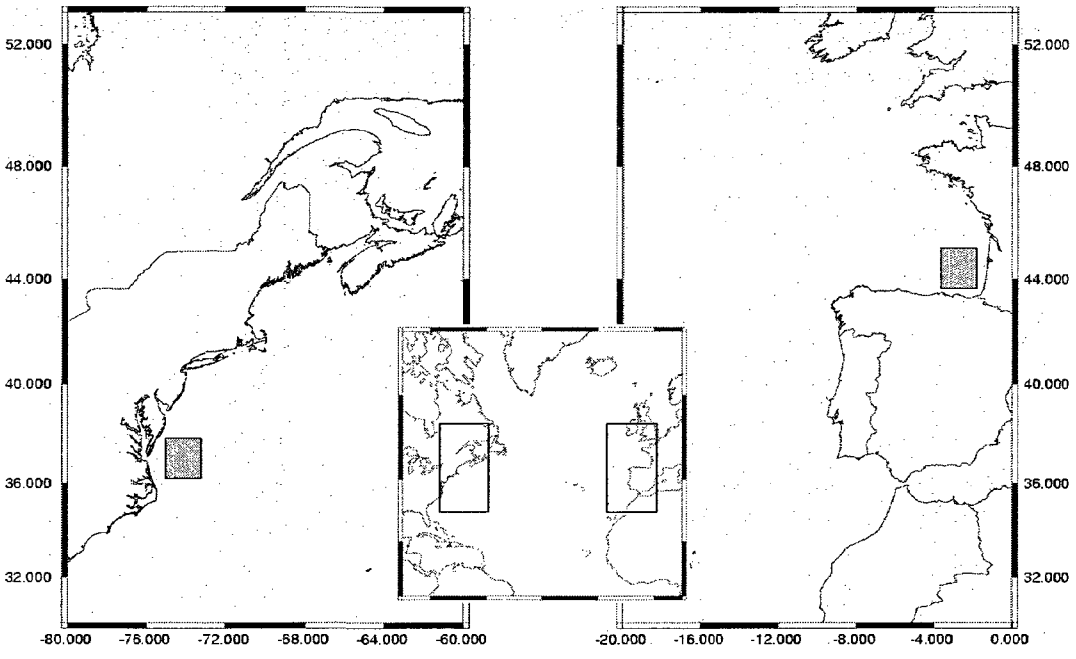

Figure 10. Maps of Mid-Atlantic Bight (MAB) and Bay of Biscay (BYB) study sites. Sampling locations are located within shaded boxed regions.

Sample collection and preservation

Biological samples were obtained from ABFT harvested by scientific surface troll surveys as well as baitboat, pelagic trawl, and recreational fisheries in the BYB (Tables 6

and 7). Live bait fishing was the primary source of stomach samples in the BYB (~ 80 %). Stomachs were removed from ABFT at sea during the scientific surveys, but most BYB samples were collected from commercial landings in which ABFT were stored on ice for ~ one to two days. Whole stomachs, dorsal white muscle, and liver samples were removed from each individual fish at sea or at dockside cleaning stations, placed on ice, then stored frozen until analysis. Curved fork length (CFL) and straight fork length (SFL) were measured (± cm), and sex and wet weight (± g) were determined for a subset of BYB samples.

Prey samples for SIA were collected from scientific trawl surveys (BYB, fall 2006) and stomach contents of ABFT and other large pelagic fishes (MAB, summer 2005, 2006). Cephalopod, teleost, and swimming crab (*Polybius henslowii*) prey samples consisted of muscle sub-samples while for all other crustacean prey, whole organisms were analyzed. All prey samples were stored frozen prior to analysis.

Tuna were grouped by size (age) or sampling season. Size classes correspond to 57-120 cm CFL (age 1-3) and 120-151 cm CFL (age 4-5). Age was estimated by direct reading on the first ray of the first dorsal fin or by applying age length curves (Rodríguez-Marín et al. 2007). Most samples from the BYB were from the smaller size class (~ 98 %). While most sampled ABFT were likely immature, the largest individuals (CFL > 110 cm) are ≥ estimated size of 50 % maturity for eastern Atlantic ABFT (Corriero et al. 2005) so the dataset could potentially include both immature and mature ABFT.

Table 6. Sample collection summary for Atlantic bluefin tuna (*Thunnus thynnus*) from the Mid-Atlantic Bight (MAB).*

	Isotope Samples			Stomach Samples				
Group	Date Range	Size (cm)**	n	Date Range	Size (cm)***	n		
						Total	Natural Prey	Empty
Age 1-3 2004	6/25 – 7/23	73.5 ± 18.5	17	NA****	NA	NA	NA	NA
Age 1-3 2005	6/29 – 7/13	75.3 ± 3.7	12	6/29 – 7/13	75 ± 4.0	10	10	0
Age 1-3 2006	7/1 – 7/4	98.1 ± 7.6	11	7/1 – 7/10	98 ± 7.3	12	10	2
Age 4-5 2004	6/25	132.1 ± 10.2	4	NA	NA	0	0	0
Age 4-5 2006	7/1 – 7/4	135.2 ± 8.5	28	7/1 – 7/4	135 ± 8.7	29	22	7
All Age 1-3	6/25 - 7/13	80.8 ± 16.6	40	6/29 – 7/13	88 ± 13.0	22	20	2
All Age 4-5	6/25 – 7/4	134.8 ± 8.6	32	7/1 – 7/4	135 ± 8.7	29	22	7

* All fish were sampled from a surface troll fishery.
** Size reported as mean curved fork length ± SD.
*** For stomach samples, size based on fish with stomachs containing natural prey (i.e., samples used in the stomach content analysis).
**** NA means not available.

Table 7. Sample collection summary for Atlantic bluefin tuna (*Thunnus thynnus*) from the Bay of Biscay (BYB).*

Group	Isotope Samples				Stomach Samples					
	Date Range	Size (cm)**	Sampling Method	n	Date Range	Size (cm)***	n			
							Total	Natural Prey	Bait Only	Empty
Summer 2001 Age 2	7/9 – 7/16	82.7 ± 2.8	BB	24	7/9 – 8/17	86 ± 3.3	48	21	16	11
Summer 2003 Age 1-2	7/14 – 8/6	73.0 ± 10.3	BB, T	7	7/7 – 8/6	70 ± 9.4	36	15	11	10
Summer 2004 Age 2-4	7/31	86.1 ± 3.5	BB	15	6/28 – 7/31	81 ± 2.8	47	18	26	3
Summer 2005 Age 1-3	NA****	NA	BB, RR	0	7/9 – 8/29	70 ± 11.1	81	46	23	22
Summer 2006 Age 1-4	NA	NA	BB, T, MWT	0	7/13 – 7/29	78 ± 21.2	22	12	0	10
Fall 2000 Age 1-2	9/25 – 9/27	71.5 ± 2.4	BB	28	9/25 – 10/24	72 ± 2.2	48	29	8	11
Fall 2002 Age 1-3	9/17 – 10/13	76.9 ± 10.8	BB	30	9/17 – 10/14	76 ± 11.7	35	13	21	1
Fall 2004 Age 1-2	10/18	70.3 ± 1.4	BB	15	10/18 – 10/26	70 ± 0.9	34	16	12	6
Fall 2006 Age 2	NA	NA	RR	0	9/24 – 10/22	75	3	1	0	2
All Summer Age 1-4	7/9 – 8/6	82.2 ± 6.3	BB, T, MWT, RR	46	6/28 – 8/29	76 ± 13.2	234	112	76	56
All Fall Age 1-3	9/17 – 10/18	73.5 ± 7.6	BB, RR	73	9/17 – 10/26	72 ± 5.9	120	59	41	20

* Size reported as mean curved fork length ± SD.

** Sampling method: Troll (T), Bait boat (BB), Rod & Reel (RR), Mid-Water Trawl (MWT).

*** For stomach samples, size based on fish with stomachs containing natural prey (i.e., samples used in the stomach content analysis).

**** NA means not available.

Stomach Content Analysis (SCA)

Whole stomachs were thawed, weighed (± 0.05 g), and contents were washed over a 1,000 micron sieve. The empty stomach was then blotted dry and re-weighed to calculate total stomach content weight as whole stomach content weight (g) minus empty stomach weight (g). Contents were identified to the lowest possible taxonomic group. Whole individuals were weighed (± 0.1 g) and measured (± mm) to estimate fork, mantle, and rostral lengths of teleost, cephalopod, and crustacean prey, respectively. Stomach samples were classified as empty, containing bait, containing bait and natural prey, and containing natural prey. Only the last two categories were used in the SCA. For "containing bait and natural prey" stomachs, prey identified as bait or chum were eliminated from the analyses. Empty stomachs and those containing only bait were excluded from analyses, since ABFT may evacuate their guts during capture (Chase 2002), and the frequency of empty stomachs is affected by the fishing method (Velasco and Quintans 2000). Collection and analyses of stomachs from the BYB were performed by Enrique Rodríguez-Marín and Santiago Barreiro of Instituto Español de Oceanografía (IEO) and Nicolas Goñi and Haritz Arrizabalaga of AZTI Tecnalia.

Stable Isotope Analysis (SIA)

Prey items and ABFT liver and white muscle samples were thawed, lightly rinsed with deionized water, and dried in glass scintillation vials at 60°C for at least 48 h. Samples were then homogenized using a Mixer/Mill® (SPEX SamplePrep, LLC Metuchen, New Jersey U.S.A) with stainless steel vials. Aliquots of homogenized sample (0.6 – 1.2 mg) were packed into 4 X 6 mm tin cups and analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen by continuous flow using a Costech ECS4010 elemental

analyzer (Costech Analytical Technologies, Inc, Valencia, CA USA) coupled with a $DELTA_{plus}$ XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory of Northern Arizona University (NAU) and the University of New Hampshire Stable Isotope Laboratory (UNH). All C:N values are reported as uncorrected percent weight calculations ($\frac{\%C}{\%N}$) as opposed to percent atomic weight ($\frac{\%C}{\%N} \times 1.16667$). Ratios of total carbon to nitrogen (C:N) are linearly related to lipid content in fish tissue (Post et al. 2007) and can be used to track changes in body condition (Estrada et al. 2005).

All sample $\delta^{13}C$ values were corrected for lipid content either *a priori* through chemical extractions or *a posteriori* using mathematical approaches. ABFT liver and white muscle samples were corrected *a posteriori* using a mass balance equation (Fry 2002) with parameters specific to ABFT liver and white muscle (Logan et al. 2008). Most prey samples were lipid extracted with 2:1 chloroform-methanol solution (see Logan et al. (2008) for detailed methods). For samples that underwent chemical extraction, a second aliquot of bulk tissue was analyzed for $\delta^{15}N$ since chemical extractions alter $\delta^{15}N$ values in some fish (Pinnegar and Polunin 1999) and crustacean tissues (Bodin et al. 2007). Some smaller prey samples had insufficient material for lipid extractions and were lipid normalized using mass balance equations (Fry 2002; Logan et al. 2008) with parameters based on common classes of organisms and tissue types (e.g. fish white muscle) (Logan et al. 2008). Carbonates were not removed from crustacean prey samples and may induce a positive bias on $\delta^{13}C$ values (Craig 1953).

All carbon and nitrogen isotope data are reported in δ notation according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] * 1000$$

where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Standard materials are Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. Standard deviations of replicate samples analyzed at both labs were 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$ (n = 45) and within-lab precision is ~ 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on the VPDB and AIR scales with IAEA CH6 (-10.4 ‰), CH7 (-31.8 ‰), N1 (0.4 ‰) and N2 (20.3 ‰).

Statistical analysis

Stomach Content Analysis

Meal size was estimated using the gut fullness index (GFI) based on the formula:

$$GFI = \frac{Full\ stomach\ weight\ (g) - \ stomach\ lining\ weight\ (g)}{Bluefin\ tuna\ weight\ (g)} * 100$$

(Berg 1979; Hyslop 1980). Full stomach weight is the wet weight of a thawed whole stomach, and the lining weight is for a rinsed, blotted dry stomach lining. ABFT weight reflects the wet weight of a whole ABFT body (i.e. round weight), and for ABFT samples from the MAB, wet weight was estimated based on length measurements. Curved fork length (CFL) data were first converted to straight fork length (SFL) using:

$$SFL\ (cm) = 0.955 * CFL\ (cm).$$

Weight was then estimated according to the formula:

$$Weight\ (kg) = 3.733 * SFL^{2.8683}$$ (Parrack and Phares 1979).

Comparisons were made using a Kruskal Wallis test and a Nemenyi-Damico-Wolfe-Dunn test when significant differences ($P<0.05$) were detected.

Adequacy of stomach content sample sizes was explored by generating cumulative prey curves (Ferry and Caillet 1996). Increasing numbers (1 to n-1) of stomach samples were randomly selected for each dataset and the total number of unique prey types was summed for each sampling. Bootstrapping techniques were used to generate 500 random samples for each sample size in order to generate means and standard deviations with the program R (R Development Core Team 2008). Sample size was then plotted against mean number of unique prey types, where a unique prey type was defined as the most specific identification for a given prey group. Where a dataset contained a prey group identifiable to species level (e.g., horse mackerel), less specific identifications would not be counted as unique prey (e.g., teleost fishes). To assess whether a sufficient number of samples had been analyzed to capture prey diversity, the slope of the final four endpoints for each prey curve was compared to a slope of zero using a Student's t-test (Bizzarro et al. 2007). All p-values were adjusted for multiple comparisons using a Holm test (Holm 1979).

Relationships between ABFT and prey size were explored using quantile regression. Regressions were performed for the median (50^{th} percentile) as well as 5^{th} and 95^{th} percentiles to test for patterns in minimum and maximum prey size in relation to ABFT length. Significance was tested using a rank sums test. All quantile regression analyses were performed in the statistics package Blossom (Cade and Richards 2005).

Percent weight and percent number were calculated for each prey group for each stomach sample to generate mean and standard deviations for each prey category.

Frequency of occurrence was also calculated for each prey group. Mean percent weight contributions were compared using a Kruskal Wallis test for crustaceans, cephalopods, and teleost fishes. Nemenyi-Damico-Wolfe-Dunn tests were performed as post-hoc analyses when significant differences ($P<0.05$) were detected.

The Morisita-Horn index was used to estimate dietary overlap between ABFT size class, sex, and sampling seasons using the formula:

$$C_H = \frac{2\sum_{i=1}^{n} P_{i,j} \times P_{i,k}}{\sum_{i=1}^{n} P^2_{i,j} + \sum_{i=1}^{n} P^2_{i,k}}$$

where C_H is the Morisita-Horn index of overlap between ABFT size classes j and k, n is the total number of species identified in all sampled stomach contents, and $P_{i,j}$ and $P_{i,k}$ are the mean percent weight contributions of prey type i to ABFT size classes j and k (Horn 1966; Krebs 1998). Comparisons were made between groups for both regions. Further comparisons were made between sexes and season-years for BYB samples. Prey were grouped at the order and family levels to explore dietary differences between groups. More general comparisons were made by grouping all prey as teleosts, cephalopods, and crustaceans due to the potential confounding effects of unidentifiable prey components. The Morisita-Horn index ranges from zero to one with higher index values reflecting greater prey overlap among predator groups and values > 0.60 considered to represent significant overlap (Zaret and Rand 1971; Cortés 1997). To include estimates of error, Morisita-Horn index calculations were made over 500 randomly selected stomach samples. Percent weight data were used for this calculation since weight information identifies the relative importance of individual prey groups to consumer nutrition to a greater extent than numeric or occurrence percentages (Bowen 1983).

Stable Isotope Analysis (SIA)

Comparisons of $\delta^{13}C$, $\delta^{15}N$, and C:N were made among seasons, sampling years, and ABFT size classes for liver and white muscle. Homogeneity of variance among groups was first tested using Levene's test. For cases where Levene's test results were non-significant, analysis of variance (ANOVA) and subsequent pairwise t-tests were performed with $\alpha = 0.05$ following a Holm test adjustment for multiple comparisons. When significant differences were detected using Levene's test, a one-way analysis of means and subsequent pairwise comparisons using t-tests with non-pooled standard deviations were performed. All tests were performed using R (R Development Core Team 2008).

Relationships between ABFT length and tissue C:N were further explored using quantile regression for the MAB dataset. Regressions were performed for the median (50^{th} percentile) as well as 5^{th} and 95^{th} percentiles to test for patterns in minimum and maximum C:N in relation to ABFT length. Significance was tested using a rank sums test. All quantile regression analyses were performed in the statistics package Blossom (Cade and Richards 2005).

To estimate relative lipid contents among prey groups, ΔC:N was calculated according to the formula:

$$\Delta C{:}N = C{:}N_{bulk} - C{:}N_{lipid-free}$$

where $C{:}N_{bulk}$ is the C:N ratio of a dried, homogenized prey sample and $C{:}N_{lipid\text{-}free}$ is the C:N ratio of a prey sample following chemical lipid extraction. Values are presented as ΔC:N rather than bulk C:N, because baseline (lipid-free) C:N values vary among fish species (Sweeting et al. 2006).

Prey dietary contributions were quantified using the package Stable Isotope Analysis in R (SIAR). ABFT liver isotope data were used for mixing model analyses since white muscle turns over more slowly (Graham et al. submitted) and may reflect a mixture of dietary information for local food webs as well as distant forage locations for this highly migratory species. While diet-tissue discrimination is often assumed to be ~ 0.4 ‰ (Peterson and Fry 1987) for $\delta^{13}C$ and 3.4 ‰ for $\delta^{15}N$ (DeNiro and Epstein 1981; Minagawa and Wada 1984), discrimination factors vary among individuals (Gaye-Siessegger et al. 2004; Barnes et al. 2008), species (Macko et al. 1982), tissue types (Pinnegar and Polunin 1999), and diet types (Adams and Sterner 2000). For mixing model calculations, diet-tissue discrimination was assumed to be 1.4 ‰ for $\delta^{13}C$ and 1.3 ‰ for $\delta^{15}N$ based on available liver data for yellowfin tuna (*Thunnus albacares*) (Graham et al. submitted) and other fish species (Pinnegar and Polunin 1999; Suzuki et al. 2005; Trueman et al. 2005; Sweeting et al. 2007a). To account for uncertainty and inherent variability in trophic discrimination values, standard deviation values of 0.43 ‰ and 0.58 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, were applied to approximate inter- and intra-species variability. Prey values were entered into SIAR models as mean values ± SD. When only a single prey sample was analyzed for a given prey group, standard deviation estimates of 0.40 ‰ were applied for each isotope to account for inter-individual variability.

Trophic Position

ABFT trophic position was estimated using TrophLab (Pauly et al. 2000) according to the following formula:

$$TROPH_i = 1 + \sum_{j=1}^{G} DC_{ij} * TROPH_j$$

where $TROPH_i$ corresponds to ABFT trophic position. $TROPH_j$ is the trophic position of a given prey group, DC_{ij} is the proportion of prey j in ABFT diet and G is the total number of prey groups. Trophic position calculations were made based on mean percent weight data from SCA and mean proportion dietary estimates from SIA. All prey types were grouped into the categories teleost fishes, crustaceans, and cephalopods, and groups were assigned trophic positions based on general data from FishBase (Froese and Pauly 2008).

Results

Stomach content characteristics

In cumulative prey curves, the slope of the final four endpoints was significantly different from zero ($P<0.05$) for all MAB (Figure 11) and fall 2002 BYB prey curves, but was not following Holm adjustments for the remaining datasets (Figure 12). Results indicate that sample size was too small for MAB but not BYB datasets.

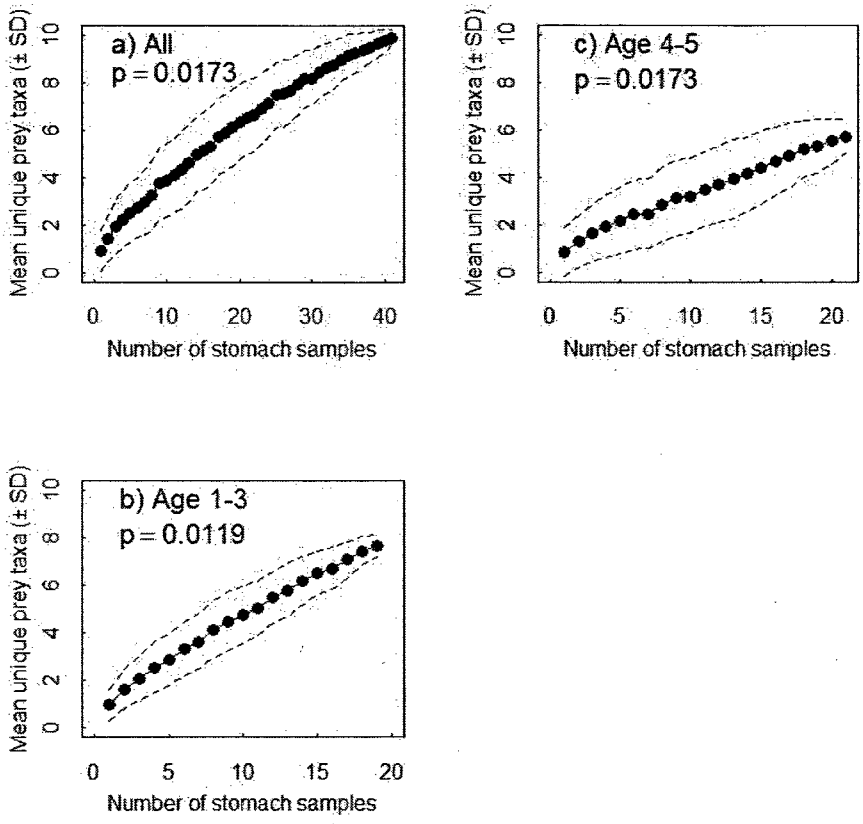

Figure 11. Cumulative prey curves (mean ± SD) for Mid-Atlantic Bight (MAB) stomach samples based on a) the entire dataset b) all age 1-3 Atlantic bluefin tuna (*Thunnus thynnus*) and c) all age 4-5 Atlantic bluefin tuna. P-values < 0.05 correspond to significant differences between the final four data points and a slope of zero and indicate insufficient sample sizes.

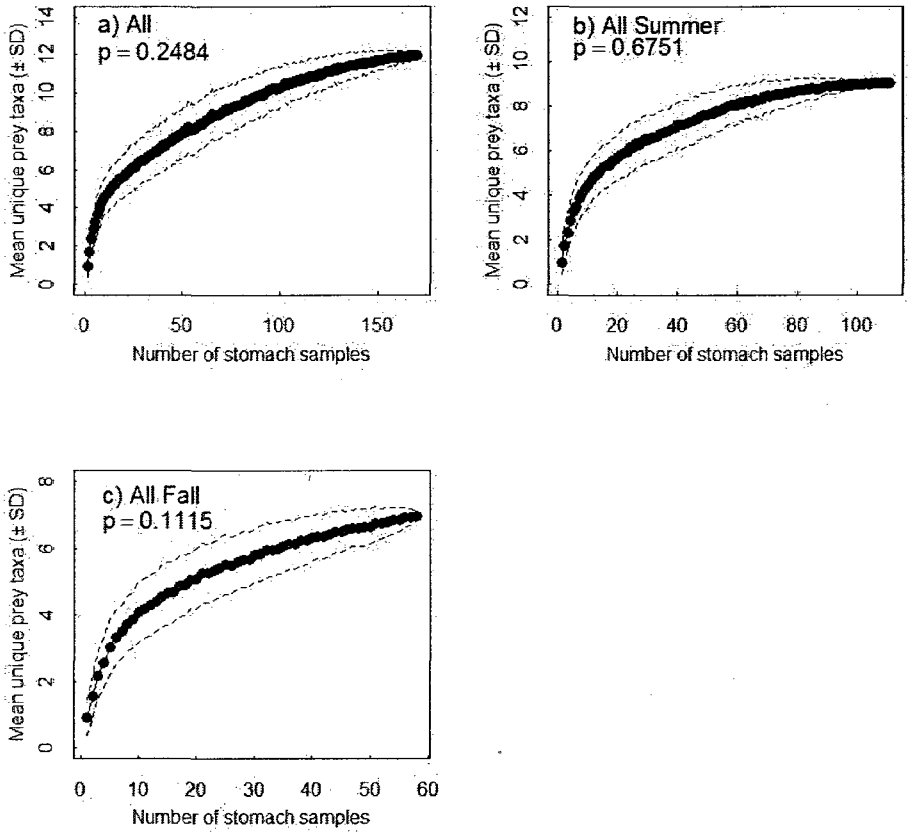

Figure 12. Cumulative prey curves (mean ± SD) for Bay of Biscay (BYB) stomach samples of Atlantic bluefin tuna (*Thunnus thynnus*) based on a) the entire dataset b) all summer and c) all fall stomach samples. P-values < 0.05 correspond to significant differences between the final four data points and a slope of zero and indicate insufficient sample sizes.

Gut fullness index (GFI) values were significantly higher in individuals from the BYB (mean ± SD: 1.6 ± 1.8) than MAB (0.5 ± 0.7 for age 1-3 yrs and 0.1 ± 0.2 for age 4-5 yrs) ($P<0.0001$). For the MAB, no significant differences were detected between size classes.

ABFT length was not significantly correlated with prey length, except for European anchovy (*Engraulis encrasicholus*) ($P<0.001$). A total of 938 prey items were measured. The largest species were Atlantic horse mackerel (*Trachurus trachurus*),

sardine (*Sardina pilchardus*), and the flying squid (*Todarodes sagittatus*). Large ABFT fed on myctophids, round herring (*Etrumeus teres*) and swimming crabs. Sand lance was consumed by a wide size range of ABFT. In the BYB, ABFT feed mainly on age-0 European anchovy and blue whiting (*Micromesistius poutassou*) as well as age-1 Atlantic horse mackerel.

Bay of Biscay

Stomach Content Analysis

For the full dataset, all three prey groups (fishes, cephalopods, and crustaceans), had significantly different dietary weight percentages ($P<0.0001$). For the summer dataset, prey group rankings were fish > crustaceans > cephalopods with significant differences among all three groups. For the fall dataset, no cephalopods were present and significantly higher proportions of fish were observed relative to crustaceans (Table 8).

Table 8. Mean ± SD prey composition for Bay of Biscay Atlantic bluefin tuna (*Thunnus* thynnus) stomach samples collected during summer and fall from 2000-2006 (percent weight (% W), percent number (% N), and frequency of occurrence (% O).

	Summer (n = 112)			Fall (n = 59)		
Prey	%W	%N	%O	%W	%N	%O
Cephalopoda	0.8 ± 8.0	0.7 ± 6.3	1.8	-	-	-
Ommastrephidae (*Todarodes sagittatus*)	0.8 ± 8.0	0.7 ± 6.3	1.8	-	-	-
Malacostraca (Crustaceans)	33.9 ± 47.1	33.9 ± 46.8	37.5	18.7 ± 39.2	20.1 ± 40.1	20.3
Unidentifiable crustaceans	5.4 ± 22.6	5.4 ± 22.6	5.4	-	-	-
Unidentifiable Decapods	0.9 ± 9.5	0.9 ± 9.5	0.9	-	-	-
Portunidae (*Polybius henslowii*)	1.8 ± 13.2	1.6 ± 12.2	1.8	-	-	-
Pasiphaeidae (*Pasiphaea multidentata*)	0.8 ± 7.6	0.2 ± 1.2	2.7	-	-	-
Natantia	-	-	-	1.7 ± 13.0	1.7 ± 13.0	1.7
Euphausiidae (Unidentifiable)	2.7 ± 16.2	2.7 ± 16.2	2.7	6.9 ± 25.3	8.2 ± 27.2	8.5
Euphasuiidae (*Meganyctiphanes norvegica*)	22.5 ± 41.6	23.2 ± 41.9	25.0	8.5 ± 28.1	8.5 ± 28.1	8.5
Isopoda	-	-	-	1.7 ± 13.0	1.7 ± 13.0	1.7
Osteichthyes	64.5 ± 47.7	65.1 ± 47.1	67.0	81.3 ± 39.2	79.9 ± 40.1	81.4
Unidentifiable Osteichthyes	12.3 ± 32.5	12.5 ± 32.6	13.4	22.2 ± 40.9	22.3 ± 41.0	23.7
Carangidae (*Trachurus trachurus*)	16.8 ± 37.3	14.4 ± 34.3	17.0	27.8 ± 43.1	27.5 ± 42.9	32.2
Scombridae (*Scomber scombrus*)	1.1 ± 9.8	1.1 ± 9.6	1.8	-	-	-
Myctophidae	1.7 ± 13.0	1.5 ± 11.5	1.8	-	-	-
Gadidae (*Micromesistius poutassou*)	18.2 ± 37.8	21.3 ± 39.6	24.1	5.1 ± 22.2	5.1 ± 22.2	5.1
Clupeidae (*Sardina pilchardus*)	-	-	-	4.1 ± 18.0	3.1 ± 15.0	5.1
Engraulidae (*Engraulus encrasicholus*)	14.4 ± 34.7	14.4 ± 34.7	15.2	22.1 ± 39.4	22.0 ± 39.1	27.1
Phyophyta	0.8 ± 7.6	0.3 ± 3.2	1.8	-	-	-

Morisita-Horn index values varied seasonally but not by size class or sex (Table 9). Based on general groupings (fishes, cephalopods, and crustaceans), summer 2004 stomach content proportions differed significantly from all other seasonal groupings (Table 9; MH<0.60), and consisted mostly of euphausiids (95.1 mean %W). Additional seasonal differences (P<0.60) were detected, but these comparisons all had relatively high P values (> 0.48) with comparable standard deviation values (> 0.45). All datasets differed significantly (P<0.60) based on family-level groupings.

Table 9. Morisita Horn (MH) values for individual prey groups of Atlantic bluefin tuna (*Thunnus thynnus*) from the Bay of Biscay and Mid-Atlantic Bight.

	Family*	General
Group	Bootstrap Mean (± SD)**	Bootstrap Mean (± SD)
Bay of Biscay (BYB)		
Males*Females	0.20 ± 0.40	0.60 ± 0.49
All Summer*All Fall	0.20 ± 0.39	0.57 ± 0.49
Fall 2000*Fall 2002	0.22 ± 0.37	0.71 ± 0.46
Fall 2000*Fall 2004	0.15 ± 0.34	0.55 ± 0.50
Fall 2000*Summer 2001	0.35 ± 0.44	0.77 ± 0.42
Fall 2000*Summer 2003	0.00 ± 0.00	0.75 ± 0.42
Fall 2000*Summer 2004	0.00 ± 0.00	0.00 ± 0.00
Fall 2000*Summer 2005	0.28 ± 0.43	0.72 ± 0.44
Fall 2000*Summer 2006	0.08 ± 0.26	1.00 ± 0.00
Fall 2002*Fall2004	0.06 ± 0.24	0.50 ± 0.50
Fall 2002*Summer 2001	0.23 ± 0.42	0.60 ± 0.49
Fall 2002*Summer 2003	0.00 ± 0.00	0.57 ± 0.49
Fall 2002*Summer 2004	0.00 ± 0.00	0.31 ± 0.46
Fall 2002*Summer 2005	0.12 ± 0.23	0.57 ± 0.49
Fall 2002*Summer 2006	0.03 ± 0.13	0.68 ± 0.47
Fall 2004*Summer 2001	0.29 ± 0.45	0.55 ± 0.50
Fall 2004*Summer 2003	0.16 ± 0.37	0.54 ± 0.49
Fall 2004*Summer 2004	0.51 ± 0.49	0.40 ± 0.48
Fall 2004*Summer 2005	0.23 ± 0.41	0.55 ± 0.49
Fall 2004*Summer 2006	0.10 ± 0.29	0.55 ± 0.49
Summer 2001*Summer 2003	0.00 ± 0.00	0.60 ± 0.48
Summer 2001*Summer 2004	0.27 ± 0.44	0.23 ± 0.42
Summer 2001*Summer 2005	0.20 ± 0.40	0.64 ± 0.48
Summer 2001*Summer 2006	0.01 ± 0.08	0.76 ± 0.43
Summer 2003*Summer 2004	0.00 ± 0.00	0.18 ± 0.38
Summer 2003*Summer 2005	0.19 ± 0.37	0.61 ± 0.48
Summer 2003*Summer 2006	0.35 ± 0.47	0.76 ± 0.42
Summer 2004*Summer 2005	0.20 ± 0.39	0.29 ± 0.45
Summer 2004*Summer 2006	0.01 ± 0.02	0.00 ± 0.02
Summer 2005*Summer 2006	0.17 ± 0.36	0.72 ± 0.44
Mid-Atlantic Bight (MAB)		
Age 1-3*Age 4-5	0.41 ± 0.39	0.87 ± 0.25

* Comparisons were made using data identifiable to the family level as well as general groupings (fishes, cephalopods, and crustaceans).

** Values > 0.60 are considered to reflect significant dietary overlap. Bootstrap mean estimates were generated from MH calculations based on 500 randomly selected individual stomach samples.

Stable isotopes

ABFT liver C:N and white muscle C:N, $\delta^{13}C$, and $\delta^{15}N$ all had significant variance differences among groups (Levene's test; $P<0.05$). Homogeneity of variance assumptions were not violated for liver $\delta^{13}C$ or $\delta^{15}N$.

For ABFT liver and muscle samples, significant differences were detected among seasons and years (Table 10). Liver $\delta^{15}N$ values for summer 2004 were significantly lower than all other seasons and years. In 2004, liver C:N, $\delta^{13}C$, and $\delta^{15}N$ and white muscle $\delta^{15}N$ all significantly increased from summer through fall (Table 10).

Isotope data loosely group common prey species into three trophic guilds (Table 11). Euphausiids (*Meganyctiphanes norvegica*), anchovies, Atlantic mackerel (*Scomber scombrus*), and swimming crabs share a low trophic level guild, with zooplantivorous grazing likely creating similarity amongst species (Fisher and Goldie 1959; Uriarte et al. 1996; Olaso et al. 2005; Signa et al. 2008). Blue whiting and myctophids, feeding on fish and crustacean prey (Geistdoerfer 1983; Stefanescu and Cartes 1992), comprise an intermediate trophic level guild, and piscivorous squids and horse mackerel constitute a high trophic level guild (Guerra and Rocha 1994; Olaso et al. 1999) (Table 11). Sardines are an outlier among prey species, with low $\delta^{13}C$ values comparable to other zooplantivores, but elevated $\delta^{15}N$ similar to piscivorous fishes. These higher $\delta^{15}N$ values likely reflect upwelled nitrogen rather than any higher trophic level prey components (Bode et al. 2004; Bode et al. 2007). Past studies detected similar $\delta^{15}N$ values in anchovies and Atlantic mackerel (Bode et al. 2004; Bode et al. 2007), and these zooplanktivorous fishes could constitute a separate isotopic guild with low $\delta^{13}C$ and high $\delta^{15}N$ values.

Table 10. Mean ± SD for Bay of Biscay (BYB) liver and muscle carbon and nitrogen values from Atlantic bluefin tuna (*Thunnus thynnus*). Values with different letter superscripts in a given column are significantly different ($P<0.05$).

			Muscle – Liver Discrimination			Liver				Muscle		
Year	Season	n	$\delta^{13}C$	$\delta^{15}N$	n	C:N	$\delta^{13}C$	$\delta^{15}N$	n	C:N	$\delta^{13}C$	$\delta^{15}N$
2001	Summer	24	$0.4 \pm 0.4^{b,d}$	$1.3 \pm 0.5^{b,c}$	24	5.2 ± 1.0^{b}	-18.0 ± 0.4^{c}	9.9 ± 0.5^{a}	24	3.2 ± 0.2^{a}	$-17.6 \pm 0.2^{a,b}$	$11.1 \pm 0.6^{a,b}$
2003	Summer	7	$0.8 \pm 0.2^{b,c}$	0.8 ± 0.6^{b}	7	$4.6 \pm 0.6^{b,c}$	-18.5 ± 0.2^{b}	9.9 ± 0.2^{a}	7	3.1 ± 0.1^{a}	-17.8 ± 0.1^{b}	$10.7 \pm 0.6^{a,b}$
2004	Summer	14	0.8 ± 0.2^{c}	1.8 ± 0.5^{a}	14	4.9 ± 0.5^{b}	-18.3 ± 0.3^{b}	8.8 ± 0.3^{b}	15	3.1 ± 0.1^{a}	$-17.6 \pm 0.2^{a,b}$	10.6 ± 0.6^{a}
2000	Fall	24	$0.1 \pm 0.3^{a,e}$	$1.5 \pm 0.4^{a,c}$	28	$6.3 \pm 1.2^{a,c}$	-17.6 ± 0.4^{a}	9.9 ± 0.3^{a}	27	3.2 ± 0.1^{a}	-17.6 ± 0.2^{a}	11.4 ± 0.3^{b}
2002	Fall	29	$0.3 \pm 0.4^{a,d}$	$1.1 \pm 0.4^{b,c}$	30	$5.6 \pm 1.2^{b,c}$	-18.0 ± 0.4^{c}	9.9 ± 0.5^{a}	30	3.2 ± 0.1^{a}	-17.7 ± 0.2^{b}	$11.0 \pm 0.8^{a,b}$
2004	Fall	14	-0.2 ± 0.4^{e}	$1.3 \pm 0.5^{b,c}$	15	8.0 ± 2.2^{a}	-17.9 ± 0.4^{a}	10.2 ± 0.7^{a}	15	3.3 ± 0.3^{a}	-17.5 ± 0.2^{a}	11.5 ± 0.6^{b}

Table 11. Mean ± SD prey item carbon, nitrogen, and C:N values from the Mid-Atlantic Bight (MAB) and Bay of Biscay (BYB).

Common Name	Scientific Name	n	$\delta^{13}C^{*}$	$\delta^{15}N^{**}$	ΔC:N	Length (mm)***
Bay of Biscay						
Northern krill	*Meganyctiphanes norvegica*	4	-19.3 ± 0.3	8.3 ± 0.5	0.8 ± 0.5	26.3 ± 2.0
Atlantic mackerel	*Scomber scombrus*	3	-19.4 ± 0.5	9.2 ± 1.3	0.6 ± 0.5	173.4 ± 35.9
European anchovy	*Engraulis encrasicholus*	1	-18.6	8.8	0.3	89.0
Henslow's swimming crab	*Polybius henslowii*	1	-19.2	9.0	1.3	44.7
Blue whiting	*Micromesistius poutassou*	2	-18.1 ± 0.3	10.2 ± 0.9	0.1 ± 0.0	153.9 ± 51.6
Jewel lanternfish	*Lampanyctus crocodilus*	1	-17.9	10.7	2.4	138.9
Atlantic horse mackerel	*Trachurus trachurus*	1	-16.4	11.3	0.3	181.2
Sardine	*Sardina pilchardus*	1	-18.4	11.4	2.0	219.7
Ommastrephid squid	*Todarodes sagitatus, Todaropsis eblanae, Illex coindetti*	4	-17.0 ± 0.6	11.7 ± 0.3	0.3 ± 0.0	160.9 ± 46.4
Mid-Atlantic Bight						
Crustaceans	Decapoda, Isopoda	3	-19.6 ± 0.4	7.7 ± 0.4	NA	19.0 ± 2.7
Squid	Teuthoidea	2	-18.6 ± 0.4	12.6 ± 0.4	0.7	11.5 ± 4.2
Sand lance	*Ammodytes* spp.	5	-20.1 ± 0.2	11.7 ± 0.1	0.7 ± 0.1	136 ± 3.5
Atlantic butterfish	*Peprilus triacanthus*	2	-20.0 ± 0.2	12.1 ± 0.5	0.7	55.0 ± 7.1
White hake	*Urophycis tenuis*	3	-19.0 ± 1.3	13.1 ± 0.7	0.1 ± 0.0	140.0 ± 7.0

* All $\delta^{13}C$ values are from lipid corrected samples.
** All $\delta^{15}N$ and C:N values are from bulk tissue samples.
*** Lengths only reported for individuals where measurement possible.

Mixing model estimates of dietary contributions were highest for euphausiid prey across all years and seasons, with Atlantic mackerel and swimming crabs of secondary importance. European anchovy, blue whiting, myctophids, horse mackerel, sardines, and ommastrephid squids represented > 10 % mean dietary proportions for any sampling season or year (Table 12).

Mid-Atlantic Bight

Stomach content analysis

Teleosts were the dominant prey group in all three dietary indices and sand lance were the main taxa for all size classes (Table 13). Teleosts had significantly greater dietary biomass than crustacean or cephalopod prey while no significant differences were detected between crustacean and cephalopod prey (Table 13). Morisita Horn index values between size classes were not significantly different (Table 9; MH>0.60).

Stable isotope analysis

Isotope values of ABFT prey were loosely grouped into three guilds: crustaceans, zooplantivorous fishes, and generalist fishes and cephalopods (Table 11). Crustacean samples had both low $\delta^{13}C$ and $\delta^{15}N$ values, indicative of a low trophic position, while gadids and cephalopods had higher $\delta^{13}C$ and $\delta^{15}N$ values, reflecting a higher trophic position. Sand lance and Atlantic butterfish (*Peprilus triacanthus*) had low $\delta^{13}C$, but correspondingly high $\delta^{15}N$ values, possibly due to upwelled (Altabet 1988) or anthropogenic sources of nitrogen (Bucci et al. 2007). Mixing model estimates of dietary contributions were highest for sand lance and crustacean prey while remaining prey groups had lower mean proportion estimates of ~ 10 % (Table 14).

Table 12. Mixing model estimates of prey contributions to Atlantic bluefin tuna (*Thunnus thynnus*) from the Bay of Biscay (BYB). Values are presented as mean proportion estimates with upper and lower 95 % confidence intervals.

Common name	Scientific name	Summer			Fall		
		Lower 95%	Upper 95%	Mean	Lower 95%	Upper 95%	Mean
Northern krill	*Meganyctiphanes norvegica*	0.45	0.89	0.67	0.45	0.70	0.57
Henslow's swimming crab	*Polybius henslowii*	0.00	0.29	0.12	0.05	0.40	0.23
Atlantic mackerel	*Scomber scombrus*	0.00	0.26	0.10	0.00	0.20	0.09
European anchovy	*Engraulis encrasicholus*	0.00	0.13	0.05	0.00	0.13	0.05
Blue whiting	*Micromesistius poutassou*	0.00	0.05	0.02	0.00	0.04	0.01
Jewel lanternfish	*Lampanyctus crocodilus*	0.00	0.04	0.01	0.00	0.03	0.01
Atlantic horse mackerel	*Trachurus trachurus*	0.00	0.02	0.01	0.00	0.02	0.01
Sardine	*Sardina pilchardus*	0.00	0.04	0.01	0.00	0.03	0.01
Ommastrephid squid	*Todarodes sagitatus, Todaropsis eblanae, Illex coindetti*	0.00	0.02	0.01	0.00	0.02	0.01

Table 13. Mean ± SD prey composition for Mid-Atlantic Bight (MAB) Atlantic bluefin tuna (*Thunnus thynnus*) stomach samples (percent weight (% W), percent number (% N), and frequency of occurrence (% O)).

	Age 1-3 (n = 20)			Age 4-5 (n = 22)		
Prey	%W	%N	%O	%W	%N	%O
Cephalopoda	1.1 ± 3.6	6.5 ± 22.5	15.0	0.3 ± 1.4	1.1 ± 5.3	4.5
Unidentifiable Teuthoidea	0.0 ± 0.1	0.8 ± 3.7	5.0	-	-	-
Loliginidae	0.7 ± 3.3	0.6 ± 2.8	5.0	-	-	-
Octopoda	0.3 ± 1.5	5.0 ± 22.4	5.0	0.3 ± 1.4	1.1 ± 5.3	4.5
Malacostraca (Crustaceans)						
Decapoda	4.9 ± 22.1	1.3 ± 5.6	5.0	0.0 ± 0.1	0.6 ± 2.7	4.5
Osteichthyes	90.9 ± 26.1	67.3 ± 45.7	100.0	86.3 ± 25.1	55.9 ± 48.6	100.0
Unidentifiable Osteichthyes	57.1 ± 39.5	0.0 ± 0.0	90.0	46.4 ± 35.0	0.0 ± 0.0	77.3
Clupeidae (*Etrumeus teres*)	4.5 ± 20.0	5.0 ± 22.4	5.0	3.8 ± 17.8	2.3 ± 10.7	4.5
Ammodytidae (*Ammodytes* spp.)	26.6 ± 35.7	57.1 ± 48.2	65.0	31.4 ± 36.3	50.8 ± 47.0	59.1
Stromateidae (*Peprillus triacanthus*)	2.7 ± 12.2	5.0 ± 22.4	5.0	-	-	-
Scorpaenidae	0.0 ± 0.0	0.2 ± 1.0	5.0	0.0 ± 0.1	0.6 ± 2.7	4.5
Gadidae	-	-	-	2.6 ± 12.2	0.8 ± 3.6	4.5
Phycidae (*Urophycis tenuis*)	-	-	-	2.6 ± 12.2	0.8 ± 3.6	4.5
Syngnathidae (*Hippocampus* sp.)	-	-	-	2.1 ± 9.6	1.5 ± 7.1	4.5
Parasites (*Hirudinella ventricosa*)	-	-	-	9.4 ± 21.1	24.2 ± 42.6	27.3
Plastic Material	-	-	-	0.0 ± 0.1	0.0 ± 0.0	4.5
Unidentifiable Material	3.0 ± 13.6	0.0 ± 0.0	5.0	4.0 ± 12.6	0.0 ± 0.0	18.2

Table 14. Mixing model estimates of prey contributions to Atlantic bluefin tuna (*Thunnus thynnus*) from the Mid-Atlantic Bight (MAB). Values are presented as mean proportion estimates with upper and lower 95% confidence intervals.

		Age 1-3			Age 4-5		
Common name	Scientific name	Lower 95 % CI	Upper 95% CI	Mean	Lower 95 % CI	Upper 95% CI	Mean
Crustaceans	Decapoda, Isopoda	0.22	0.45	0.33	0.11	0.39	0.24
Sand lance	*Ammodytes* spp.	0.07	0.40	0.24	0.01	0.29	0.15
Round herring	*Etrumeus teres*	0.00	0.22	0.11	0.00	0.23	0.12
Butterfish	*Peprilus triacanthus*	0.00	0.20	0.10	0.00	0.20	0.09
Sea horse	*Hippocampus* sp.	0.01	0.18	0.09	0.05	0.27	0.17
Hake	*Urophycis tenuis*	0.00	0.15	0.07	0.00	0.18	0.08
Shortfin squid	*Loligo pealeii*	0.00	0.14	0.06	0.03	0.26	0.15

Liver values varied in C:N and $\delta^{13}C$, but not $\delta^{15}N$. Minimum and median liver C:N values increased significantly ($P<0.05$) while maximum values did not change with ABFT length, but significant differences were not detected among size classes or years (Table 15).

Muscle values varied in C:N, $\delta^{13}C$, and $\delta^{15}N$ (Table 15). All muscle C:N value metrics significantly increased with ABFT length ($P<0.05$). C:N values differed between age 1-3 fish in 2005 and age 4-5 fish in 2006, but not among any other size classes, years, or seasons. For age 1-3 fish in 2004, muscle $\delta^{15}N$ differed significantly from all other years and size classes. For muscle-liver discrimination, significantly lower values of $\delta^{15}N$ were measured for age 1-3 ABFT in 2004 (0.5 ± 0.6 ‰) than all other size classes and years (1.0 ± 0.3 ‰ to 1.5 ± 0.4 ‰) (Table 15).

Trophic position estimates

Trophic position (TP) estimates based on SCA data were higher than those based on SIA diet proportion values. For BYB fish, there was little seasonal variation in TP (± SE) from SIA (3.6 ± 0.6, summer and 3.8 ± 0.6, fall), and from SCA data (4.1 ± 0.7, summer and 4.3 ± 0.7, fall). For MAB fish, TP based on SCA did not vary with size (4.4 ± 0.8, age 1-3 and 4.5 ± 0.8, age 4-5). With SIA, age 1-3 and 4-5 TP were estimated as 4.1 ± 0.7 and 4.2 ± 0.7, respectively.

Table 15. Mean ± SD for Mid-Atlantic Bight (MAB) liver and muscle carbon and nitrogen values from Atlantic bluefin tuna (*Thunnus thynnus*). Values with different letter superscripts in a given column are significantly different (P<0.05).

			Muscle – Liver Discrimination		Liver				Muscle	
Age class	Year	n	$\delta^{13}C$	$\delta^{15}N$	C:N	$\delta^{13}C$	$\delta^{15}N$	C:N	$\delta^{13}C$	$\delta^{15}N$
1-3 Years	2004	17	1.2 ± 0.3^{a}	0.5 ± 0.6^{a}	4.7 ± 0.8^{a}	-18.9 ± 0.5^{b}	11.2 ± 0.4^{a}	$3.2 \pm 0.1^{a,b}$	$-17.7 \pm 0.4^{a,b}$	11.7 ± 0.9^{a}
	2005	12	1.1 ± 0.3^{a}	1.0 ± 0.3^{c}	4.7 ± 0.6^{a}	$-18.8 \pm 0.3^{a,b}$	11.6 ± 0.3^{a}	3.1 ± 0.1^{b}	$-17.7 \pm 0.4^{a,b}$	12.6 ± 0.4^{b}
	2006	11	1.4 ± 0.4^{a}	$1.2 \pm 0.3^{b,c}$	5.5 ± 1.4^{a}	-19.5 ± 0.5^{a}	11.4 ± 0.3^{a}	$3.3 \pm 0.3^{a,b}$	-18.0 ± 0.3^{a}	12.7 ± 0.3^{b}
4-5 Years	2004	4	1.3 ± 0.4^{a}	$1.3 \pm 0.6^{b,c}$	4.8 ± 0.5^{a}	$-18.6 \pm 0.3^{a,b}$	11.8 ± 0.2^{a}	$3.2 \pm 0.1^{a,b}$	-17.3 ± 0.2^{b}	13.1 ± 0.6^{b}
	2006	28	1.1 ± 0.4^{a}	1.5 ± 0.4^{b}	5.0 ± 0.7^{a}	-18.6 ± 0.6^{b}	11.4 ± 0.4^{a}	3.4 ± 0.4^{a}	-17.5 ± 0.3^{b}	12.9 ± 0.2^{b}

Discussion

Young Atlantic bluefin tuna in eastern and western north Atlantic forage grounds mainly consumed zooplanktivorous fishes and crustacean prey. SCA and SIA estimates of teleost and crustacean prey importance varied with SCA showing higher teleost prey proportions, but both techniques indicated that cephalopods are minor prey components. Stomach content composition reflected variable forage patterns, leading to high standard deviations in percent prey weight and number calculations and low Morisita-Horn index values. In contrast, isotope values in each region were closely grouped across seasons and size classes, reflecting a consistency in diet and trophic position over longer periods. Isotope data also reflected slightly lower trophic positions than stomach contents. ABFT from the Mid-Atlantic Bight (MAB) fed at a slightly higher trophic position than Bay of Biscay (BYB) fish, because they consumed fewer crustaceans. Prey size generally did not change across ABFT size classes sampled in this study, as noted in previous studies on bluefin tuna species (Young et al. 1997; Chase 2002). Ram feeding on dense prey aggregations like sand lance or euphausiids could explain the wide predator size range in comparison with prey size (Chase 2002).

Caveats

Gut fullness values suggest higher feeding success in the BYB, but this could be due to sampling biases related to capture methods. Fish sampled from the MAB came from surface troll fisheries that could have induced regurgitation and selection for "hungry" fishes with lower gut fullness (Velasco and Quintans 2000). Most BYB fish came from the baitboat fishery, where fish are landed rapidly. Feeding on live bait would positively bias gut fullness. To account for this potential bias, the size and color of the

gall bladder as well as the texture of the stomach wall were observed for samples collected from the baitboat fishery to estimate whether stomach contents had been consumed during fishing operations. These criteria removed > 40 % of stomach samples collected from the baitboat fishery, but based on differences between SCA and SIA results, some bait were likely still included in SCA results. Distinction between recent diet derived from the baitboat fishery and past feeding on natural prey was difficult due to rapid ABFT digestion rates.

The importance of teleosts in ABFT diet varied between techniques, especially for horse mackerel and blue whiting in the BYB. Horse mackerel is the most common bait in local baitboat fisheries (Rodríguez-Marín et al. 2003) and was only observed in stomach samples collected from this fishery. SCA results are at least partly biased by consumption of chum and may account for their higher proportions in stomach contents relative to isotope results. Blue whiting is not used as live bait, but is a major discard from local trawl fisheries and a diet source for many fish species (Olaso et al. 1998; Lema et al. 2006). Large numbers of ABFT stomachs contained calcified structural remains of blue whiting. Opportunistic feeding on fishery discards has previously been documented for ABFT, including offal from albacore tuna (*Thunnus alalunga*) in the BYB (Priol 1944) and trawl discards in the NW Atlantic (Chase 2002).

The importance of crustacean prey varied between SCA and SIA results in both study areas, although SCA in the MAB was based on small sample sizes that may not adequately reflect prey diversity. Lower crustacean proportions in stomach samples could also be due to differential digestion rates, although sand lance are rapidly digested (Hilton et al. 1998), but still abundant in MAB stomach samples. Given the prevalence

of regurgitation in large pelagic fishes during fishing operations, smaller prey (i.e., crustaceans) might be preferentially removed from stomach contents.

A potential source of bias in these results is that BYB isotope proportion estimates were based on small prey sample sizes. Similar $\delta^{15}N$ values for sardines and horse mackerel have been reported, but higher values have been reported for Atlantic mackerel and anchovies (Bode et al. 2004; Bode et al. 2007). Similar $\delta^{13}C$ (-19.2 ‰) but lower $\delta^{15}N$ (7.6 ‰) values were reported for swimming crabs (Cartes et al. 2007) relative to present values. If these higher $\delta^{15}N$ values are used for Atlantic mackerel and anchovies, their proportion estimates are further reduced while euphausiids increase. Alternatively, lower $\delta^{15}N$ values for swimming crabs would increase their estimated contribution to diet, although cephalopods and fishes in swimming crab diet (Signa et al. 2008) are consistent with higher $\delta^{15}N$ values measured in this study.

If liver tissues were not in isotopic equilibrium with local diet, isotope values could also be biased by previous diet. For example, transits from offshore regions would likely produce lower $\delta^{15}N$ values due to baseline shifts (Fry and Quiñones 1994; Montoya et al. 2002), causing overestimation of lower trophic level prey. ABFT arrive in the MAB in late May (Richards 1965), but sampling ranged from 25 June to 23 July, and no significant differences in liver $\delta^{15}N$ values were detected. With the exception of summer 2004, liver $\delta^{15}N$ values did not differ seasonally in the BYB (Table 10).

Diet-tissue discrimination for liver $\delta^{15}N$ is assumed to be ~ 1.3 ‰ based on studies from other fish species. If actual values for ABFT are lower, results would also be biased towards lower trophic level prey, but experimental results support $\delta^{15}N$

discrimination ≥ 1.3 ‰ (Pinnegar and Polunin 1999; Suzuki et al. 2005; Trueman et al. 2005; Sweeting et al. 2007a).

Bluefin Diet and Trophic Position

Based on isotope results ABFT appear to feed on low trophic level prey on eastern and western Atlantic forage grounds. King mackerel (*Scomberomorus cavalla*), a piscivore (Bowman et al. 2000) from the MAB, had higher $\delta^{13}C$ and $\delta^{15}N$ values relative to ABFT. King mackerel liver samples had $\delta^{13}C$ and $\delta^{15}N$ (± SD) values of -17.9 ± 0.3 ‰ and 12.5 ± 0.3 ‰ (n = 3), respectively, while the complete ABFT dataset had $\delta^{13}C$ and $\delta^{15}N$ values of -18.8 ± 0.6 ‰ and 11.4 ± 0.4 ‰. Consequently, isotope discrimination values assumed in this study would place king mackerel ~ 1 TL above ABFT. Stable isotope results showing higher proportions of crustacean prey may also reflect other prey types (e.g. larval fishes: $\delta^{15}N$ = 8.1 ‰) with similar isotopic values that were overlooked in SCA samples from the MAB due to small sample sizes. BYB isotope results that crustaceans are the main prey base are generally supported by SCA results when sampling biases are assumed.

ABFT diet in the MAB contained high proportions of sand lance, consistent with previous studies (Eggleston and Bochenek 1990; Barr 1991) and indicative of the stability and abundance of this food resource over time (Nelson and Ross 1991; Ostrand et al. 2005). Sand lance abundance was inversely related on a decadal scale to that of co-occurring herring (*Clupea harengus*) and mackerel (Sherman et al. 1981; Nelson and Ross 1991). Large population fluctuations have been recorded in the MAB over recent decades (Nelson and Ross 1991), but young ABFT fed on sand lance during both high (Barr 1991) and low (Eggleston and Bochenek 1990) abundance periods. Despite this

variability, no major prey group has filled the sand lance niche during periods of lower abundance. Atlantic mackerel, herring, butterfish, and longfin squid (*Loligo pealeii*), which occupy similar trophic positions to sand lance (Bowman et al. 2000), are secondary prey items in this and in prevous studies (Eggleston and Bochenek 1990; Barr 1991). Thus, age 1-5 yrs ABFT occupy a common niche along these coastal forage grounds, as further supported by similar trophic position estimates (TL: ~ 4.5 (SCA) and 4.0 (SIA)) and liver isotope values (Table 15).

In a previous study, anchovies were the dominant prey of ABFT in the Bay of Biscay with euphausiids as a secondary prey group (Ortiz de Zárate and Cort 1986), while the opposite was found in this study. Although this could result from differences in sampling, it may reflect inter-annual shifts in available prey or dietary preferences. In trawl surveys within the southern BYB, horse mackerel and blue whiting were the dominant pelagic forage fishes, with minor contributions from sardines, Atlantic mackerel, and anchovies (Mahe et al. 2007; Preciado et al. 2008). Anchovy populations in the BYB have recently declined (Ibaibarriaga et al. 2008; ICES 2008), and their low representation in stomach samples could reflect reduced availability and a consequent dietary shift for ABFT. Anchovy recruitment was relatively high for 2000, but then declined for the remaining years of the study (Ibaibarriaga et al. 2008). The highest proportion of anchovies was observed for fall 2000 (relative to all other sampling years) while euphauiids were absent from 2000 stomach samples, providing further support for a dietary shift in relation to prey availability. Diurnally migratory euphausiids such as *M. norvegica* (Mauchline and Fisher 1969) may provide a concentrated prey source for ABFT and other marine predators (Brown et al. 1979) in the Bay of Biscay.

In both regions, summer 2004 showed anomalous isotope values relative to other seasons and years. Significant differences in muscle $\delta^{15}N$ and muscle-liver $\delta^{15}N$ discrimination for age 1-3 ABFT in the MAB in 2004 likely reflect recent immigration from offshore waters. These isotopic differences could be due to previous foraging on lower trophic level prey or regional isotopic baseline shifts (Hobson 1999). Since local diets consist primarily of zooplanktivorous fishes and crustaceans, past foraging at lower trophic levels (e.g., zooplankton) is unlikely, and offshore forage grounds dominated by N_2 fixation and recycled nitrogen could produce such low $\delta^{15}N$ values (Wada and Hattori 1991). Differences in oceanographic conditions could have influenced timing of arrivals to shelf regions. For example, in 2004, increased cold, fresh Scotian shelf water entered the eastern Gulf of Maine, which was then advected later in the year onto George's Bank and into the MAB (Taylor et al. 2005). For the BYB, muscle-liver $\delta^{13}C$ and $\delta^{15}N$ discrimination for summer 2004 was inconsistent with other years. Similar to outlier MAB isotope values, BYB summer 2004 values could be indicative of past feeding in offshore gyres.

Summer 2004 samples from the BYB reflected a higher proportion of euphausiid prey based on SCA and $\delta^{15}N$ values, and were significantly different from all other seasons. Isotope results may also mirror this unique diet. Euphausiid abundance in the BYB was high in 2004 (Lezama et al. 2008; Irigoien et al. 2009) while anchovy recruitment was lowest among sampling years (Ibaibarriaga et al. 2008). In the BYB, 2004 was unique, because it had extreme and prolonged winter and summer climatic conditions with minimal transitional spring and fall seasons (Fontán et al. 2008).

Anomalous climatic conditions may thus have contributed to the high and low abundances of euphausiids and anchovies, respectively, in ABFT diet that year.

Prey and ABFT Condition

In the NW Atlantic, lipid content estimates of bluefin prey range from ~ 3 – 7 % for sand lance, butterfish, and squid (Lawson et al. 1998; Budge et al. 2002). Based on prey sample ΔC:N values, gadid prey have low lipid content while butterfish, squid, and sand lance have higher values (Table 11). Despite high intra-species variability, sand lance has relatively high energetic content, which combined with dense schooling behavior (Nizinski 2002) provides an ideal prey for a ram-feeder like ABFT.

Available Bay of Biscay prey items vary widely in quality, with anchovies and Atlantic mackerel generally having higher lipid content than euphausiids, blue whiting, and horse mackerel (Soriguer et al. 1997; Mayzaud et al. 1999). Euphausiid lipid content varies widely depending on season, sex, and reproductive state (Mauchline and Fisher 1969; Mayzaud et al. 1999; Albessard et al. 2001). Prey ΔC:N was lowest for blue whiting, squids, horse mackerel, and anchovies, intermediate for Atlantic mackerel and euphausiids, and highest for swimming crabs, sardines, and myctophids (Table 11). Since prey isotope sample sizes were limited and their lipid contents highly variable, in the absence of higher sample sizes and duration, prey energetic cannot be fully assessed from this study. While further studies are needed to compare the nutritional quality of euphausiid and anchovy prey, differences in their quality could affect ABFT energetics as ABFT appear to feed preferentially on whichever prey group is most abundant.

For age 1-3 ABFT, muscle tissues from both regions had minimal lipid stores (mean C:N ~ 3.2, lipid-free value ~ 3.1 (Logan et al. 2008)), while age 4-5 ABFT from

the MAB had slightly higher C:N values (~3.4). ABFT increase in length rather than girth at smaller sizes (< 110 cm), then gain greater mass at larger sizes (Mather et al. 1995; Fromentin and Powers 2005), and a corresponding increase in lipid stores was observed for age 4-5 yr ABFT in the MAB. No ABFT > 110 cm were analyzed for muscle C:N in the Bay of Biscay, but summer biomass gain for age 1-3 ABFT in this region is ~ 5-6 times faster than winter growth (Cort 1991), suggesting that energy stores should increase during this period of elevated growth. During 2004 when data were collected across the feeding season (BYB), liver C:N significantly increased from summer to fall, suggesting that juveniles might instead store energy reserves in the liver. In adults, lipid content changes seasonally in muscle tissue in adults (Estrada et al. 2005; Golet et al. 2007), and increases in somatic condition may have been missed because of limited sample size. In a more comprehensive study in the BYB, there was a linear increase in muscle lipid content with size and both inter and intra-annual variability (Goñi and Arrizabalaga *In press*).

Young ABFT occupy a lower trophic position than co-occurring marine mammal and some fish predators like bluefish and albacore tuna in both Atlantic shelf regions (Ortiz de Zárate 1987; Bowman et al. 2000; Spitz et al. 2006). This result is due to their higher predation rates on crustaceans and other lower trophic level prey species. In the Bay of Biscay, 1 to 2 year old ABFT and individuals up to 80 cm FL in the BYB have low $\delta^{15}N$ values, corresponding to values of smaller primary consumers (e.g., sardines) (Bode et al. 2007). It is noteworthy that adult ABFT can reach a trophic level similar to top pelagic predators such as odontocetes and sharks (Estrada et al. 2003; Estrada et al. 2005; Sara and Sara 2007), suggesting that ABFT undergo an ontogenetic dietary shift of

several trophic levels. Nonetheless, young ABFT fed on similar prey items to ABFT in NW Atlantic forage grounds (Estrada et al. 2005), but lower TL prey than ABFT of the same size classes in the Mediterranean Sea (Sanz Brau 1990; Sinopoli et al. 2004; Sara and Sara 2007).

Young ABFT feed mainly on zooplanktivorous fishes and crustaceans in forage grounds in the eastern and western Atlantic. Results generally support past findings that ABFT in the MAB forage mainly on sand lance, but isotope results suggest similar contributions of lower trophic level prey. Further studies comparing the quality and abundance of these prey groups would better define potential impacts on ABFT feeding in this region, and long term monitoring of trophic relationships will be necessary to understand ecosystem changes related to climate change and human exploitation.

Diet of Atlantic Bluefin Tuna in NW Atlantic Forage Grounds

Introduction

Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) occupy productive forage grounds in the Gulf of Maine from May through October (Crane 1936; Mather et al. 1995; Wilson et al. 2005), feeding on available crustacean, cephalopod, and fish prey (Crane 1936; Chase 2002; Estrada et al. 2005). Bluefin tuna have historically supported recreational and commercial fisheries in the Gulf of Maine (Mather et al. 1995). In recent decades, catch landings have shifted, with commercial landings (> 185 cm curved fork length (CFL)) decreasing and recreational landings of smaller individuals (69 – 185 cm) increasing (B. McHale, Pers. Comm.). The somatic condition of adult bluefin tuna

has also decreased in recent decades (Golet et al. 2007). These local shifts in bluefin tuna abundance, size distribution, and condition could be linked to changes in the available forage base (Polovina 1996; Benson and Trites 2002).

Bluefin tuna in the Gulf of Maine consume a variety of prey species with adults feeding preferentially on fishes and cephalopods and juveniles also consuming high proportions of crustaceans (Crane 1936; Chase 2002; Estrada et al. 2005). Most recent assessments of adult bluefin tuna diet showed a prevalence of Atlantic herring (*Clupea harengus*) and sand lance (*Ammodytes* spp.) (Chase 2002; Estrada et al. 2005), as well as Atlantic mackerel (*Scomber scombrus*), bluefish (*Pomatomus saltatrix*), and cephalopods (Chase 2002). Juvenile bluefin tuna fed at a lower trophic position, likely consuming crustacean prey (Estrada et al. 2005) in addition to cephalopods, Atlantic butterfish (*Peprilus triacanthus*), and mackerel (Chase 2002).

Many seasonal migrants to the Gulf of Maine leave this region in the fall for winter shelf forage grounds in the Mid-Atlantic Bight (Block et al. 2001; Wilson et al. 2005; Walli et al. 2009), where they feed on dense aggregations of Atlantic menhaden (*Brevoortia tyrannus*) and swimming crabs (Butler 2007). This forage ground could offer an important energy source for Gulf of Maine migrants during winter months, providing additional nutrition for spring migrations to spawning grounds or returns to summer forage grounds.

Carbon and nitrogen stable isotopes in consumer tissues are derived from dietary sources and can provide longer term feeding histories in addition to the detailed short term records of stomach contents (Peterson and Fry 1987). Carbon ($\delta^{13}C$; $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$; $^{15}N/^{14}N$) isotopes in consumer tissues are offset from dietary values by a

discrimination factor (Martínez del Rio and Wolf 2004). Using isotope values of consumer tissue and potential prey species and estimates of diet-tissue isotope discrimination, dietary proportions can be estimated through mixing models (Phillips and Gregg 2001; Moore and Semmens 2008). Isotope turnover rates differ among tissues and compounds; liver and white muscle reflect short (weeks) and intermediate (months) timescales, respectively, in fishes (Logan et al. 2006; MacNeil et al. 2006). Tissue total carbon to nitrogen ratios (C:N) provide a proxy for lipid content (Post et al. 2007) that can be used to track body condition (Estrada et al. 2005).

Stomach samples were collected from bluefin tuna and isotope samples from bluefin tuna and prey to test for temporal shifts in diet and trophic position. Liver and white muscle C:N ratios were also measured to assess seasonal trends in condition.

Methods

Sample Collection

Atlantic bluefin tuna stomach and isotope samples were collected from commercial and recreational landings as well as scientific troll surveys in the Gulf of Maine (Table 16; Figure 13) and commercial landings in the Mid-Atlantic Bight (Table 17; Figure 14). For stomach content analyses, samples were grouped based on size class as school (< 140 cm CFL) and large (small-medium, large-medium, and giant; > 165 cm CFL). For isotope analyses, school and large size classes correspond to curved fork lengths < 155 cm and > 175 cm, respectively. Whole stomach samples were removed by cutting the lining above the pylorus. White muscle samples were collected from the dorsal musculature anterior to the caudal fin while liver samples were collected from the tip of the anterior lobe. All samples were temporarily stored on ice after collection then

frozen until preparation for analysis. Curved fork length (CFL) ± cm was measured for each fish. For some commercially landed fish (CFL > 185 cm), dressed length (DL), curved fork length with head removed, was first measured, then converted to CFL using the equation: CFL = DL * 1.35 (S. Turner, unpublished data). For commercially landed fish, dressed weight, round weight (RW) minus head and internal organs, was recorded and converted to round weight using the equation: RW = DW * 1.25 (Anonymous 2003).

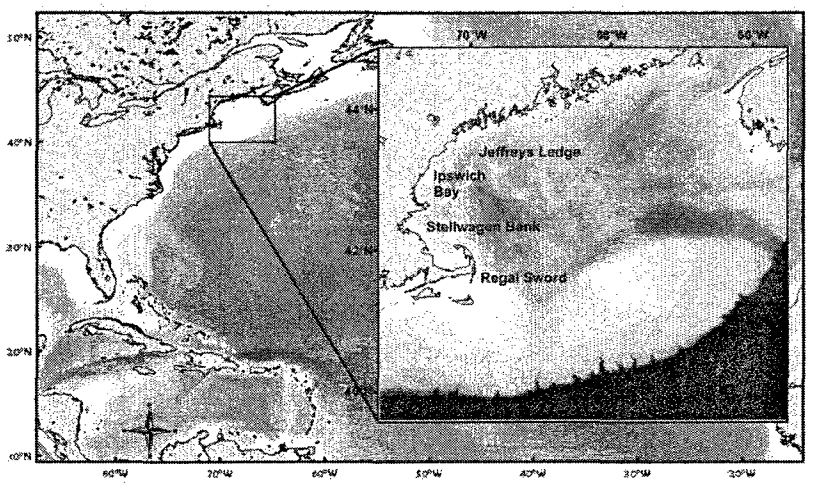

Figure 13. Map of Gulf of Maine study site showing locations of fishing areas where Atlantic bluefin tuna (*Thunnus thynnus*) were sampled.

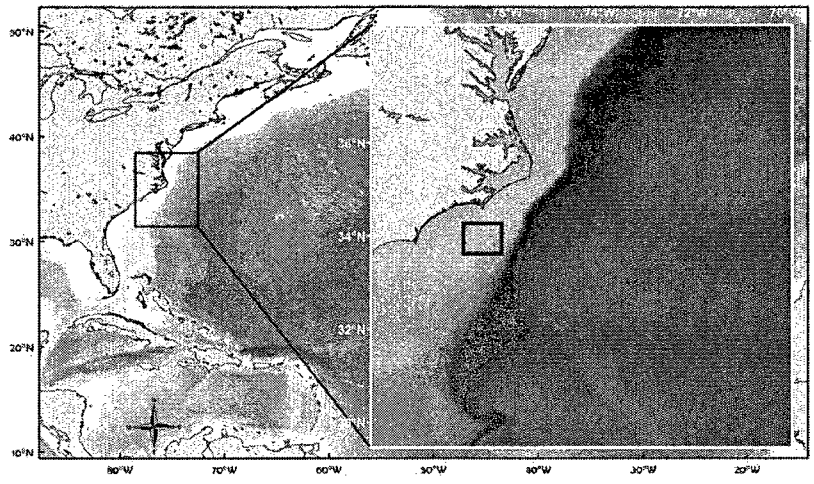

Figure 14. Map of Mid-Atlantic Bight study site. Samples of Atlantic bluefin tuna (*Thunnus thynnus*) liver and muscle were collected from the boxed region south of Cape Lookout, North Carolina.

Prey samples for stable isotope analysis were obtained from bluefin tuna stomach contents, fishery landings, or scientific surveys. Prey samples were measured ± 0.5 cm by straight fork length (fishes), mantle length (cephalopods), and rostral length (crustaceans). Whole samples were stored for analysis for all prey except spiny dogfish (*Squalus acanthias*), sand lance (*Ammodytes* spp.), Atlantic cod (*Gadus morhua*), and cusk (*Brosme brosme*), from which sub-samples of dorsal muscle were obtained. Sand lance isotope data were obtained from literature values (Kaufman and Brown 2009).

Table 16. Sampling summary of liver, muscle, and stomach samples from Atlantic bluefin tuna (*Thunnus thynnus*) in the Gulf of Maine.

	Early Season (June – July)			Mid-Season (August)			Late Season (September – October)		
	Liver	Muscle	Stomach	Liver	Muscle	Stomach	Liver	Muscle	Stomach
School[*]									
2004	0	0	0	0	0	0	0	4	0
2005	1	2	1	2	2	3	7	2	8
2006	0	0	0	0	0	1	2	2	2
2007	0	1	0	1	1	1	1	1	1
2008	0	0	0	0	1	0	1	1	0 (1)
Total	**1**	**3**	**1**	**3**	**4**	**5**	**11**	**10**	**12**
Large[**]									
2004	13	35	23 (2)[***]	1	7	2	12	138	13 (1)
2005	5	22	9 (1)	31	15	8 (3)	8	30	10 (2)
2006	8	17	8	4	18	6	3	14	2 (1)
2007	3	16	1 (1)	6	21	2 (2)	14	35	12 (2)
2008	8	18	9 (1)	0	0	1 (2)	0	0	4 (1)
Total	**37**	**108**	**52**	**42**	**61**	**23**	**37**	**217**	**45**

* For liver and muscle samples, school bluefin tuna refers to fish < 155 cm CFL while for stomach samples, school bluefin tuna refers to fish < 140 cm CFL.

** For liver and muscle samples, large bluefin tuna are > 175 cm and for stomach samples, large bluefin tuna are > 165 cm CFL.

*** For stomach samples, values in parentheses represent stomachs that were empty or only contained chum.

Table 17. Sampling summary of liver and muscle samples from Atlantic bluefin tuna (*Thunnus thynnus*) from the Mid-Atlantic Bight (North Carolina).

	December		January	
	Liver	Muscle	Liver	Muscle
2004	1	3	0	0
2005	42	1	0	0
2006	0	0	9	0
Total	43	4	9	0

Stomach Content Analysis (SCA)

Whole stomachs were thawed, weighed (± 0.05 g), and contents were washed over a 1,000 micron sieve. The stomach lining was then blotted dry and re-weighed to calculate total stomach content weight as whole stomach content weight (g) – lining weight (g). Contents were identified to the lowest possible taxonomic group using keys for external morphometrics as well as beak and otolith morphology (Clarke 1986; Collette et al. 2002; Campana 2004). Whole individuals were weighed (± 0.1 g) and measured (± 0.5 cm) to estimate fork, mantle and rostral lengths of teleost, cephalopod, and crustacean prey, respectively. Chum, consisting of cut pieces of Atlantic herring and other fishes, was separated from remaining contents and not included in any analyses. Stomachs containing only chum and empty stomachs were excluded from analyses, since ABFT may evacuate their guts during capture (Chase 2002). Loose cephalopod beaks and teleost otoliths were analyzed separately from soft tissue samples since these hard parts degrade more slowly (Jobling and Breiby 1986; Van Heezik and Seddon 1989). Beaks and otoliths were identified and measured to generate estimates of prey size (Jobling and Breiby 1986; Hunt 1992; Bowen and Harrison 1994; Staudinger et al. 2009).

Stable Isotope Analysis (SIA)

Prey items and ABFT liver and white muscle samples were thawed and lightly rinsed with deionized water. Liver and muscle samples were then transferred to glass scintillation vials while whole prey were finely minced and transferred to aluminum weigh boats. For whole prey samples, all gut contents were also removed. All samples were then dried at 60°C for at least 48 h. Dried samples were then homogenized with a Wig-L-Bug® ball and capsule amalgamator (Crescent Industries, Auburn, Maine, USA), Mixer/Mill® (SPEX SamplePrep, LLC Metuchen, New Jersey U.S.A) and stainless steel grinding vials or a mortar and pestle, depending on tissue volume.

Aliquots of homogenized sample (0.6 – 1.2 mg) were packed into 4 X 6 mm tin cups and analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen by continuous flow using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies, Inc, Valencia, CA USA) coupled with a $DELTA_{plus}$ XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory of Northern Arizona University (NAU) and the University of New Hampshire Stable Isotope Laboratory (UNH). All C:N values are reported as uncorrected percent weight calculations ($\frac{\%C}{\%N}$) as opposed to percent atomic weight ($\frac{\%C}{\%N} \times 1.16667$).

All sample $\delta^{13}C$ values were corrected for lipid content either *a priori* through chemical extractions or *a posteriori* using mathematical approaches. ABFT liver and white muscle samples were corrected *a posteriori* using a mass balance equation (Fry 2002) with parameters specific to ABFT liver and white muscle (Logan et al. 2008). All prey samples were lipid extracted with 2:1 chloroform-methanol solution (Logan et al. 2008) except sand lance, which were normalized with mathematical corrections (Logan et

al. 2008). Crustacean prey also underwent a carbonate extraction (Jacob et al. 2005). For samples that underwent chemical extractions, a second aliquot of bulk tissue was analyzed for $\delta^{15}N$ to avoid potential error induced by the extraction process (Bunn et al. 1995; Pinnegar and Polunin 1999; Bodin et al. 2007).

All carbon and nitrogen isotope data are reported in δ notation according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] * 1000$$

where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Standard materials are Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. Standard deviations of replicate samples analyzed at both labs were 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$ (n = 45) and within-lab precision is ~ 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on the VPDB and AIR scales with IAEA CH6 (-10.4 ‰), CH7 (-31.8 ‰), N1 (0.4 ‰) and N2 (20.3 ‰).

Statistical Analyses

Adequacy of stomach content sample sizes were evaluated by generating cumulative prey curves (Ferry and Caillet 1996). Increasing numbers (1 to n-1) of stomach samples were randomly selected for each dataset, and the total number of unique prey types was summed for each sampling. Bootstrapping techniques were used to generate 500 random samples for each sample size in order to generate means and standard deviations with the program R (R Development Core Team 2008). Sample size was then plotted against mean number of unique prey types, where a unique prey type was defined as the most specific identification for a given prey group. Where a dataset contained a prey group identifiable to species level (e.g., Atlantic herring), less specific

identifications would not be counted as unique prey (e.g., teleost fishes). To assess whether a sufficient number of samples had been analyzed to capture prey diversity, the slope of the final four endpoints for each prey curve was compared to a slope of zero using a Student's t-test (Bizzarro et al. 2007). All p-values were adjusted for multiple comparisons using a Holm test (Holm 1979).

Relationships between ABFT and prey size were explored using quantile regression. Regressions were performed for the median (50^{th} percentile) as well as 5^{th} and 95^{th} percentiles to test for patterns in minimum and maximum prey size in relation to ABFT length. Significance was tested using a rank sums test. All quantile regression analyses were performed in the statistics package quantreg in R (R Development Core Team 2008). Prey lengths were based on measurements of whole prey and regression estimates from beak rostral length (cephalopods) and otolith length (teleost fishes) (Jobling and Breiby 1986; Hunt 1992; Bowen and Harrison 1994; Staudinger et al. 2009). Comparisons were made for all prey pooled as well as for sand lance, Atlantic herring, and shortfin squid (*Illex illecebrosus*), species that were found in stomach samples of both bluefin tuna size classes.

Percent weight and percent number were calculated for each prey group for each stomach sample to generate mean and standard deviations for each prey category. Frequency of occurrence was also calculated for each prey group. Mean percent weight contributions were compared using a Kruskal Wallis test for crustaceans, cephalopods, and teleost fishes. Nemenyi-Damico-Wolfe-Dunn tests were performed as post-hoc analyses when significant differences ($P<0.05$) were detected.

Stable Isotope Analysis (SIA)

Large ABFT liver and muscle samples were grouped by sampling date: early (mid-June – July), mid- (August), and late (September – October), while school ABFT samples were pooled across seasons. Homogeneity of variance among groups was assessed using Levene's test. Comparisons of tissue C:N, $\delta^{13}C$, and $\delta^{15}N$ were made using analysis of variance (ANOVA) when assumptions of homogeneity of variance were not violated and a one-way analysis of means when Levene's test results were significant. When significant differences were detected among groups, pairwise t-tests were performed with $\alpha = 0.05$ following a Holm test adjustment for multiple comparisons. For cases where homogeneity of variance was violated, pairwise comparisons using t-tests with non-pooled standard deviations were performed.

To estimate relative lipid contents among prey groups, ΔC:N was calculated according to the formula:

$$\Delta C{:}N = C{:}N_{bulk} - C{:}N_{lipid-free}$$

where CN_{bulk} is the C:N ratio of a dried, homogenized prey sample and $CN_{lipid\text{-}free}$ is the C:N ratio of a prey sample following chemical lipid extraction. Values are presented as ΔC:N rather than bulk C:N, because baseline (lipid-free) C:N values vary among fish species (Sweeting et al. 2006; Logan et al. 2008).

Prey dietary contributions were quantified using the SIAR (Stable Isotope Analysis in R) package in R (R Development Core Team 2008). For the Gulf of Maine, ABFT liver isotope data from fish sampled during late season (September to October) were used for mixing model analyses since white muscle turns over more slowly (Graham et al. submitted) and may reflect a mixture of dietary information for local food

webs as well as distant forage locations for this highly migratory species. For prey sources, species found in stomach samples at > 3 % weight that represented distinct trophic guilds were used (Table 18). For species found in stomach samples of both ABFT size classes, prey isotope data were segregated based on the size range found in stomach samples for this study (Tables 18 and 19) and a previous study (Chase 2002). For the Mid-Atlantic Bight, all ABFT liver samples (December and January) were used. Prey items identified as important dietary components based on biomass or frequency of occurrence in stomach samples (Butler 2007) were included as sources (Table 20).

Mixing models can only generate absolute solutions when the number of prey sources is $\leq n + 1$ isotopes. As source number increases, uncertainty in individual source contributions also increases (Phillips and Gregg 2003; Moore and Semmens 2008). To reduce such error, prey sources that did not significantly differ were combined *a priori* (Phillips et al. 2005). Prey species' isotope values were first plotted for visual comparison (Figures 15 and 16), then statistically compared with ANOVA followed by pairwise t-tests with Holm adjustment for multiple comparisons. Nitrogen isotope values were first compared among individual prey species. A subsequent statistical comparison of carbon isotope values was performed for any pairs that did not have significantly different nitrogen isotope values.

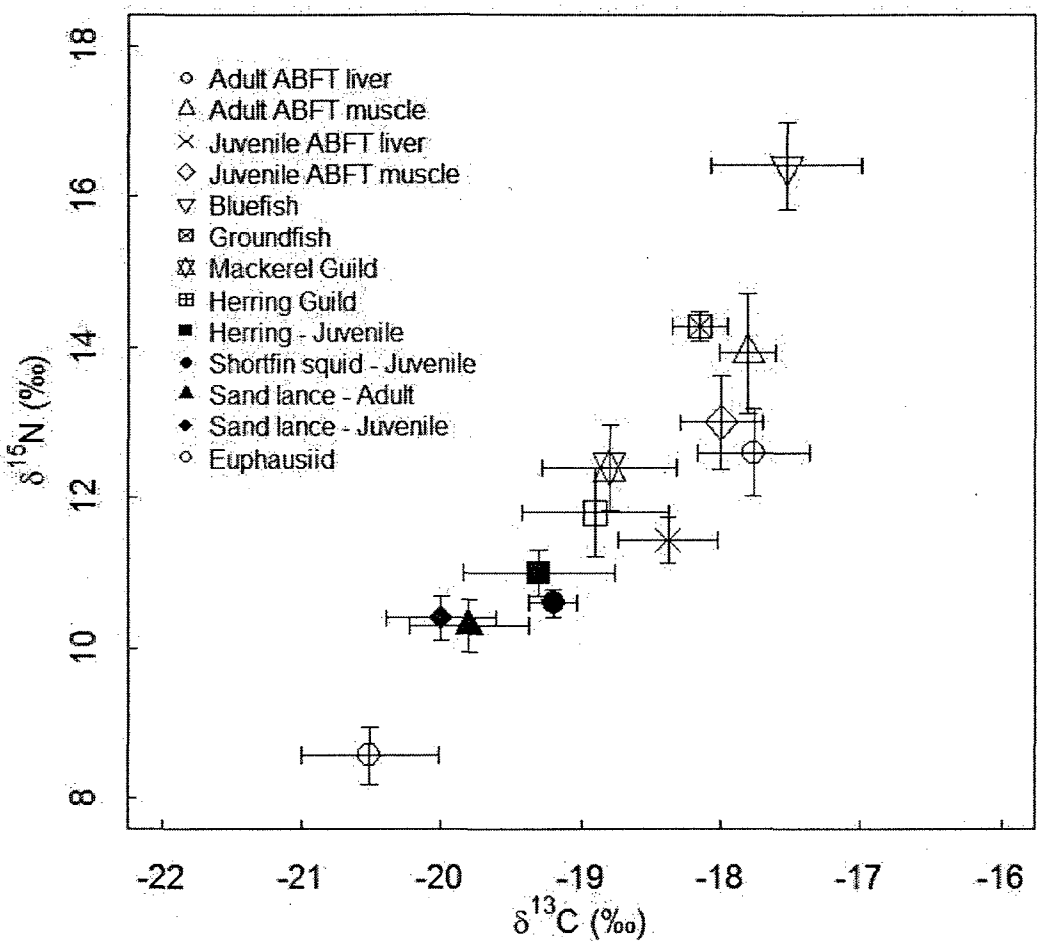

Figure 15. Mean ± SD carbon and nitrogen isotope values for Atlantic bluefin tuna (*Thunnus thynnus*) and representative prey species in the Gulf of Maine. School bluefin tuna are all < 155 cm CFL while large individuals are > 175 cm CFL. Among prey species, the herring guild consists of Atlantic herring (*Clupea harengus*), adult shortfin squid (*Illex illecebrosus*), and spiny dogfish (*Squalus acanthias*). The mackerel guild consists of Atlantic mackerel (*Scomber scombrus*) and silver hake (*Merluccius bilinearis*). Groundfish refers to Atlantic cod (*Gadus morhua*) and cusk (*Brosme brosme*). These groups were pooled due to non-significant differences in isotope values among individual species.

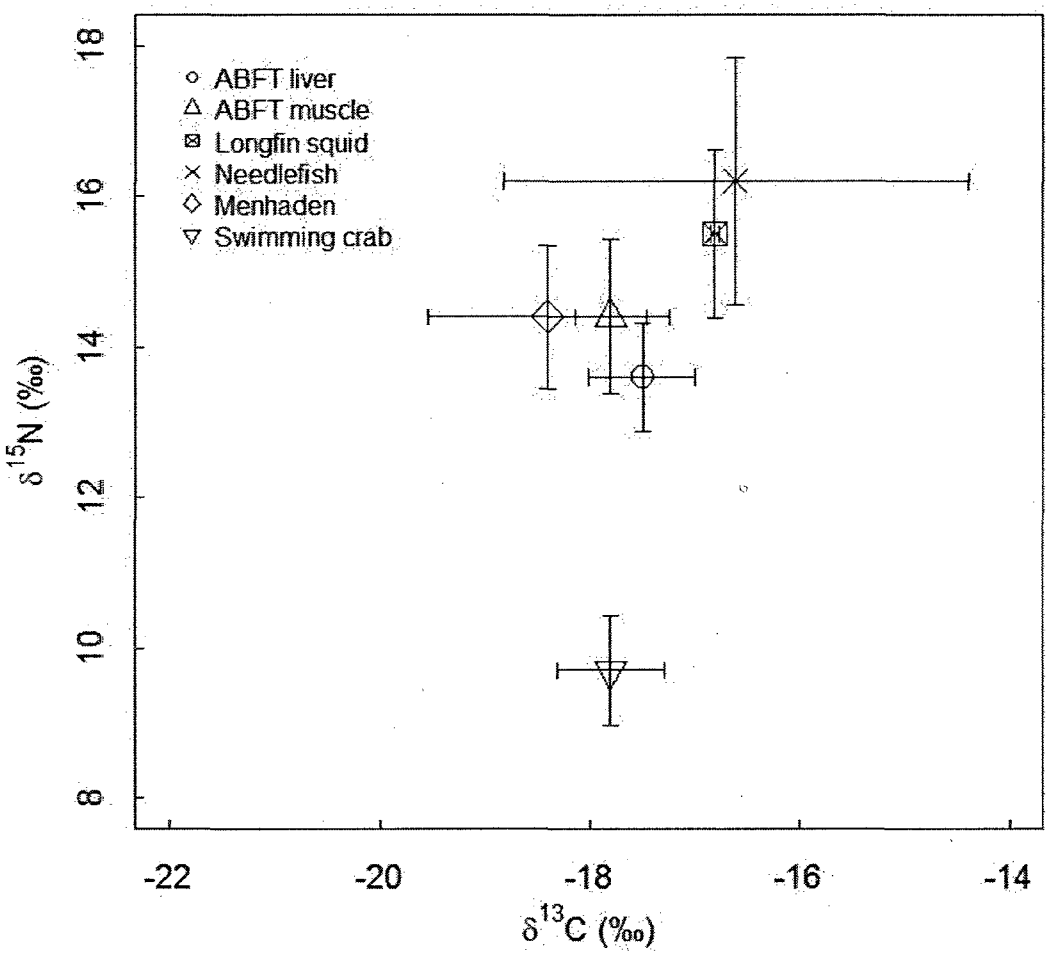

Figure 16. Mean ± SD carbon and nitrogen isotope values for Atlantic bluefin tuna (*Thunnus thynnus*) and representative prey species in the Mid-Atlantic Bight.

While diet-tissue discrimination is often assumed to be ~ 0.4 ‰ (Peterson and Fry 1987) for $\delta^{13}C$ and 3.4 ‰ for $\delta^{15}N$ (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987; Post 2002), discrimination factors vary among individuals (Gaye-Siessegger et al. 2004; Barnes et al. 2008), species (Macko et al. 1982), tissue types (Pinnegar and Polunin 1999), and diet types (Adams and Sterner 2000; Focken 2004; Aberle and Malzahn 2007). For mixing model calculations, diet-tissue

discrimination was assumed to be 1.4 ‰ for $\delta^{13}C$ and 1.3 ‰ for $\delta^{15}N$ based on available liver data for yellowfin tuna (*Thunnus albacares*) (Graham et al. submitted) and other fish species (Pinnegar and Polunin 1999; Suzuki et al. 2005; Trueman et al. 2005; Sweeting et al. 2007a). To account for uncertainty and inherent variability in trophic discrimination values, we applied standard deviation values of 0.43 ‰ and 0.58 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, to approximate inter and intra-species variability (Pinnegar and Polunin 1999; McCutchan Jr et al. 2003; Suzuki et al. 2005; Trueman et al. 2005; Sweeting et al. 2007a; Sweeting et al. 2007b; Graham et al. submitted). Prey values were entered into SIAR models as mean values ± SD. When only a single prey sample was analyzed for a given prey group, standard deviation estimates of 0.40 were applied for each isotope to account for inter-individual variability.

Table 18. Gulf of Maine prey item carbon, nitrogen, and CN values (± SD). Prey names with different superscript letters significantly differ for at least one isotope.

Common Name	Scientific Name	n	$\delta^{13}C^{**}$	$\delta^{15}N^{***}$	ΔCN^{****}	Length (cm)
Adult Prey						
Sand lance[a]	*Ammodytes* spp.	121	-19.8 ± 0.4	10.3 ± 0.3	1.9 ± 0.8*	14.8 ± 2.5
Shortfin squid[b]	*Illex illecebrosus*	9	-19.1 ± 0.3	11.4 ± 0.2	1.0 ± 0.1	15.0 ± 4.0
Atlantic herring[b,c]	*Clupea harengus*	25	-19.0 ± 0.5	11.9 ± 0.6	3.3 ± 2.0	24.5 ± 2.8
Spiny dogfish[b,c]	*Squalus acanthias*	8	-18.5 ± 0.4	12.0 ± 0.8	1.6 ± 1.1	78.6 ± 6.9
Atlantic mackerel[c]	*Scomber scombrus*	11	-19.0 ± 0.3	12.5 ± 0.7	1.8 ± 1.6	23.6 ± 3.9
Silver hake[c]	*Merluccius bilinearis*	25	-18.8 ± 0.5	12.4 ± 0.5	0.6 ± 0.3	21.1 ± 8.5
Groundfish[d]	*Gadus morhua, Brosme brosme*	6	-18.2 ± 0.3	14.3 ± 0.2	NA	48.3 ± 7.6
Bluefish[e]	*Pomatomus saltatrix*	13	-17.5 ± 0.5	16.4 ± 0.6	2.3 ± 0.9	55.7 ± 3.7
Juvenile Prey						
Krill[a]	Euphausiidae	11	-20.5 ± 0.5	8.6 ± 0.4	0.5 ± 0.2	1.6 ± 0.6
Sand lance[b]	*Ammodytes* spp.	63	-20.0 ± 0.4	10.4 ± 0.3	2.4 ± 0.3*	12.7 ± 1.4
Shortfin Squid (juv.)[c]	*Illex illecebrosus*	6	-19.2 ± 0.2	10.6 ± 0.2	0.9 ± 0.2	8.9 ± 1.0
Longfin squid[NA]	*Loligo pealeii*	1	-18.8	12.3	0.7	6.5
Atlantic herring (juv.)[c]	*Clupea harengus*	4	-19.3 ± 0.5	11.0 ± 0.3	1.1 ± 0.3	13.7 ± 1.1

* Sand lance values from literature (Kaufman et al. 2009) with $\delta^{13}C$ based on lipid normalization (Logan et al. 2008). Sand lance ΔCN estimates are based on additional samples obtained from adult (length: 19.5 ± 0.0 cm; n = 2) and school ABFT stomachs (11.2 ± 1.7; n = 8).

** All $\delta^{13}C$ values are from lipid corrected samples.

*** All $\delta^{15}N$ and CN values are from bulk tissue samples.

**** ΔCN represents the difference in CN between bulk and lipid extracted sub-samples.

Table 19. Size distributions of prey from school (< 140 cm CFL) and large (>165 cm CFL) Atlantic bluefin tuna (*Thunnus thynnus*) in the Gulf of Maine (GOM).

Prey Species	Common name	n*	Prey Length (cm)** Mean ± SD	Minimum	Maximum
	School Atlantic bluefin tuna (<140 cm CFL)				
Ammodytes spp.	Sand lance	711 (9)	10.5 ± 1.2	6.3	15.5
Selene setapinnis	Atlantic moonfish	1 (1)	4.9	4.9	4.9
Clupea harengus	Atlantic herring	18 (1)	10.9 ± 9.2	4.5	28.4
Euphausiidae	Krill	3 (1)	4.0 ± 4.4	3.7	4.5
Loligo pealeii	Longfin squid	3 (1)	5.5 ± 2.4	2.8	7.4
Illex illecebrocus	Shortfin squid	3 (3)	9.5 ± 2.3	7.0	11.5
	Large Atlantic bluefin tuna (>165 cm CFL)				
Ammodytes spp.	Sand lance	1 (1)	13.1	13.1	13.1
Cyclopterus lumpus	Lumpfish	2 (2)	13.9 ± 14.0	4.0	23.8
Clupea harengus	Atlantic herring	185 (51)	23.1 ± 2.7	16.4	29.0
Euphausiidae	Krill	1 (1)	6.4	6.4	6.4
Melanogrammus aeglefinus	Haddock	1 (1)	41.2	41.2	41.2
Pollachius virens	Pollock	32 (9)	24.1 ± 5.6	14.7	36.6
Urophycis chuss	Red hake	3 (3)	27.7 ± 5.4	22.7	33.3
Merluccius bilinearis	Silver hake	112 (30)	19.7 ± 6.0	10.6	40.5
Illex illecebrocus	Shortfin squid	312 (34)	12.6 ± 2.2	5.1	22.4
Pseudopleuronectes americanus	Winter flounder	13 (1)	25.2 ± 2.5	22.5	30.2
Pomatomus saltatrix	Bluefish	4 (3)	39.2 ± 20.5	20.8	63.0
Rajidae	Skate	1 (1)	16.0	16.0	16.0
Scomber scombrus	Atlantic mackerel	51 (7)	21.7 ± 3.8	18.3	31.2
Sebastes fasciatus	Redfish	15 (8)	15.4 ± 3.8	9.3	22.6
Squalus acanthias	Spiny dogfish	3 (2)	70.8 ± 2.8	68.0	73.5
Peprilus triacanthus	Atlantic butterfish	2 (1)	11.8 ± 1.1	11.0	12.5

* Sample size (n) refers to the number of prey samples followed by stomach sample size in parentheses.

** Lengths are based on whole prey and otolith and beak regressions.

Table 20. Mid-Atlantic Bight (MAB; North Carolina) prey item carbon, nitrogen, and CN values (± SD). Prey names with different superscript letters significantly differ for at least one isotope.

Common Name	Scientific Name	n	$\delta^{13}C^{*}$	$\delta^{15}N^{**}$	ΔCN^{***}	Length (cm)
Atlantic needlefish[a]	*Strongylura marina*	8	-16.6 ± 2.2	16.2 ± 1.6	1.3 ± 1.2	NA
Atlantic menhaden[b]	*Brevoortia tyrannus*	14	-18.4 ± 1.2	14.4 ± 1.0	2.3 ± 1.2	27.2 ± 2.3
Longfin squid[a]	*Loligo pealeii*	2	-16.8 ± 0.0	15.5 ± 1.1	0.7 ± 0.4	11.5
Iridescent shore crab[c]	*Portunus gibbesii*	8	-17.8 ± 0.5	9.7 ± 0.7	1.4 ± 0.8	5.3 ± 0.7

* All $\delta^{13}C$ values are from lipid corrected samples.
** All $\delta^{15}N$ and CN values are from bulk tissue samples.
*** ΔCN represents the difference in CN between bulk and lipid extracted sub-samples.

Results

Prey Curves

The slopes of endpoints for all datasets were significantly different from zero, indicating inadequate sample size for all datasets (Figure 17).

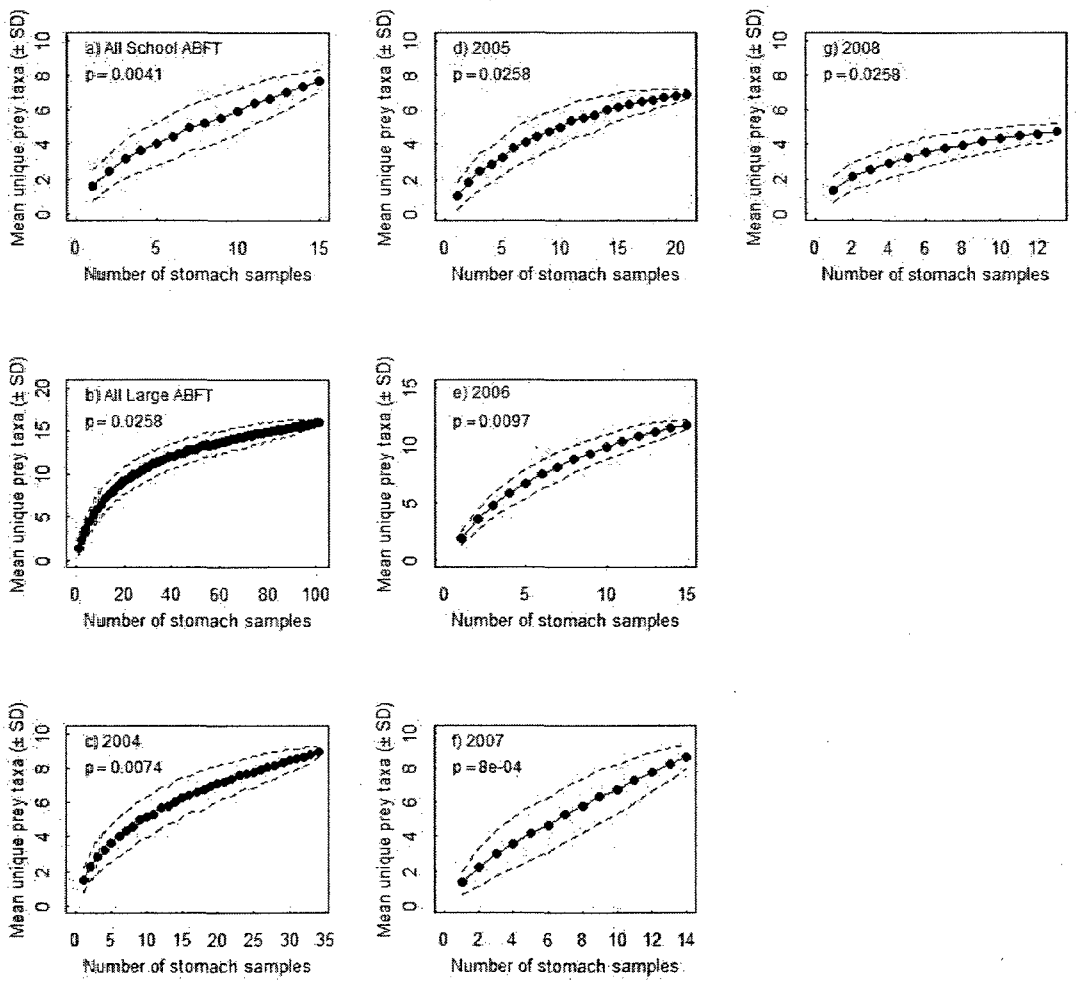

Figure 17. Cumulative prey curves showing estimated numbers of unique prey species observed across increasing sample sizes for a) school bluefin tuna (< 140 cm CFL) b) all large bluefin tuna (>165 cm CFL) pooled across sampling years and c-g) for individual sampling years (2004 – 2008). P-values refer to comparisons of the slope of the final four sample sizes to a slope of zero. Significant values indicate insufficient sample size to adequately capture prey diversity.

Prey Size

For all measured prey, median and maximum prey size increased significantly with predator size, but minimum prey size did not change (Table 19). For shortfin squid, no size relationships were detected. For herring, minimum prey size increased with ABFT size, but maximum and median sizes did not change. This is likely due to the presence of a single school size class fish that contained many small and large herring. For sand lance, median and maximum prey size increased with ABFT size, but not minimum prey size, although only one sand lance was measured for adult bluefin tuna.

Prey Composition

ABFT diet composition was made up mainly of fishes, which comprised a significantly greater weight percentage of stomach contents than cephalopod or crustacean prey for both ABFT size classes (Tables 21 and 22). No differences were detected between cephalopod and crustacean prey for either size class. School ABFT stomach samples contained mostly sand lance and euphausiids and to a lesser degree Atlantic herring (Table 21). Large ABFT samples contained mostly Atlantic herring (Table 22). Secondary prey groups (~ 3 – 5 % weight) in order of increased weight proportions were Atlantic mackerel, pollock (*Pollachius virens*), silver hake, sand lance, bluefish, and cephalopods.

Table 21. Stomach contents from school Atlantic bluefin tuna (*Thunnus thynnus*; <140 cm CFL). Contents are reported as mean wet weight and number percentages ± SD (%W and %N) and by frequency of occurrence (%O).

Prey	%W	%N	%O
Cephalopoda	0.3 ± 1.0	0.4 ± 1.4	18.8
Unidentifiable Teuthoidea	0.0 ± 0.2	0.0 ± 0.0	6.3
Ommastrephidae (*Illex illecebrosus*)	0.0 ± 0.2	0.0 ± 0.0	6.3
Loliginidae (*Loligo pealeii*)	0.2 ± 1.0	0.3 ± 1.4	6.3
Malacostraca (Crustaceans)	27.1 ± 41.9	48.3 ± 46.7	62.5
Amphipoda	0.5 ± 2.1	2.1 ± 8.3	6.3
Decapoda (Unidentifiable)	0.0 ± 0.1	0.0 ± 0.0	6.3
Euphausia (Euphausiidae)	26.5 ± 41.0	46.2 ± 45.0	62.5
Osteichthyes	72.4 ± 41.7	41.2 ± 43.8	93.8
Unidentifiable teleosts	20.4 ± 35.3	0.6 ± 2.1	62.5
Clupeidae (*Clupea harengus*)	9.4 ± 25.8	12.2 ± 33.2	12.5
Ammodytidae (*Ammodytes* spp.)	41.0 ± 46.2	28.0 ± 37.9	50.0
Carangidae (*Selene setapinnis*)	0.2 ± 0.8	0.0 ± 0.0	6.3
Monacanthidae	1.3 ± 5.3	0.5 ± 2.1	6.3
Heterokontophyta (*Ascophyllum* and unidentifiable)	0.1 ± 0.4	0.0 ± 0.0	12.5
Foreign Material (Wood, gravel, plastic)	0.1 ± 0.5	0.0 ± 0.0	12.5

Table 22. Stomach contents from large Atlantic bluefin tuna (*Thunnus thynnus*; >165 cm CFL). Contents are reported as mean wet weight and number percentages ± SD (%W and %N) and by frequency of occurrence (%O).

Prey	%W	%N	%O
Bivalvia	0.0 ± 0.0	1.0 ± 8.4	2.0
Cephalopoda	5.1 ± 18.7	8.2 ± 21.4	28.4
Unidentifiable Teuthoidea	1.8 ± 10.5	2.5 ± 11.6	19.6
Ommastrephidae (*Illex illecebrosus*)	3.4 ± 15.3	5.7 ± 17.9	18.6
Malacostraca (Crustaceans)	1.1 ± 9.9	3.6 ± 15.6	8.8
Amphipoda	0.1 ± 0.8	2.0 ± 12.0	3.9
Decapoda (Unidentifiable Decapoda)	0.0 ± 0.1	0.7 ± 4.0	3.9
Decapoda (*Homarus americanus*)	0.0 ± 0.0	0.1 ± 0.7	1.0
Euphausiidae	1.0 ± 9.9	1.0 ± 9.9	1.0
Fishes	92.9 ± 21.3	74.1 ± 34.6	96.1
Chondrichthyes	2.2 ± 12.6	0.6 ± 4.1	5.9
Squalidae (*Squalus acanthias*)	2.2 ± 12.6	0.6 ± 4.1	4.9
Rajidae	0.0 ± 0.0	0.0 ± 0.5	1.0
Osteichthyes	90.8 ± 24.1	73.5 ± 34.7	96.1
Unidentifiable teleosts	24.5 ± 31.6	13.4 ± 29.6	76.5
Clupeidae (*Clupea harengus*)	39.2 ± 38.7	39.5 ± 39.5	64.7
Ammodytidae (*Ammodytes* sp.)	4.4 ± 19.7	1.2 ± 10.0	4.9
Pomatomidae (*Pomatomus saltatrix*)	5.3 ± 19.5	3.2 ± 10.8	10.8
Scombridae (*Scomber scombrus*)	3.1 ± 11.7	3.1 ± 14.1	10.8
Stromateidae (*Peprilus triacanthus*)	0.0 ± 0.3	0.0 ± 0.5	1.0
Pleuronectidae (*Pseudopleuronectes americanus*)	0.8 ± 8.3	0.7 ± 6.8	1.0
Ophichthidae (*Ophichthus cruentifer*)	0.0 ± 0.0	0.1 ± 1.0	1.0
Unidentifiable Gadiformes	2.3 ± 11.9	1.4 ± 10.8	6.9
Merluccidae (*Merluccius bilinearis*)	3.8 ± 14.9	5.0 ± 19.5	10.8
Phycidae (Unidentifiable)	0.5 ± 4.8	0.3 ± 3.3	1.0
Phycidae (*Urophycis chuss*)	0.8 ± 6.7	0.8 ± 5.9	2.0
Gadidae (*Pollachius virens*)	3.4 ± 13.4	3.7 ± 14.9	6.9
Gadidae (*Melanogrammus aeglefinus*)	0.4 ± 4.3	0.1 ± 0.9	1.0
Sebastidae (*Sebastes fasciatus*)	1.5 ± 10.3	1.0 ± 6.9	3.9
Cyclopteridae (*Cyclopterus lumpus*)	0.8 ± 8.4	0.3 ± 2.9	2.0
Heterokontophyta (*Ascophyllum* and unidentifiable)	0.4 ± 3.3	0.4 ± 1.9	12.7
Parasites (Trematoda)	0.2 ± 1.0	6.9 ± 16.2	29.4
Foreign Material (Wood, gravel, plastic)	0.3 ± 1.9	0.0 ± 0.0	11.8

Isotopes

Gulf of Maine

For liver and muscle, both inter-season and inter-size class differences were detected (Table 23). Differences in $\delta^{13}C$ were detected between school and large size classes; school ABFT liver values were significantly lower than all seasonal groups while muscle values were lower than those for early and late season fish. For both tissue types, mid- and late season $\delta^{15}N$ values were significantly higher than early season fish. School ABFT liver $\delta^{15}N$ values were lower than all adult season groups, while muscle values were lower than mid- and late season, but not early season adults. For C:N, adult early and mid-season liver values were significantly lower than late season while school ABFT did not differ from any season group. Muscle C:N values for early season large ABFT and school ABFT were lower than mid and late season fish (Table 23).

Table 23. Mean ± SD Atlantic bluefin tuna (*Thunnus thynnus*) carbon and nitrogen data. Values in the same column for a given tissue type with different superscript letters are significantly different ($P < 0.05$). *

	n	CN	$\delta^{13}C$	$\delta^{15}N$
Liver				
Early	37	7.6 ± 2.1^{a} (0.28)	-17.8 ± 0.4^{a} (0.02)	12.1 ± 0.8^{a} (0.06)
Mid	39	7.9 ± 2.2^{a} (0.27)	-17.7 ± 0.4^{a} (0.02)	12.6 ± 0.5^{b} (0.04)
Late	36	10.8 ± 4.9^{b} (0.45)	-17.8 ± 0.4^{a} (0.02)	12.6 ± 0.7^{b} (0.05)
School	15	$9.3 \pm 2.8^{a,b}$ (0.19)	-18.4 ± 0.4^{b} (0.03)	11.4 ± 0.3^{c} (0.02)
Muscle				
Early	107	3.3 ± 0.3^{a} (0.10)	-17.8 ± 0.3^{a} (0.02)	13.5 ± 0.9^{a} (0.07)
Mid	61	4.4 ± 1.0^{b} (0.22)	$-17.8 \pm 0.3^{a,b}$ (0.02)	13.8 ± 0.9^{b} (0.06)
Late	217	4.4 ± 1.0^{b} (0.23)	-17.8 ± 0.3^{a} (0.01)	13.9 ± 0.8^{b} (0.06)
School	18	3.3 ± 0.3^{a} (0.01)	-18.0 ± 0.3^{b} (0.02)	13.0 ± 0.6^{a} (0.04)

* Data are grouped by season as early (mid-June – July), mid- (August), and late (September-October) season for large (> 175 cm CFL) ABFT. School (< 155 cm CFL) ABFT are pooled across seasons. Values in parentheses are coefficient of variation (CV, %).

Mixing Model Estimates

Gulf of Maine

Large ABFT

Isotope values for bluefish, groundfish (cod and cusk), and sand lance all differed significantly from other prey sources, allowing them to be entered into mixing models as individual sources (Table 24). Mid-trophic level prey isotope values overlapped, with no significant differences among herring, shortfin squid, and spiny dogfish or Atlantic mackerel and silver hake. Additional overlap was detected among individual prey groups between herring, dogfish, mackerel, and silver hake. Spiny dogfish overlapped significantly with all mid-trophic level species, but were assigned to the lower trophic level guild with shortfin squid and Atlantic herring since their nitrogen isotope values more closely reflected these lower $\delta^{15}N$ species.

Sand lance and the herring guild comprised the highest mean proportions followed by the mackerel guild. Groundfish and bluefish had minor contributions (< 10%). Exact proportion estimates were sensitive to assumptions of isotope discrimination, but across a broad range of assumed discrimination factors, the lower trophic level guilds (sand lance, herring guild, mackerel guild) were the dominant prey sources while higher trophic level prey (groundfish and bluefish) were minor contributors (Table 24).

School ABFT

Mixing model estimates showed similar dietary contributions among the three prey groups, although close spacing of isotope values also made results sensitive to discrimination factor estimates (Table 25). ABFT liver values were intermediate among

prey sources after correction for trophic discrimination, which creates uncertainty in mixing model estimates of diet proportions (Phillips and Gregg 2003). While proportion estimates were similar among prey groups, ABFT values were aligned more closely with fish and cephalopod prey than euphausiids, and sand lance, herring, and squid had higher proportion estimates (Table 25).

Table 24. Mixing model estimates of prey contributions for adult Atlantic bluefin tuna (*Thunnus thynnus*) for the Gulf of Maine. Values are presented as mean proportion estimates with upper and lower 95% confidence intervals.

Common name	Scientific name	Lower 95%	Upper 95%	Mean
Sand lance	*Ammodytes* spp.	0.35	0.58	0.46
Herring guild*	*Clupea harengus, Illex illecebrosus, Squalus acanthias*	0.11	0.57	0.36
Mackerel guild**	*Merluccius bilinearis, Scomber scombrus,*	0.00	0.28	0.12
Groundfish***	*Gadus morhua, Brosme brosme*	0.00	0.11	0.04
Bluefish	*Pomatomus saltatrix*	0.00	0.06	0.02

* The herring guild consists of pooled data for Atlantic herring, shortfin squid, and spiny dogfish since their isotope values did not significantly differ.

** The mackerel guild consists of pooled data for silver hake and Atlantic mackerel.

*** Groundfish consists of Atlantic cod and cusk.

Table 25. Mixing model estimates of prey contributions for school Atlantic bluefin tuna (*Thunnus thynnus*) for the Gulf of Maine. Values are presented as mean proportion estimates with upper and lower 95% confidence intervals.

Common name	Scientific name	Lower 95%	Upper 95%	Mean
Sand lance	*Ammodytes* spp.	0.15	0.58	0.36
Atlantic herring, shortfin squid*	*Clupea harengus, Illex illecebrosus*	0.23	0.54	0.38
Krill	Euphausiidae	0.15	0.37	0.26

* Atlantic herring and shortfin squid isotope values were pooled to form a single group, since their isotope values did not significantly differ.

Mid-Atlantic Bight

Menhaden and swimming crabs each differed significantly from all remaining prey species, while needlefish and swimming crabs were statistically indistinguishable (Table 26). Needlefish and squid isotope data were then pooled as a common prey source in the mixing model. Menhaden were the dominant prey source with a mean proportion of 56 %. Swimming crabs comprised a slightly lower proportion (43 %), while squid and needlefish contributed only 1 % (Table 26).

Table 26. Mixing model estimates of common Atlantic bluefin tuna (*Thunnus thynnus*) prey species in Mid-Atlantic Bight (North Carolina) forage grounds.

Common name	Scientific name	Lower 95%	Upper 95%	Mean
Atlantic needlefish, Longfin squid	*Strongylura marina*, *Loligo pealeii*	0.00	0.04	0.01
Atlantic menhaden	*Brevoortia tyrannus*	0.50	0.61	0.56
Iridescent shore crab	*Portunus gibbesii*	0.38	0.48	0.43

* Atlantic needlefish and longfin squid isotope values were pooled to form a single group, since their isotope values did not significantly differ.

Discussion

Gulf of Maine

Both stomach content and stable isotope results are generally consistent with previous feeding studies in the Gulf of Maine; adults feed primarily on zooplanktivorous fishes and juveniles on fishes and euphausiids. Given the apparent stability in diet across several decades, observed declines in adult condition (Golet et al. 2007) and shifts in local abundance do not appear to be driven by shifts in diet composition. Both liver and muscle C:N values increased significantly across seasons, indicating a general improvement in body condition. School bluefin tuna fed at a lower trophic level than larger conspecifics, which in turn fed at a lower level than other piscivorous fishes.

Median and maximum prey sizes increased with bluefin tuna size, but small prey were found across all size classes.

Large bluefin tuna stomach contents were composed mainly of Atlantic herring, a keystone prey species in the Gulf of Maine (Collette et al. 2002; Overholtz 2006). Atlantic herring comprised about half of contents biomass in both this study and a past assessment (Chase 2002). Bluefin tuna diet varies spatially within the Gulf of Maine (Chase 2002). While the specific forage ground was not documented for most stomach samples, all of the commercial size class samples were obtained through a single fishermen's cooperative, whose fleet largely targets the forage grounds in Ipswich Bay and Jeffreys Ledge (R. Campbell, Pers. Comm.). When compared with past stomach content results for this region (Chase 2002), results from this study are remarkably similar, showing Atlantic herring as the major prey component and small contributions from cephalopods, bluefish, Atlantic mackerel, and sand lance. A large proportion of stomach contents from this study were not identified beyond the class level. Much unclassified soft tissue was found in stomachs also containing herring remains and probably was largely composed of highly digested herring. If much of the unidentifiable teleost category is assumed to be herring remains, herring dietary proportions estimates are about 70 % for both studies.

School bluefin tuna stomach contents consisted mostly of sand lance and euphausiid prey, with lesser contributions (< 10 % weight) from juvenile herring and cephalopods. Results agree with past studies on juvenile bluefin tuna in Mid-Atlantic Bight forage grounds, where sand lance was their major prey (Eggleston and Bochenek 1990; Barr 1991). A previous isotope assessment of juvenile bluefin tuna diet in the Gulf

of Maine showed a reliance on crustacean prey (Estrada et al. 2005), which is supported by the prevalence of euphausiids in stomach samples.

Isotope results generally support stomach content findings, with mid-trophic level prey representing the main diet sources for large bluefin tuna. Higher trophic level prey species like bluefish and demersal predators like cod and cusk were minor prey components. Bluefish prey samples actually had higher $\delta^{13}C$ and $\delta^{15}N$ values than large bluefin tuna, indicative of a higher average trophic position. Bluefish are piscivores that consume many of the same prey species as bluefin tuna (e.g., herring, sand lance, cephalopods, and other bluefish (Link and Almeida 2000)), and their elevated trophic status is either a product of their predation on higher trophic level fishes (e.g., gadids and other demersal predators) or feeding by bluefin tuna on lower trophic level prey.

Bluefin tuna liver isotope values closely match isotope values for schooling zooplanktivorous fishes once adjusted for assumed isotope discrimination factors. Previous isotope analyses demonstrated a seasonal shift in carbon isotope values reflective of a dietary shift from silver hake to sand lance and herring (Estrada et al. 2005). While mixing model results generally reflect the same local prey, the absence of a similar seasonal shift in carbon isotope values in lipid-normalized samples suggests that past findings were an artifact of seasonal shifts in tissue lipid content.

The herring guild that comprised a major prey base also contained dogfish and shortfin squid, so prey contributions cannot be distinguished among these three prey species based solely on isotope results. The prevalence of herring in stomach samples and the less frequent and smaller amounts of dogfish and squid suggest that herring are the main prey group within this guild. Herring and cephalopods are both digested rapidly

in marine predator stomachs (Bigg and Fawcett 1985), and dogfish would likely break down more slowly, given their large size. In addition to differential digestion rates, stomach content data could be biased by sampling location and the inclusion of chum. While herring are more prevalent in samples from Jeffreys Ledge (Chase 2002) and commonly used as chum in the local commercial fishery, herring were also major prey components in other major forage grounds (Stellwagen Bank and the Great South Channel (Chase 2002)), and all contents identified as chum were excluded from analysis.

Isotope results show higher proportions of sand lance than stomach contents, which may be due to regional sampling biases. Sand lance are the main prey on Stellwagen Bank (Chase 2002), a region that was likely underrepresented in this study. Adult bluefin tuna travel between local forage grounds within the Gulf of Maine on a timescale of days (Lutcavage et al. 2000) and likely seek available forage across most local regions within a feeding season. Isotopes, which incorporate dietary information over timescales of weeks in tuna liver (Graham et al. submitted), would capture these patterns while stomach contents would not, given that they only incorporate daily dietary information (Butler and Mason 1978; Carey et al. 1984; Butler 2007).

Juvenile ABFT isotope mixing model outputs did not distinguish any prey group as a dominant prey source, but did indicate slightly higher contributions from fish and cephalopods than crustacean prey. Sample sizes of juvenile ABFT were limited for both SCA and SIA, but combined results suggest a mixed diet of sand lance, juvenile herring, cephalopods, and euphausiids. Juveniles overall fed at a lower TP and on smaller prey than adults.

Mid-Atlantic Bight

Isotope results support stomach content data showing menhaden as the main prey group in the Mid-Atlantic Bight (Butler 2007). Menhaden had a high ΔC:N, indicative of a high lipid content. Dense aggregations of menhaden occupy shelf waters off North Carolina during winter months (Ahrenholz 1991), providing a concentrated, high energy forage base for migrating ABFT. Like herring in the Gulf of Maine, menhaden are a keystone species in the Mid-Atlantic Bight that transfer nutrients from estuaries and coastal waters to the pelagic environment (Deegan 1993).

Mixing model estimates also indicated a major dietary input from swimming crabs whereas previous stomach content analyses found swimming crabs in < 10 % of samples constituting < 1 % of prey biomass (Butler 2007). Inter-study differences could be due to biases associated with each method; stomach data may be overestimating larger prey with slower digestion rates like menhaden while isotope data may be overestimating lower trophic level prey due to incomplete equilibration. Small swimming crabs would likely degrade more rapidly than menhaden, resulting in underestimation in stomach content analyses. Local prey species, particularly menhaden, needlefish, and squid, had elevated $\delta^{15}N$ values relative to most prey in other regional forage grounds (see Movement section). These elevated $\delta^{15}N$ values could be incorporated during past residency in coastal and estuarine habitats, where anthropogenic nitrogen sources may elevate baseline $\delta^{15}N$ values (Schlacher et al. 2005; Bucci et al. 2007; Hadwen and Arthington 2007). Recent migration to the Mid-Atlantic Bight from Gulf of Maine or open ocean forage grounds would cause tissue $\delta^{15}N$ values to be low relative to many local prey items. Arrival of ABFT to Mid-Atlantic Bight forage grounds occurs as early

as October, but peak residency is delayed until December and January (Wilson et al. 2005; Walli et al. 2009). Since all isotope samples were collected in December and January, some fish may have been recent immigrants with isotope values reflecting both local and past diets. Estimates of swimming crab isotope values may also have been artificially low, as whole prey samples likely contained chitin, which is known to have lower $\delta^{15}N$ values than protein (Schimmelmann and DeNiro 1988; Macko et al. 1990; Webb et al. 1998). While differences in lower trophic level prey between studies cannot be resolved, both techniques show a prevalence of menhaden in ABFT diet. ABFT are clearly making use of this regional aggregation as a prey base during winter months.

Past studies on bluefin tuna species have found variable relationships between tuna and prey sizes. No relationships exist between southern bluefin tuna (*Thunnus maccoyii*) size and prey size (Young et al. 1997). For Atlantic bluefin tuna, the minimum and median size but not the maximum size of the dominant prey in Mid-Atlantic Bight forage grounds, menhaden (*Brevoortia tyrannus*), increases with tuna size (Butler 2007). A past study of bluefin tuna in the Gulf of Maine found a positive relationship between tuna and prey sizes (Chase 2002). A significant increase in median and maxium prey size was found with bluefin tuna size in this study, due to the inclusion of large prey in adult samples like spiny dogfish and bluefish. Minimum prey size did not vary across sizes, due to the presence of small prey like sand lance and crustaceans. For the Gulf of Maine, bluefin tuna size classes appear to overlap in prey size and species composition, with all size classes consuming smaller prey and many of the same prey species (e.g., herring, sand lance, squids), but with adult bluefin tuna occupying a wider niche, consuming larger individuals of these common species as well as additional larger prey species.

Tissue lipid content increased seasonally for large bluefin tuna for both liver and muscle while school bluefin tuna had high liver lipid content but low levels in muscle tissue. Past analyses of body condition have also showed a seasonal increase in condition for adult bluefin tuna in the Gulf of Maine (Crane 1936; Rivas 1955; Estrada et al. 2005; Goldstein et al. 2007). Bluefin tuna generally arrive in spring and early summer in poor condition (Crane 1936), then attain high lipid content as summer and fall progress (Estrada et al. 2005). In recent decades, this pattern has diminished, with fish arriving in leaner condition and putting on less fat stores as the season progresses (Golet et al. 2007). Diet analyses agree with past assessments, suggesting that adult bluefin tuna still rely mainly on sand lance and herring prey bases in the Gulf of Maine. Condition assessments also show a general increase in tissue lipid content across seasons, although in the absence of a comparable dataset of C:N values from previous decades, relative seasonal increases in lipid content cannot be determined. For school bluefin tuna, muscle lipid content was low while liver had elevated lipid levels similar to late season adults, suggesting that juvenile bluefin tuna might preferentially use liver as a lipid storage site over white muscle.

This assessment of bluefin tuna diet and condition in Gulf of Maine forage grounds demonstrates a consistency in diet and trophic position of adult bluefin tuna relative to previous studies performed over the past twenty years (Chase 2002; Estrada et al. 2005). Observed changes in condition over this time period (Golet et al. 2007) cannot be explained by major diet shifts. A thorough analysis of prey condition was not performed in this study, and bluefin tuna could potentially be consuming the same prey species but of a lower lipid content than previous decades. Comparisons of prey C:N

values showed herring to have the highest lipid content among sampled prey species, although these values could still potentially be lower than past values for herring. Observed patterns in bluefin tuna condition might instead be linked to differences in migratory routes and arrival times to Gulf of Maine forage grounds (Golet et al. 2007) or different reproductive schedules (Goldstein et al. 2007). Delayed arrival of higher proportions of fish to the Gulf of Maine in diminished condition could manifest itself as an overall decline in condition for the local assemblage. Further studies of prey condition in the Gulf of Maine and past movements and arrival times to the Gulf of Maine should improve understanding of observed dramatic declines in condition.

CHAPTER 3

USING STABLE ISOTOPES TO TRACK ATLANTIC BLUEFIN TUNA MOVEMENTS

Spatial gradients in stable isotope ratios occur across the marine environment, resulting in distinct isotope provinces (Hobson 1999; Rubenstein and Hobson 2004; Hobson 2007a; Hobson 2007b). Isotopic values vary among different marine habitat types (France 1995) and across latitudinal gradients (Rau et al. 1982; Rau et al. 1989). Nitrogen isotope values should increase towards higher latitudes in the North Atlantic (Montoya 2007) whereas carbon values should decrease (Rau et al. 1982; Rau et al. 1989; Sharp 2007).

Geographic and habitat-specific isotope gradients in aquatic systems have been used to track animal movements for a range of species (Hobson 1999). Movement between inshore and offshore regions and across latitudinal gradients have been measured for pinnipeds (Burton and Koch 1999), whales (Best and Schell 1996; Mendes et al. 2007), and sea turtles (Reich et al. 2007). Fish movements have been tracked between mangroves and coral reefs (Nakamura et al. 2008), within estuaries (McMahon et al. 2005; Suzuki et al. 2008; Haas et al. 2009), and between coastal and open ocean systems (Rodgers and Wing 2008).

Isotopic analysis of multiple tissues with different turnover rates can provide migratory information over different times scales. A multiple tissue approach is useful in that comparison of tissue types for an individual can provide information on arrival times

to local forage grounds. Fry et al. (2003) described an isotopic classification system for brown shrimp movement and residency based on multiple tissue analyses. If isotope values are similar among tissue types and reflect local values, an individual would be classified as a long term resident. If the faster turnover tissue reflects local prey values, but the slower turnover tissue does not, an individual would be classified as a recent migrant to the region. If neither tissue type reflects local prey values, an individual would be classified as a transient or very recent migrant to the region.

In fishes, liver turns over rapidly relative to white muscle (Suzuki et al. 2005; Logan et al. 2006; MacNeil et al. 2006); comparison of these tissue types can provide both short and long term records of movement and residency. Fairly slow turnover tissues, such as white muscle (Hesslein et al. 1993; MacAvoy et al. 2001), may retain isotopic values representative of past feeding and migration in addition to local conditions (MacNeill et al. 2005; Perga and Gerdeaux 2005), and may be used to infer fish migratory histories (Haas et al. 2009). In a validation of the Fry et al. (2003) classification system, Haas et al. (2009) manipulated movements of estuarine fish and demonstrated that the different migration classifications matched known movement patterns. In these field experiments, fish liver rapidly matched local baseline isotope values while muscle lagged behind, providing a record of past migrations (Haas et al. 2009).

Bluefin tuna occupy forage grounds throughout the north Atlantic including both coastal and offshore areas (Mather et al. 1995), where geographic variations in stable isotope values are known to occur (Hobson et al. 1994; Montoya et al. 2002). They should then provide a good test for identifying the merits of stable isotope analysis as a

chemical tag for tracing large scale movements, and determine the relative contributions of different forage bases and habitats (e.g. inshore vs. offshore) to bluefin tuna diet.

Electronic tagging projects applied to Atlantic bluefin tuna (Lutcavage et al. 1999; Block et al. 2001; Block et al. 2005; Wilson et al. 2005) as well as co-occurring sharks (Skomal et al. 2004), billfishes (Prince et al. 2005) and tunas (Arrizabalaga et al. 2008) in the north Atlantic have increased our understanding of the movements of these highly migratory species (Block et al. 1998a; Block et al. 1998b; Gunn and Block 2001). Electronic tags provide detailed records of geolocation, depth, and temperature and can record information for a single (pop-up satellite archival tag) or multiple years (implantable archival tags) (Gunn and Block 2001). While these tag technologies can answer many questions regarding the movements of large pelagic fishes, they also possess limitations including premature tag shedding, satellite transmission failure, and high costs that generally limit sample size (Gunn and Block 2001; Fromentin and Powers 2005).

Chemical tags like stable isotopes involve relatively inexpensive, routine analyses (Brand 1996; Kelly 2000), and can be measured from frozen tissue samples collected from fishery landings (e.g. Estrada et al. 2005; MacNeill et al. 2005) or non-lethal biopsies (e.g., Kelly et al. 2006; Church et al. 2009). The relative logistical simplicity of stable isotope analyses could make these chemical tracers a useful compliment to more detailed electronic tag records. Chemical tags are also beneficial in that 100 % of the population is labeled by this method, so results will not be biased by spatial differences in tag recapture (Lucas and Baras 2000) or deployment locations.

Tracking Migratory Routes of Atlantic Bluefin Tuna into Gulf of Mexico Spawning Grounds

Introduction

While electronic tagging studies have been performed on Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) for more than a decade, only a small percentage of fish tagged on forage grounds have traveled to Gulf of Mexico spawning grounds (Lutcavage et al. 1999; Block et al. 2001; Stokesbury et al. 2004; Block et al. 2005; Wilson et al. 2005; Stokesbury et al. 2007). Migration records continue to raise the possibility of alternative spawning grounds (e.g., Lutcavage et al. 1999; Galuardi et al. submitted). Understanding connectivity between forage and spawning grounds is important for management since fishing effort and quotas vary widely across Atlantic forage grounds (Fromentin and Powers 2005), and ICCAT management policies seek to limit take of spawning fish.

Bluefin tuna spawn in the Gulf of Mexico from April to June (Baglin 1982; Mather et al. 1995; Schaefer 2001) in water $\geq 24°C$ (Mather et al. 1995; Schaefer 2001; Teo et al. 2007b), with variable arrival, departure, and residence from December to July (Block et al. 2001; Stokesbury et al. 2004; Block et al. 2005; Teo et al. 2007a; Galuardi et al. submitted). Many fish arrive as early as December (Stokesbury et al. 2004; Galuardi et al. submitted) and February (Teo et al. 2007a), several months before the presumed spawning period. Many other large pelagic fishes feed in the Gulf of Mexico (Rooker et al. 2006; Cai et al. 2007), and bluefin tuna arriving during winter are likely using this area to feed prior to spawning. Later arrivals range from late March (Stokesbury et al. 2004; Galuardi et al. submitted) to June (Block et al. 2001; Teo et al. 2007a). Individual

residency varies from one month (Block et al. 2001; Teo et al. 2007a) to more than five months (Galuardi et al. submitted).

Chemical tracers can provide a novel alternative to track migration routes to Gulf of Mexico spawning grounds. Isotopic analysis of slower turnover tissues like muscle would provide a record of previous winter forage grounds while complementary analysis of rapid turnover tissue such as liver would provide information on arrival times and residency in Gulf of Mexico spawning grounds. I generated a habitat and region-based isotope map by analyzing tissue samples from bluefin tuna and other large pelagic fishes sampled throughout the Atlantic (Figure 18). Observed gradients between shelf and offshore regions (Figure 19) were then used to identify previous habitats of bluefin tuna sampled in Gulf of Mexico spawning grounds.

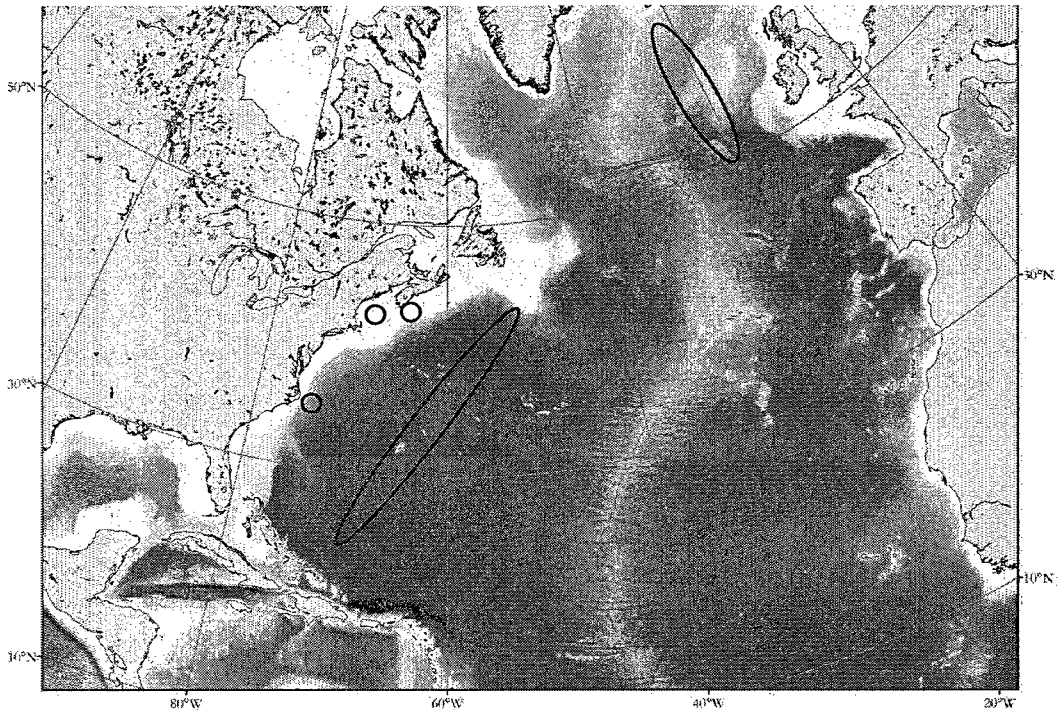

Figure 18. Map of sampling locations from western Atlantic shelf (Nova Scotia (NS), Gulf of Maine (GOM), and Mid-Atlantic Bight (MAB)) and offshore (western central Atlantic (WCA) and eastern central Atlantic (ECA)). WCA samples are from Atlantic swordfish (*Xiphias gladius*). All other samples are from adult Atlantic bluefin tuna (*Thunnus thynnus*).

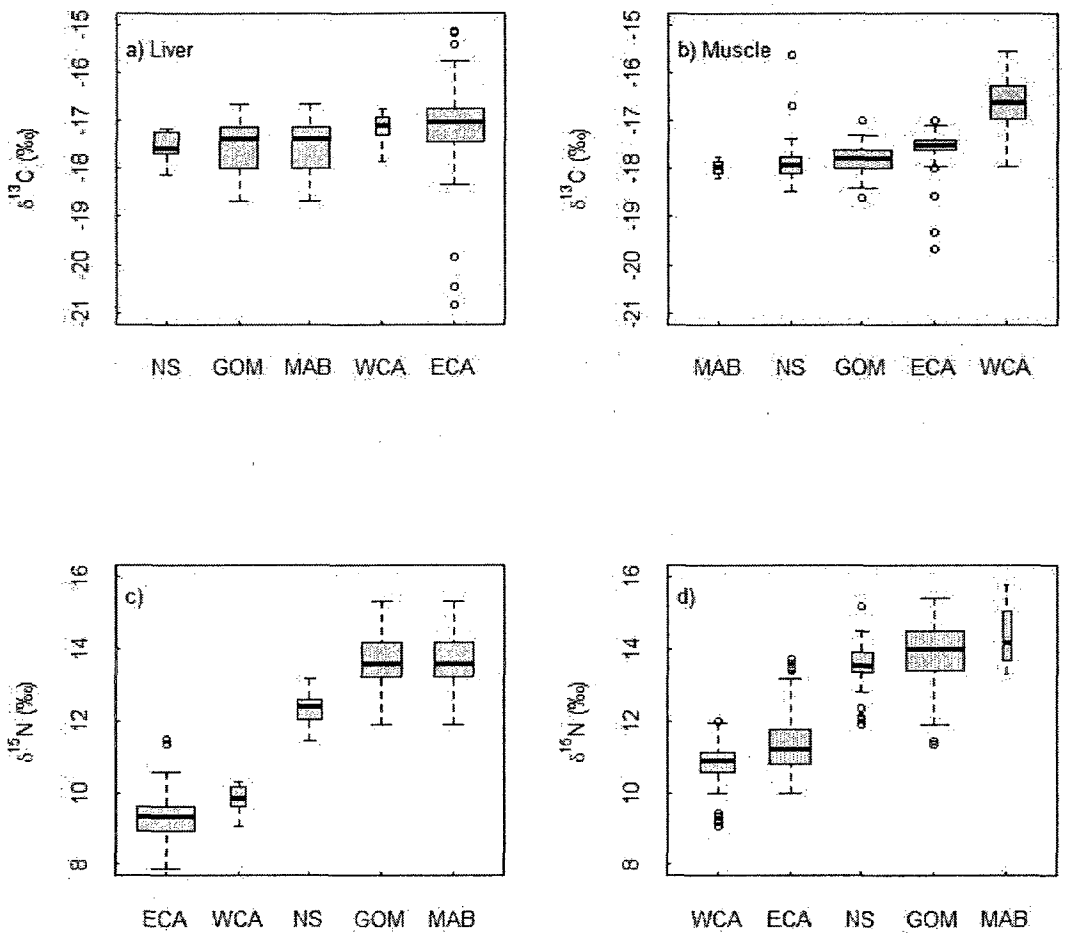

Figure 19. Box plots of $\delta^{13}C$ and $\delta^{15}N$ data from adult bluefin tuna (*Thunnus thynnus*) grouped by forage ground. Samples are from western Atlantic shelf (Nova Scotia (NS), Gulf of Maine (GOM), and Mid-Atlantic Bight (MAB)) and eastern central Atlantic (ECA). WCA samples are from Atlantic swordfish (*Xiphias gladius*). Data are presented for a) liver $\delta^{13}C$ b) muscle $\delta^{13}C$ c) liver $\delta^{15}N$ and d) muscle $\delta^{15}N$.

Following the residency classification system of Fry et al. (2003), isotope differences between liver and muscle for individual fish should indicate its equilibration state. In fishes, muscle $\delta^{15}N$ values in equilibrium with diet are generally higher than liver values (Pinnegar and Polunin 1999; Suzuki et al. 2005; Trueman et al. 2005;

Sweeting et al. 2007a), possibly due to differential amino acid composition between tissue types (Pinnegar and Polunin 1999; McClelland and Montoya 2002). Consequently, a fish in long-term equilibrium with local diet (both liver and muscle equilibrated) would be expected to have slightly higher muscle values relative to liver. In a bluefin tuna migrating from shelf (high $\delta^{15}N$) to offshore regions (low $\delta^{15}N$), isotope differences between tissue types should initially increase as the liver rapidly incorporates the lower pelagic $\delta^{15}N$ baseline (Figure 20a). For bluefin tuna traveling from offshore onto the shelf, isotope differences should remain fairly low, since liver initially has a lower $\delta^{15}N$ than muscle. As liver incorporates the higher shelf baseline, it eventually meets and surpasses $\delta^{15}N$ muscle values, until muscle achieves equilibrium, reflecting the shelf baseline (Figure 20b).

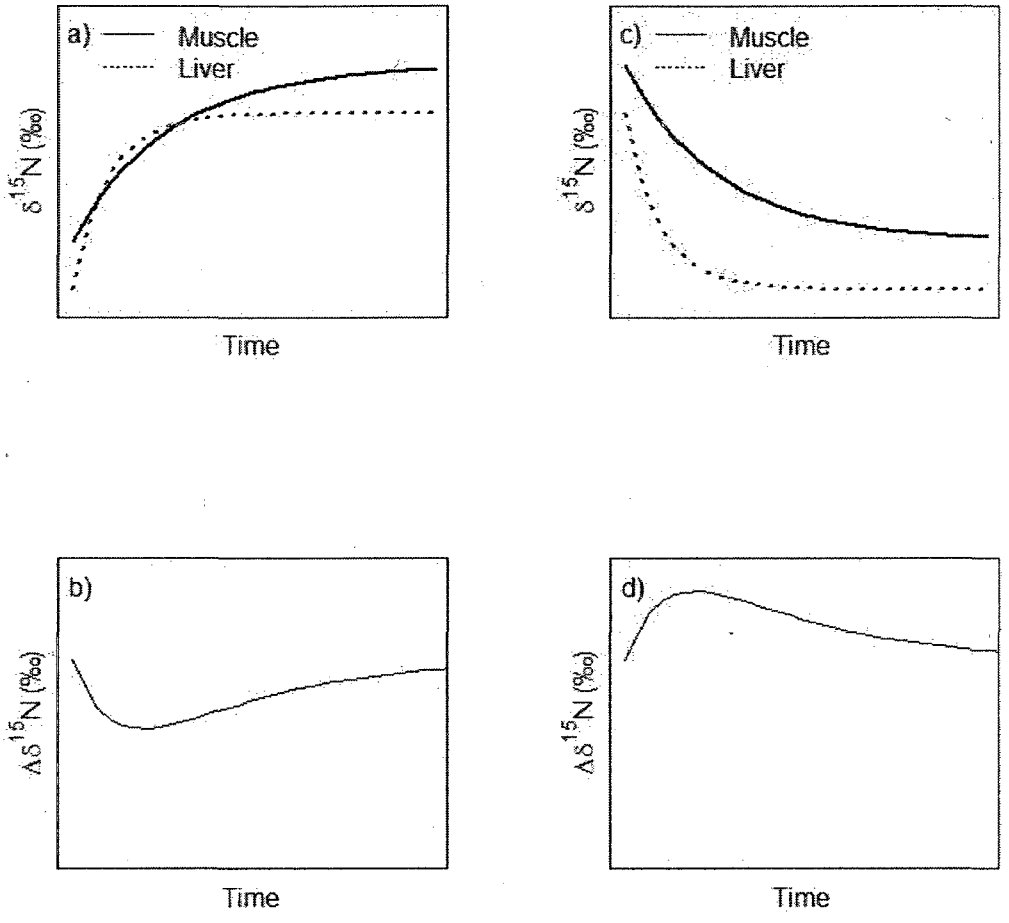

Figure 20. Theoretical changes in liver and muscle $\delta^{15}N$ values and tissue isotope discrimination for a fish shifting from equilibration with a, b) offshore to shelf and c,d) shelf to offshore ecosystems. Isotope differences during equilibration are reduced for fish shifting from offshore (low $\delta^{15}N$ baseline) (offshore) to the shelf (elevated baseline), and elevated for the opposite scenario.

To estimate previous movements of bluefin tuna on Gulf of Mexico spawning grounds, I examined liver and muscle $\delta^{13}C$ and $\delta^{15}N$ values from these fish and compared them with values from individuals sampled in different foraging areas in the north

Atlantic. Muscle, a slow turnover tissue, was used to estimate past movements while liver (rapid turnover) was used to estimate equilibration state and possible biases.

Methods

Samples of liver and muscle were collected by NOAA fisheries observers aboard commercial longline vessels in the Gulf of Mexico in 2007 and 2008 (Table 27; Figure 21). Samples were stored on ice at sea then stored frozen until analysis. Samples were then sub-sampled, lightly rinsed with deionized water, and dried in glass scintillation vials at 65°C for at least 48 hours. Dried samples were homogenized using a Mixer/Mill® (SPEX SamplePrep, LLC Metuchen, New Jersey U.S.A) with stainless steel vials. Aliquots of homogenized sample (0.6 – 1.2 mg) were packed into 4 X 6 mm tin cups and analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen by continuous flow using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies, Inc, Valencia, CA USA) coupled with a $DELTA_{plus}$ XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at the University of New Hampshire Stable Isotope Laboratory (UNH). All C:N values are reported as uncorrected percent weight calculations ($\frac{\%C}{\%N}$) as opposed to percent atomic weight ($\frac{\%C}{\%N} \times 1.16667$).

All sample $\delta^{13}C$ values were corrected for lipid content *a posteriori* using a mass balance equation (Fry 2002) with parameters specific to ABFT liver and white muscle (Logan et al. 2008). All carbon and nitrogen isotope data are reported in δ notation according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000$$

where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Standard materials are Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. Precision is ~ 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on the VPDB and AIR scales with IAEA CH6 (-10.4 ‰), CH7 (-31.8 ‰), N1 (0.4 ‰) and N2 (20.3 ‰).

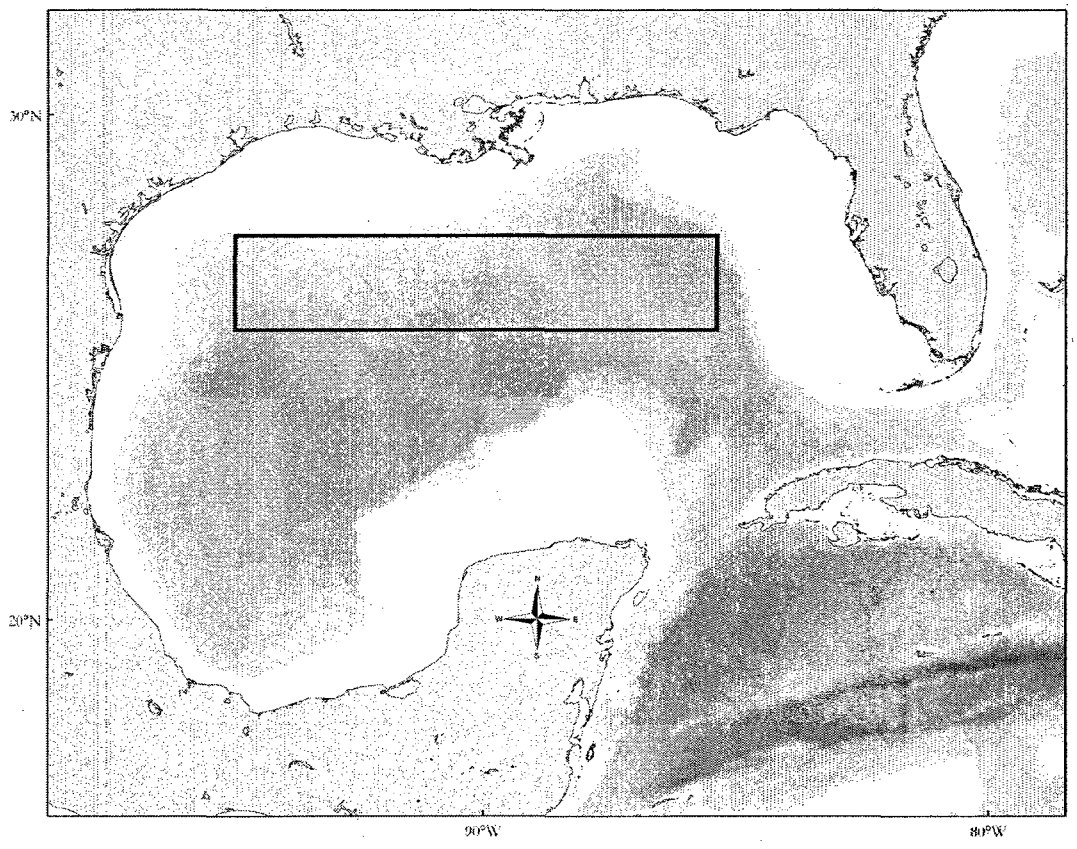

Figure 21. Map of locations from the Gulf of Mexico for Atlantic bluefin tuna (*Thunnus thynnus*) liver and muscle samples.

Table 27. Sampling summary for Atlantic bluefin tuna (*Thunnus thynnus*) from the Gulf of Mexico.

Year	Month	Liver				Muscle			
		Male	Female	Unknown	All	Male	Female	Unknown	All
2007	March	4	2	0	6	0	0	0	0
	April	5	7	0	12	6	2	0	8
	May	0	0	0	0	5	7	0	12
	June	0	0	0	0	1	0	0	1
	All	9	9	0	18	12	9	0	21
2008	March	4	5	3	12	4	5	2	11
	April	6	11	1	18	7	13	1	21
	May	4	16	0	20	11	22	0	33
	June	0	0	0	0	0	0	0	0
	All	14	32	4	50	22	40	3	65
Unknown	Unknown	0	0	24	24	1	1	26	28
Combined	All	23	41	28	92	35	50	29	114

Statistical Analyses

Comparisons were made among pooled shelf, offshore, and Gulf of Mexico liver and muscle samples for $\delta^{13}C$ and $\delta^{15}N$ (Table 28). Homogeneity of variance among groups was first assessed using Levene's test. Isotope comparisons among groups were then made using analysis of variance (ANOVA) followed by pairwise t-tests with $\alpha = 0.05$ following a Holm test adjustment for multiple comparisons if variance assumptions were not violated. If significant differences were detected among variances, a one-way analysis of means without assumptions of equal variances followed by pairwise comparisons using t-tests with non-pooled standard deviations was performed. Using the same statistical procedures, isotope and C:N values were compared for each tissue type among sampling months (March, April, and May) for the Gulf of Mexico.

Table 28. Mean ± SD carbon and nitrogen data from Atlantic bluefin tuna (*Thunnus thynnus*) and Atlantic swordfish (*Xiphias gladius*) pooled by sampling region. *

	n	$\delta^{13}C$	$\delta^{15}N$
Liver **			
Shelf	117	-17.6 ± 0.5^{a}	12.9 ± 1.1^{a}
Offshore	113	-17.1 ± 0.8^{b}	9.3 ± 0.7^{b}
Gulf of Mexico	92	-18.1 ± 0.4^{c}	11.3 ± 1.1^{c}
Muscle			
Shelf	252	-17.8 ± 0.3^{a}	13.9 ± 0.8^{a}
Offshore	194	-17.1 ± 0.6^{b}	11.1 ± 0.8^{b}
Gulf of Mexico	114	-17.8 ± 0.4^{a}	13.3 ± 0.9^{c}

* Values in the same column for a given tissue type with different superscript letters are significantly different ($P < 0.05$).
** Data are grouped by NW Atlantic shelf, open ocean, and Gulf of Mexico.

To further explore patterns in carbon and nitrogen data within the Gulf of Mexico, quantile regressions were performed for C:N, $\delta^{13}C$, and $\delta^{15}N$ based on sampling date.

Simple linear regressions were performed for tissue $\delta^{15}N$ relative to C:N to explore possible effects of condition on tissue $\delta^{15}N$.

Using the MASS package in R (R Development Core Team 2008), linear discriminant analysis (LDA) was first applied to the shelf and offshore isotope datasets to estimate classification accuracy using these two groups. Resubstitution error was used to estimate accuracy, where individual samples are removed from the dataset and the discriminant function used to classify the excluded sample is estimated from the remaining samples. LDA was then applied to liver and muscle tissue from the Gulf of Mexico (the mixed stock) to classify individual fish according to past residency (shelf or offshore).

Further comparisons were performed for individual fish that had liver and muscle data. Isotope differences were calculated between tissue types ($\delta^{15}N_{muscle}$ - $\delta^{15}N_{liver}$). Samples were then grouped according to tissue origin classification for liver and muscle (shelf – shelf, offshore – offshore, and offshore – shelf), and isotope differences, $\delta^{13}C$, and $\delta^{15}N$ values were compared among groups using the ANOVA methods described for other group comparisons.

Results

For liver and muscle, ABFT sampled in the NW Atlantic shelf had significantly lower $\delta^{13}C$ and higher $\delta^{15}N$ values than individuals sampled offshore (Figure 19; Table 28). These significant differences support the use of stable isotopes to distinguish onshore-offshore movements. Liver samples from the Gulf of Mexico had significantly lower $\delta^{13}C$ values than either region, while muscle $\delta^{13}C$ values were significantly lower than offshore values but not different from shelf samples. Liver and muscle $\delta^{15}N$ values

for Gulf of Mexico samples were intermediate between shelf and offshore values, and significantly different from each region (Table 28).

Based on quantile regression, significant relationships were detected with ordinal date for C:N, $\delta^{13}C$, and $\delta^{15}N$ (Figure 22). For liver, maximum C:N and median $\delta^{13}C$ decreased while maximum $\delta^{13}C$ increased with sampling date. For muscle, median C:N decreased while median and minimum $\delta^{15}N$ values increased with sampling date (Figure 22). Based on monthly groupings, mean C:N values decreased across months but differences were not significant, while May muscle $\delta^{15}N$ isotope values were significantly higher than in March and April (Table 29). No other significant differences were detected. Using linear regression, $\delta^{15}N$ was not correlated with C:N for liver or muscle (Figure 23).

Table 29. Mean ± SD monthly Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) carbon and nitrogen data from the Gulf of Mexico. *

	n	CN	$\delta^{13}C$	$\delta^{15}N$
Liver				
March	12	7.9 ± 3.1^{a} (0.26)**	-18.1 ± 0.4^{a} (0.03)	11.0 ± 1.4^{a} (0.11)
April	24	7.0 ± 2.4^{a} (0.10)	-18.1 ± 0.5^{a} (0.02)	11.0 ± 1.3^{a} (0.05)
May	32	6.7 ± 1.7^{a} (0.05)	-18.0 ± 0.5^{a} (0.01)	11.3 ± 0.8^{a} (0.03)
Muscle				
March	11	4.6 ± 1.2^{a} (0.11)	-17.8 ± 0.3^{a} (0.03)	12.8 ± 1.2^{a} (0.11)
April	29	4.1 ± 0.9^{a} (0.03)	-17.7 ± 0.4^{a} (0.01)	12.9 ± 1.1^{a} (0.04)
May	46	3.9 ± 1.1^{a} (0.02)	-17.8 ± 0.4^{a} (0.01)	13.4 ± 0.7^{b} (0.01)

* Values in the same column for a given tissue type with different superscript letters are significantly different ($P < 0.05$).

** Values in parentheses are coefficient of variation (CV, %).

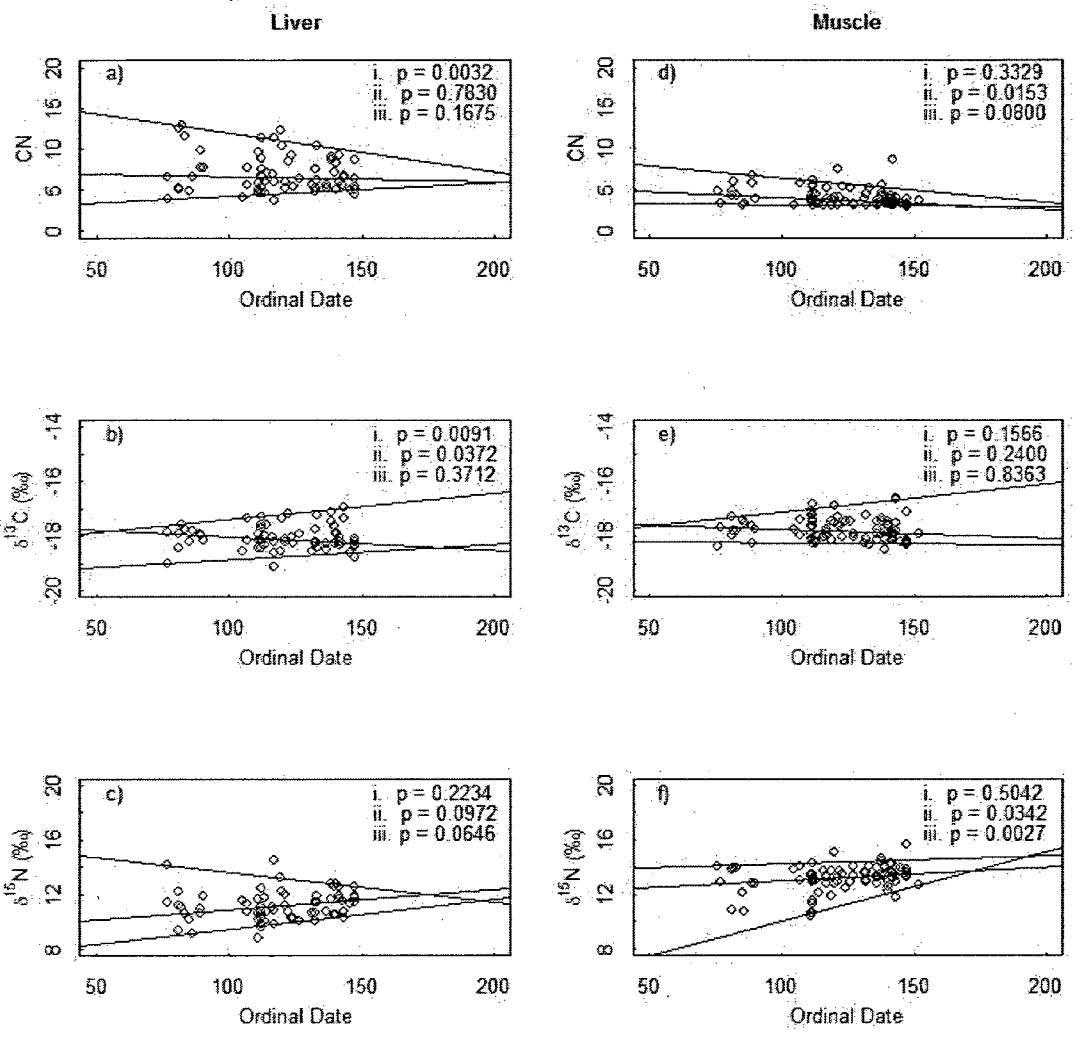

Figure 22. Quantile regressions of a) liver C:N b) liver $\delta^{13}C$ c) liver $\delta^{15}N$ d) muscle C:N e) muscle $\delta^{13}C$, and f) muscle $\delta^{15}N$ values from Atlantic bluefin tuna (*Thunnus thynnus*) sampled in the Gulf of Mexico relative to sampling date. Regressions were performed for the i. 95th, ii. 50th, and iii. 5th percentiles.

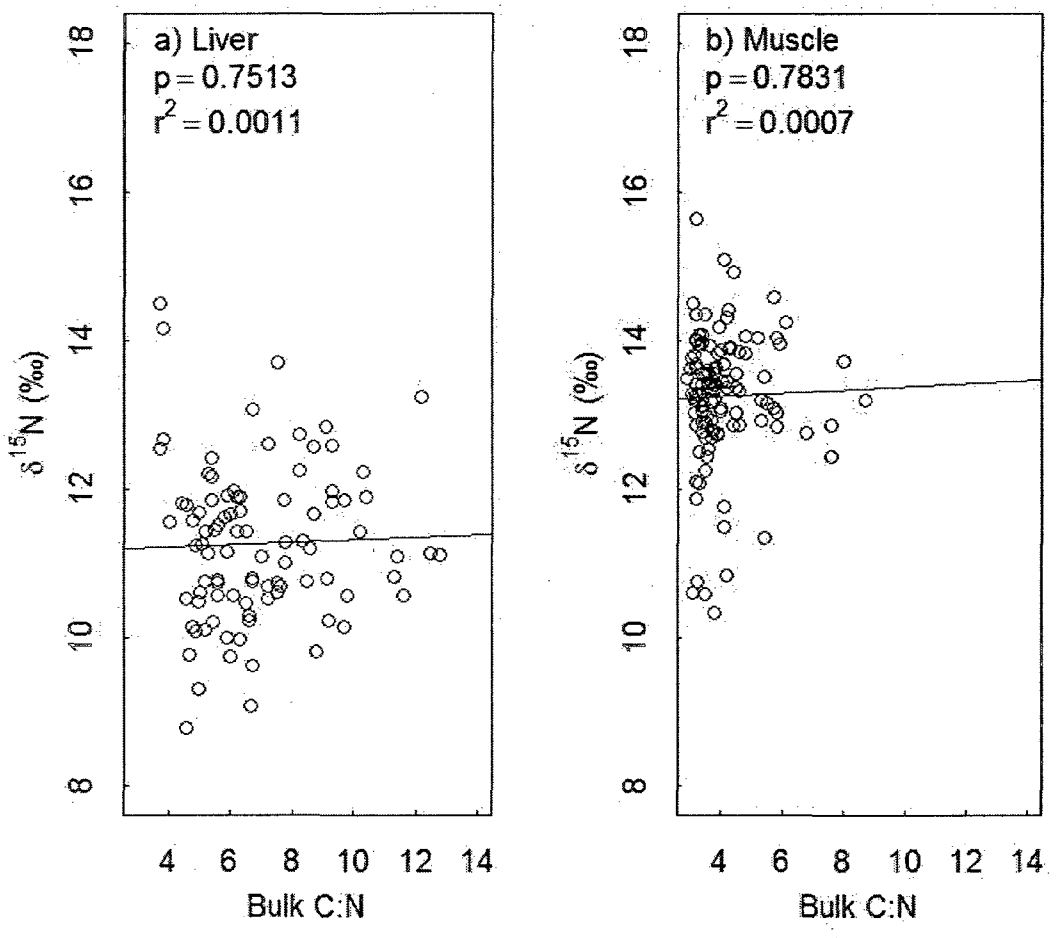

Figure 23. Linear regressions of a) liver and b) muscle $\delta^{15}N$ values relative to tissue C:N for Atlantic bluefin tuna (*Thunnus thynnus*) sampled in the Gulf of Mexico.

Classification success was 99.1 % for liver and 94.6 % for muscle (Table 30), mainly due to discrimination based on $\delta^{15}N$, since similar classification percentages of 98.7 % (liver) and 93.7 % (muscle) were generated using only $\delta^{15}N$ data (Table 30). For Gulf of Mexico samples, liver and muscle values showed that fish more likely occupied coastal rather than offshore forage grounds (Table 31). Shelf assignment was higher for muscle (87.7 %) than liver (60.9 %), and increased across sampling months (Table 31).

Classification of individual fish showed three groupings for liver and muscle: shelf – shelf, offshore – offshore, and offshore – shelf (Table 32). No fish had liver classified as offshore and muscle as shelf. Fish classified as offshore (liver) – shelf (muscle) migrants had significantly higher muscle – liver $\delta^{15}N$ discrimination than fish with common classifications across tissues (i.e., offshore – offshore and shelf – shelf) (Table 32). Muscle $\delta^{15}N$ values significantly differed between groups with liver classified as offshore and shelf migrants (Table 32).

Table 30. Classification success among shelf and offshore region isotope values of Atlantic bluefin tuna (*Thunnus thynnus*) based on linear discriminant analysis (LDA).

Region	Tissue	Isotope	% Classification
All	Liver	$\delta^{13}C$	69.1
		$\delta^{15}N$	98.7
		$\delta^{13}C$ and $\delta^{15}N$	99.1
Offshore	Liver	$\delta^{13}C$	69.4
		$\delta^{15}N$	99.2
		$\delta^{13}C$ and $\delta^{15}N$	100.0
Shelf	Liver	$\delta^{13}C$	68.9
		$\delta^{15}N$	98.2
		$\delta^{13}C$ and $\delta^{15}N$	98.2
All	Muscle	$\delta^{13}C$	83.9
		$\delta^{15}N$	93.7
		$\delta^{13}C$ and $\delta^{15}N$	94.6
Offshore	Muscle	$\delta^{13}C$	91.8
		$\delta^{15}N$	93.2
		$\delta^{13}C$ and $\delta^{15}N$	95.2
Shelf	Muscle	$\delta^{13}C$	80.0
		$\delta^{15}N$	94.1
		$\delta^{13}C$ and $\delta^{15}N$	94.2

Table 31. Classification of Gulf of Mexico Atlantic bluefin tuna (*Thunnus thynnus*) as migrants from shelf or offshore regions.

Date*	N	Tissue	Shelf	Offshore
All	92	Liver	60.9	39.1
April 2007	6		60.7	39.1
May 2007	12		66.7	33.3
March 2008	12		58.3	41.7
April 2008	18		38.9	61.1
May 2008	20		60.0	40.0
Males	23		78.3	21.7
Females	67		56.7	43.3
All	114	Muscle	87.7	12.3
April 2007	8		100.0	0.0
May 2007	13		100.0	0.0
March 2008	11		81.8	18.2
April 2008	21		85.7	14.3
May 2008	33		100.0	0.0
Males	47		100.0	0.0
Females	50		90.0	10.0

* Sample size for "All" does not equal sum of months, because some samples did not have date information.

Table 32. Tissue isotope discrimination and C:N grouped by migratory classification for individual Atlantic bluefin tuna (*Thunnus thynnus*) from the Gulf of Mexico with both liver and muscle data. *

Migratory Classification					Liver		Muscle				
Liver	Muscle	n	% Classification	$\delta^{13}C$ Discrimination**	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{15}N$ Discrimination	Liver CN	Muscle CN
Shelf	Shelf	53	60.9	$0.3 \pm 0.33^{a,c}$	-18.1 ± 0.4^{a}	11.8 ± 0.8^{a}	-17.9 ± 0.4^{a}	13.7 ± 0.6^{a}	1.8 ± 0.9^{a}	7.1 ± 2.3^{a}	4.3 ± 1.4^{a}
Offshore	Offshore	12	13.8	0.5 ± 0.22^{b}	-17.8 ± 0.4^{b}	10.0 ± 0.6^{b}	-17.2 ± 0.3^{b}	11.6 ± 0.9^{b}	1.7 ± 0.4^{a}	6.0 ± 1.3^{a}	3.9 ± 0.7^{a}
Offshore	Shelf	22	25.3	0.1 ± 0.38^{c}	$-18.0 \pm 0.4^{a,b}$	10.4 ± 0.4^{b}	-17.9 ± 0.3^{a}	13.2 ± 0.3^{c}	2.8 ± 0.5^{b}	7.1 ± 2.0^{a}	4.2 ± 0.8^{a}
Shelf	Offshore	0	0	NA	NA	NA	NA	NA	NA	NA	NA

* Values in a given column with different letter superscripts are significantly different ($P < 0.05$).

** Isotope and CN data are presented as mean ± SD.

Discussion

Significant isotope baseline shifts were detected in Atlantic bluefin tuna sampled on shelf and offshore forage grounds, indicating that stable isotopes can provide a novel tracer of their movements between these regions. More pronounced baseline shifts in $\delta^{15}N$ between shelf and offshore regions match gradients observed for zooplankton (Graham et al. 2009), with lower values in offshore food webs likely driven by nitrogen fixation (Montoya et al. 2002; Montoya 2007) or condensed food chains associated with less productive systems (Jenkins et al. 1992). The majority (87.7 %) of Atlantic bluefin sampled in the Gulf of Mexico had isotope values suggesting recent occupation of coastal shelf forage grounds.

These results are consistent with conventional and PSAT tagging results showing movements of some adult ABFT from the New England, Canadian, and Carolina shelves (Mather et al. 1995; Block et al. 2001; Stokesbury et al. 2004; Block et al. 2005; Teo et al. 2007a; Walli et al. 2009; Galuardi et al. submitted) to the Gulf of Mexico, although others traveled there via offshore routes (Galuardi et al. submitted). Analysis of catch records in the 1950's suggested a connectivity between seasonal forage grounds in the NW Atlantic and Gulf of Mexico spawning grounds (Rivas 1955), and conventional tagging studies in the 1970's later provided direct evidence (Mather et al. 1995). While results reflect recent and historical findings from catch records and tracking studies, isotope validation is important, because isotope results are not biased by sampling location. All electronic tagging efforts focused on NW Atlantic shelf fishing grounds and can only provide information on linkages between that region and the Gulf of Mexico. Isotopes provide a means of detecting past movements from previously unsampled

regions (e.g., central Atlantic) and offer a more complete representation of bluefin tuna habitats throughout the north Atlantic.

Stable isotope values in soft tissues change with growth, turnover, and nutritional status (Hobson et al. 1993; Gannes et al. 1998), and these characteristics could bias movement estimates. The Atlantic bluefin tuna's range extends from the Mediterranean Sea and eastern Atlantic to the south equatorial Atlantic (Mather et al. 1995). Movements from regions not included in the analysis could also affect classification estimates, although past electronic tagging results have not shown movements from these regions (i.e., eastern Atlantic and Mediterranean Sea).

Starvation during spawning has been proposed based on observations of bluefin tuna in lean condition in the Gulf of Mexico (Rivas 1954; Rivas 1955) and can cause increases in tissue $\delta^{15}N$ (Hobson et al. 1993; Doucett et al. 1999; Cherel et al. 2005; Boag et al. 2006) as ^{14}N is preferentially excreted during the catabolic breakdown of body tissues (Hobson et al. 1993; Gannes et al. 1997). In fishes, increases in $\delta^{15}N$ as a result of starvation have been observed for metabolically active tissues (i.e., liver) but not in white muscle (Doucett et al. 1999; Guelinckx et al. 2007). These results suggest that bluefin tuna muscle $\delta^{15}N$ should not be affected by possible starvation during migrations or spawning. For salmonids, observed increases in $\delta^{15}N$ were related to decreases in tissue C:N (Doucett et al. 1999), as lipid stores were diminished. Correlations were not detected between $\delta^{15}N$ and bulk C:N for bluefin tuna liver or muscle, indicating that isotope differences were being driven mainly by baseline shifts rather than nutritional status.

Unlike isotope values of otoliths, used to establish natal origin of ABFT (Rooker et al. 2008a; Rooker et al. 2008b), isotope values in liver and muscle tissues change in relation to growth and metabolic turnover (Gannes et al. 1998). Isotope dynamics in soft tissues pose challenges to their use as migratory tracers. Tissue isotopes will eventually shift towards values more indicative of local diet. The extent of such biases can be assessed through comparison and calibration with fisheries or electronic tagging information indicating bluefin arrival times and residency in the Gulf of Mexico, as well as expected isotope baselines for this region.

Electronic tagging results show fish entering the Gulf of Mexico from December to June (Teo et al. 2007a; Galuardi et al. submitted). Mean residency in the Gulf of Mexico was reported as slightly greater than one month (Teo et al. 2007a), although some exceeded five months (Galuardi et al. submitted). Given the variability in arrival and residence times as well as small sample size of tagged bluefin tuna in the Gulf of Mexico (n = 12), bias of local isotope values is difficult to assess. For a maximum estimated residence time (e.g., fish sampled in late May following a five month residence), tissue isotope values would likely be heavily influenced by local values.

Because prey samples from the Gulf of Mexico were not available, their expected baseline isotope values could not be determined, but SIA analysis of other large pelagic fishes (Rooker et al. 2006; Cai et al. 2007) had values similar to those sampled in the western central Atlantic (Logan et al. 2007b). If bluefin tuna diet in the Gulf of Mexico is similar to these large pelagic fishes, bluefin tuna residency there would be expected to be biased towards an offshore classification. The only fish that had different migratory classifications between tissue types had shelf muscle values and offshore liver values.

These fish also had increased $\delta^{15}N$ differences between tissues, indicative of isotopic disequilibrium, and their liver values are likely biased by local baseline values. Muscle $\delta^{15}N$ values for mixed-classification fish were significantly lower than fish identified as shelf migrants, indicating a potential bias from local feeding. Since muscle values were still classified as shelf migrants, muscle data for these fish would not show any classification bias.

Muscle, which has a lower metabolic turnover rate than liver in fishes (Suzuki et al. 2005; Logan et al. 2006; MacNeil et al. 2006; Guelinckx et al. 2007), is affected more by growth, with new tissue reflecting the isotope values of local diet. Bluefin tuna growth tends to be elevated during summer and fall and lower in winter (Cort 1991). This heterogeneous growth pattern likely biases bulk muscle isotope values towards periods of increased growth, and thus provides a marker of seasonal forage grounds for fish sampled in winter and spring.

Classification results could also be biased by failure to incorporate values from samples taken across the appropriate geographic range in this analysis. Shelf classification is based on a bluefin tuna dataset from NW Atlantic forage grounds and offshore classification is based on bluefin tuna and swordfish sampled from the western and eastern central Atlantic (Figure 18). Adult Atlantic swordfish in the western central Atlantic feed mainly on ommastrephid squids (Logan et al. 2007a), and occupy a similar trophic position to bluefin tuna in this region based on historical records (Matthews et al. 1977). As such, they should provide a reliable estimate of bluefin tuna isotope values for the western central Atlantic. If fish traveled to the Gulf of Mexico from the eastern

Atlantic or Mediterranean Sea (regions not included in this study), either dispersal route would be falsely classified as western shelf or offshore.

Results from other studies (Estrada et al. 2005; Sara and Sara 2007) provide support for combining eastern and western shelf regions as a common isotope group and offshore and Mediterranean Sea regions as a second group. Isotope results for bluefin tuna sampled in the western Atlantic and the Mediterranean Sea show a similar $\delta^{15}N$ baseline shift to the observed gradient between western shelf and pelagic regions (Estrada et al. 2005; Sara and Sara 2007). Prey isotope studies of the eastern Atlantic shelf and Mediterranean Sea regions (Bode et al. 2004; Bode et al. 2007; Sara and Sara 2007; Navarro et al. 2009) also show higher values for the shelf relative to the Mediterranean Sea. This baseline shift appears to propagate to higher trophic levels; albacore tuna (*Thunnus alalunga*) in the Bay of Biscay have higher $\delta^{15}N$ values than in the Mediterranean Sea (Goñi et al. In Prep).

Muscle isotope data from bluefin tuna in Gulf of Mexico spawning grounds link them to known coastal feeding grounds such as the New England and Carolina shelves, and are consistent with fisheries and other ecological information. Bluefin tuna utilize productive shelf waters to generate the lipid stores required for migration and spawning (Rivas 1955; Mather et al. 1995; Goldstein et al. 2007; Golet et al. 2007). Their prey in western Atlantic coastal areas consist mainly of densely schooling, planktivorous fishes with high lipid content (Chase 2002; Butler 2007) that provide diet resources to fuel migratory and reproductive activities. Analysis of organochlorine tracers in bluefin tuna from the Gulf of Mexico also indicated previous foraging in western Atlantic shelf waters (Dickhut et al. 2009). Similarly, adult bluefin tuna sampled in Mediterranean Sea

spawning areas had elevated muscle $\delta^{15}N$ values indicative of past movements from shelf forage grounds (Sara and Sara 2007). Analyses of $\delta^{18}O$ values in otolith cores showed a strong connectivity between NW Atlantic shelf forage grounds and the Gulf of Mexico, with most (94.8 %) fish sampled from the Gulf of Maine and all fish sampled from the Gulf of St. Lawrence showing Gulf of Mexico origin. Connection to the Gulf of Mexico was lower and variable across size classes for fish from Mid-Atlantic Bight forage grounds (Rooker et al. 2008b). Since $\delta^{15}N$ values do not appear to be distinct between eastern and western shelf regions, their resolution is insufficient to provide evidence for dispersal patterns in relation to the 45° W ICCAT management boundary, but can demonstrate a link between spawning areas and coastal forage grounds.

A smaller percentage of fish sampled in the Gulf of Mexico (12.3 %) were classified as offshore migrants (Table 31), providing evidence for multiple migratory routes into the spawning area, consistent with PSAT tagging results (Galuardi et al. submitted). Possible bias of local Gulf of Mexico isotope values on these offshore classifications cannot be ruled out, although these fish would need to have resided in the Gulf of Mexico for several months to affect muscle values. If fish traveled from the central Atlantic, they were most likely feeding on ommastrephid squids (Matthews et al. 1977; Logan et al. 2007a). Since electronic tagging efforts are mainly focused on shelf fishing areas, movement of fish with longer periods in pelagic regions would be underrepresented by tagging results. Combined stable isotope and PSAT tagging results (Block et al. 2001; Stokesbury et al. 2004; Block et al. 2005; Teo et al. 2007a; Walli et al. 2009; Galuardi et al. submitted) for Gulf of Mexico bluefin tuna indicate complex migratory patterns.

Current results would likely benefit from the inclusion of additional chemical tracers. Analysis of additional isotope tracers (i.e., $\delta^{18}O$) could distinguish between western and Mediterranean Sea values (Rooker et al. 2008a; Rooker et al. 2008b), since regional differences in $\delta^{18}O$ are also detectable in fish muscle tissue (Church et al. 2008). Additional chemical tracers, like fatty acids (e.g., Baduini et al. 2006) or organochlorines (e.g., Dickhut et al. 2009), should also provide added resolution for identifying dispersal patterns and foraging grounds of Atlantic bluefin tuna.

How long do Atlantic bluefin tuna spend in the Gulf of Maine?

Introduction

Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) occur in the Gulf of Maine from May to October (Lutcavage and Kraus 1995; Mather et al. 1995; Wilson et al. 2005), feeding on Atlantic herring, sand lance, mackerel, and bluefish (Chase 2002). Somatic condition of adult bluefin tuna in this seasonal foraging region has significantly declined in recent decades, possibly as a result of shifts in diet composition or quality (Golet et al. 2007). Alternatively, trends in condition could reflect differences in arrival times and migratory routes to the Gulf of Maine, as fishermen have long believed that changes in quality and appearance of fish landed throughout the commercial fishing season (June-November) are due to different arrival times and migration histories. Comparisons between individual ABFT liver and muscle isotope values provide additional information on estimates of residency time in the Gulf of Maine. These estimates are compared with measurements of muscle condition using C:N ratios as proxies for lipid content (Estrada et al. 2005). This approach offers the advantage of examining condition and migratory

data from a single sample, and may help to explain the basis of inter and intra-seasonal changes in somatic condition.

Methods

Samples of liver and muscle were collected from local fishery landings from 2004 to 2008 (Table 33). Bluefin tuna tissue samples were obtained from the Yankee Fisherman's Cooperative in Seabrook, New Hampshire U.S.A. where they were held in cold storage for < five days, then stored frozen until preparation for analysis. Samples were sub-sampled, lightly rinsed with deionized water, and dried in glass scintillation vials at 65°C for at least 48 hours. Dried samples were homogenized using a Mixer/Mill® (SPEX SamplePrep, LLC Metuchen, New Jersey U.S.A) with stainless steel vials. Aliquots of homogenized sample (0.6 – 1.2 mg) were packed into 4 X 6 mm tin cups and analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen by continuous flow using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies, Inc, Valencia, CA USA) coupled with a $DELTA_{plus}$ XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory of Northern Arizona University (NAU) and the University of New Hampshire Stable Isotope Laboratory (UNH). All C:N values are reported as uncorrected percent weight calculations ($\frac{\%C}{\%N}$) as opposed to percent atomic weight ($\frac{\%C}{\%N} \times 1.16667$).

All sample $\delta^{13}C$ values were corrected for lipid content *a posteriori* using a mass balance equation (Fry 2002) with parameters specific to ABFT liver and white muscle (Logan et al. 2008). All carbon and nitrogen isotope data are reported in δ notation according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000$$

where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Standard materials are Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. Standard deviations of replicate samples analyzed at both labs were 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$ (n = 45) and within-lab precision is ~ 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on the VPDB and AIR scales with IAEA CH6 (-10.4 ‰), CH7 (-31.8 ‰), N1 (0.4 ‰) and N2 (20.3 ‰).

Table 33. Sampling summary of liver and muscle samples from Atlantic bluefin tuna (*Thunnus thynnus*) from the Gulf of Maine.

	June		July		August		September		October	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
2004	2	6	11	29	1	7	11	87	1	51
2005	1	6	4	16	31	15	7	20	1	10
2006	7	7	1	10	4	18	3	14	0	0
2007	0	10	3	6	6	21	9	26	5	9
2008	8	18	0	0	0	0	0	0	0	0
Total	18	47	19	61	42	61	30	147	7	70

Statistical Analyses

Seasonal trends in bluefin tuna liver and muscle C:N, $\delta^{13}C$, and $\delta^{15}N$ were assessed through quantile regression. Annual comparisons were made for the median (50^{th} percentile) as well as 5^{th} and 95^{th} percentiles to test for patterns in minimum and maximum condition and isotope value in relation to date. Significance was tested using a rank sums test. All quantile regression analyses were performed in the statistics package quantreg in R (R Development Core Team 2008).

To test for possible explanations of the presence of fish in late season in poor somatic condition, adult ABFT liver and muscle samples were grouped by sampling date (Early Season: mid-June – July, Late Season: September-October). August ("Mid-Season") samples were not included in this analysis, as differences were only compared between groups presumed to be recent arrivals and long-term residents. Samples for each seasonal grouping were pooled across sampling years to provide adequate sample sizes. Samples in each season ("Early" and "Late") were grouped based on muscle lipid content (low: C:N $\leq$ 3.5 (lipid content: 2.5 %) and high: C:N $\geq$ 5.0 (lipid content: 8.5 %)).

Three groups were created based on season and condition: "Early Low Lipid (ELL)", "Late Low Lipid (LLL)", and "Late High Lipid (LHL)." No "Early" season fish had high lipids. Muscle lipid content was used as a grouping factor, because white muscle is a major lipid storage site for adult ABFT (Mourente et al. 2001), and recent studies have shown significant changes in these lipid stores in Gulf of Maine bluefin tuna (Golet et al. 2007). For bluefin tuna samples with low early and high late season muscle C:N values, liver and muscle isotope values were compared for both ELL and LHL groups. For muscle, these two groups were also compared with the LLL group. Due to

an inadequate number of liver samples (n = 2), similar comparisons for the LLL group were not performed for liver. Isotope differences (muscle isotope – liver isotope) for individual fish were also compared for early season low C:N and late season high C:N groups. Remaining groups were not included due to inadequate sample sizes.

Homogeneity of variance among groups was assessed using Levene's test. Comparisons of tissue C:N, $\delta^{13}C$, and $\delta^{15}N$ were made using analysis of variance (ANOVA) followed by pairwise t-tests with $\alpha = 0.05$ following a Holm test adjustment for multiple comparisons. When assumptions of homogeneity of variance were violated, pairwise comparisons using t-tests with non-pooled standard deviations were performed.

Results

Muscle isotope values were similar for "Early" and "Late" season groups (Table 34). Both groups had a $\delta^{13}C$ range of ~ 1.5 ‰ (early season: -18.5 to -16.8 ‰, late season: -18.6 to -17.0 ‰) and $\delta^{15}N$ range of ~ 4 ‰ (early season: 11.2 to 15.2 ‰, late season: 11.4 to 15.4 ‰). Coefficient of variation and standard deviation values were also similar between groups for each isotope (Table 34).

Table 34. Mean ± SD adult Atlantic bluefin tuna (*Thunnus thynnus*) carbon and nitrogen data from the Gulf of Maine grouped by muscle lipid content. *

	n	CN	$\delta^{13}C$	$\delta^{15}N$
Liver				
Early low lipid (ELL) C:N	27	$7.8 \pm 2.1^{a**}$ (0.27)***	-17.9 ± 0.4^{a} (0.02)	11.9 ± 0.6^{a} (0.05)
Late high lipid (LHL) C:N	14	13.3 ± 6.0^{b} (0.45)	-17.6 ± 0.4^{a} (0.02)	12.6 ± 0.7^{b} (0.06)
Muscle				
Early low lipid (ELL) C:N	98	3.2 ± 0.1^{a} (0.04)	-17.8 ± 0.3^{a} (0.02)	13.4 ± 0.9^{a} (0.06)
Late low lipid (LLL) C:N	39	3.3 ± 0.2^{a} (0.05)	-17.7 ± 0.3^{a} (0.01)	13.4 ± 0.8^{a} (0.06)
Late high lipid (LHL) C:N	55	5.8 ± 1.0^{b} (0.17)	-17.9 ± 0.3^{b} (0.01)	14.2 ± 0.7^{b} (0.05)

* Data are grouped by season as early (mid-June – July) and late (September-October) season and by muscle C:N content as low (≤ 3.5) and high (≥ 5.0).
** Values in the same column for a given tissue type with different superscript letters are significantly different ($P<0.05$).
*** Values in parentheses are coefficient of variation (CV, %).

Regression results revealed inter-annual variability in trends (Figures 24 to 29). While median muscle C:N values were positively correlated with sampling date, minimum C:N values were not for either tissue, indicating the presence of low lipid fish across all seasons (Figure 25). Significant relationships were not observed for most $\delta^{13}C$ datasets for either tissue type (Figures 26 and 27). Relationships between tissue $\delta^{15}N$ and sampling date varied across years, with minimum and median values positively correlated with date in 2004. Median muscle $\delta^{15}N$ values also increased across sampling dates in 2004 as well as 2006 (Figure 29).

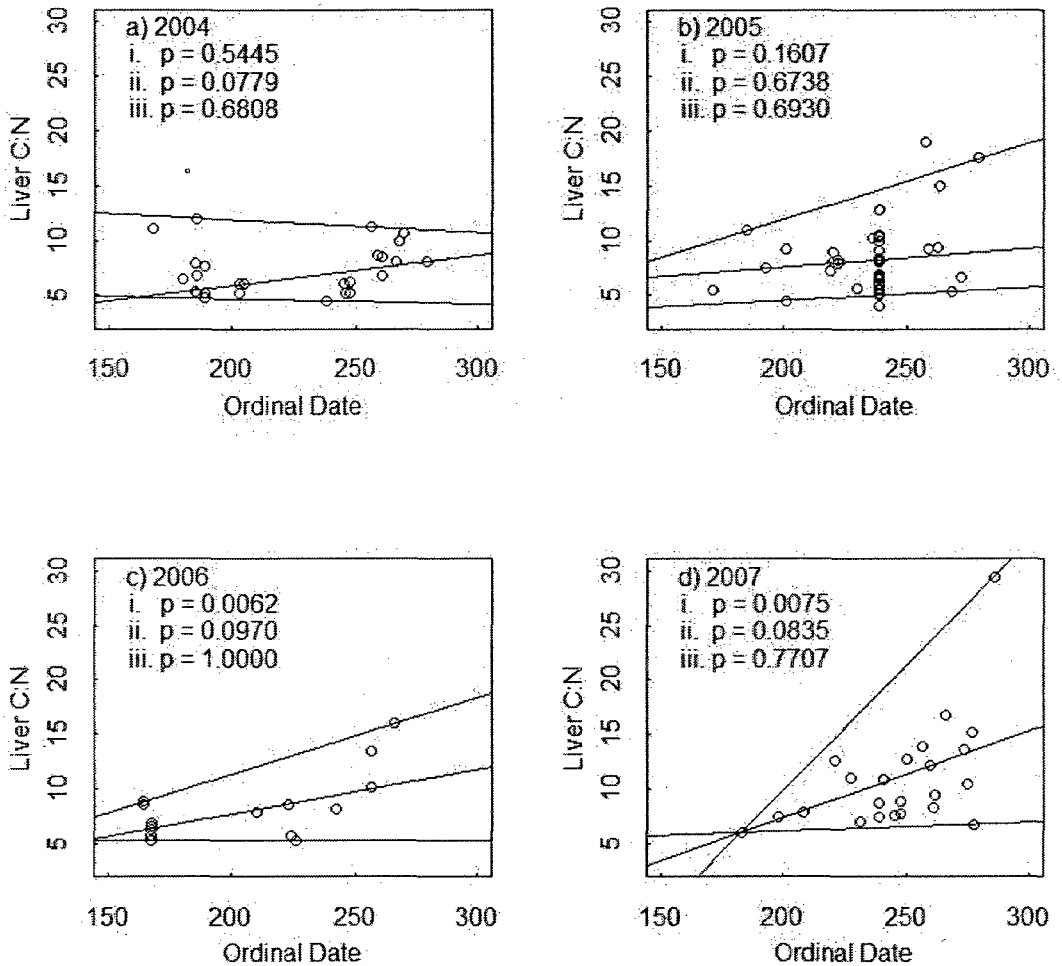

Figure 24. Quantile regressions for Atlantic bluefin tuna (*Thunnus thynnus*) liver C:N values for a) 2004 b) 2005 c) 2006 and d) 2007. P-values refer to regressions for the i. 95th percentile, ii. 50th percentile, and iii. 5th percentile.

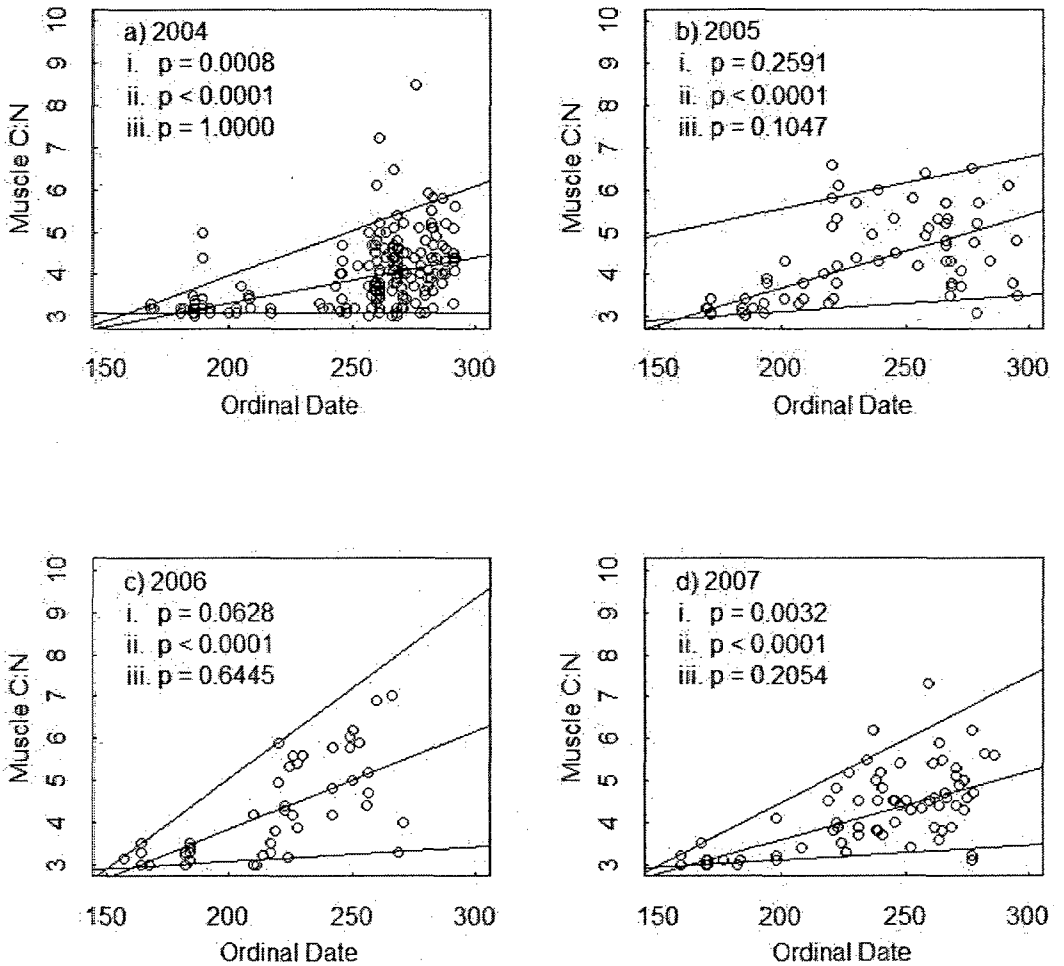

Figure 25. Quantile regressions for Atlantic bluefin tuna (*Thunnus thynnus*) muscle C:N values for a) 2004 b) 2005 c) 2006 and d) 2007. P-values refer to regressions for the i. 95th percentile, ii. 50th percentile, and iii. 5th percentile.

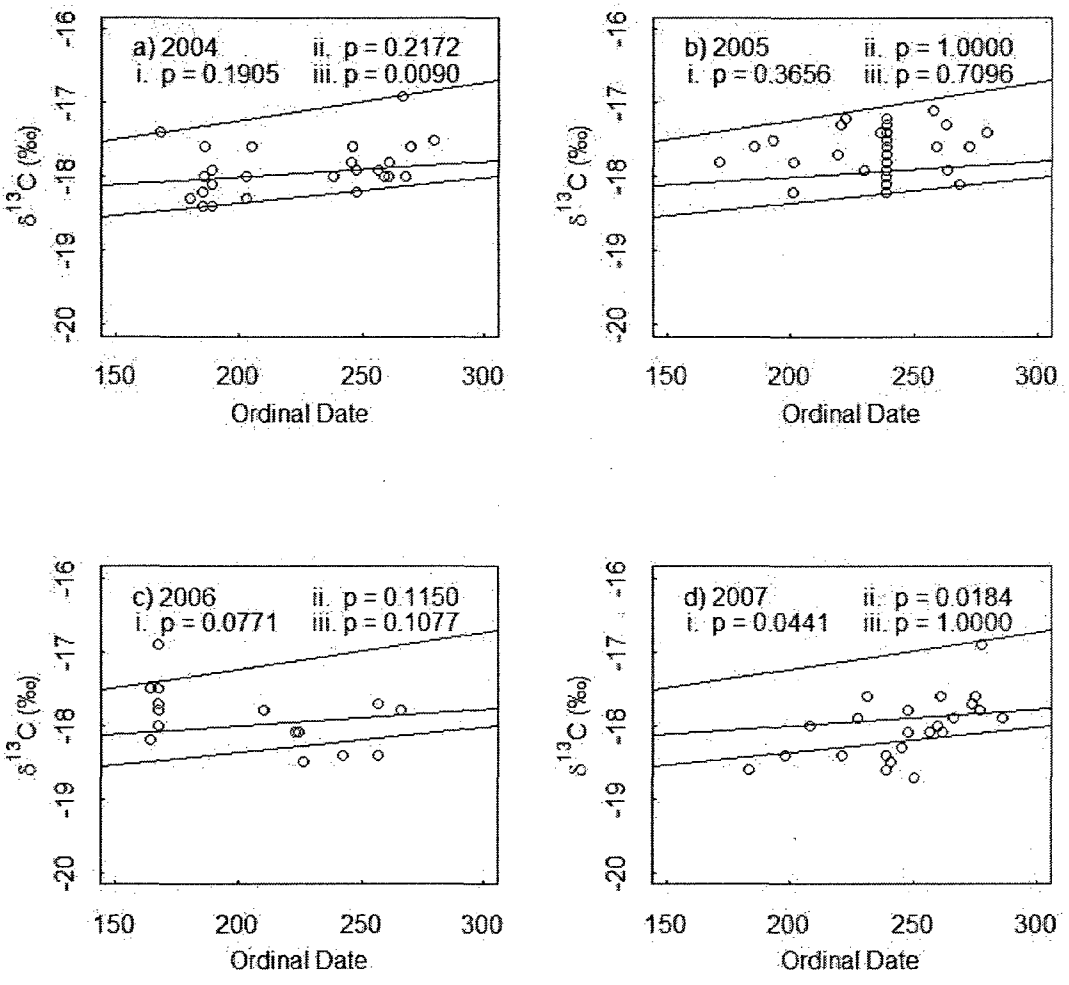

Figure 26. Quantile regressions for Atlantic bluefin tuna (*Thunnus thynnus*) liver $\delta^{13}C$ values for a) 2004 b) 2005 c) 2006 and d) 2007. P-values refer to regressions for the i. 95th percentile, ii. 50th percentile, and iii. 5th percentile.

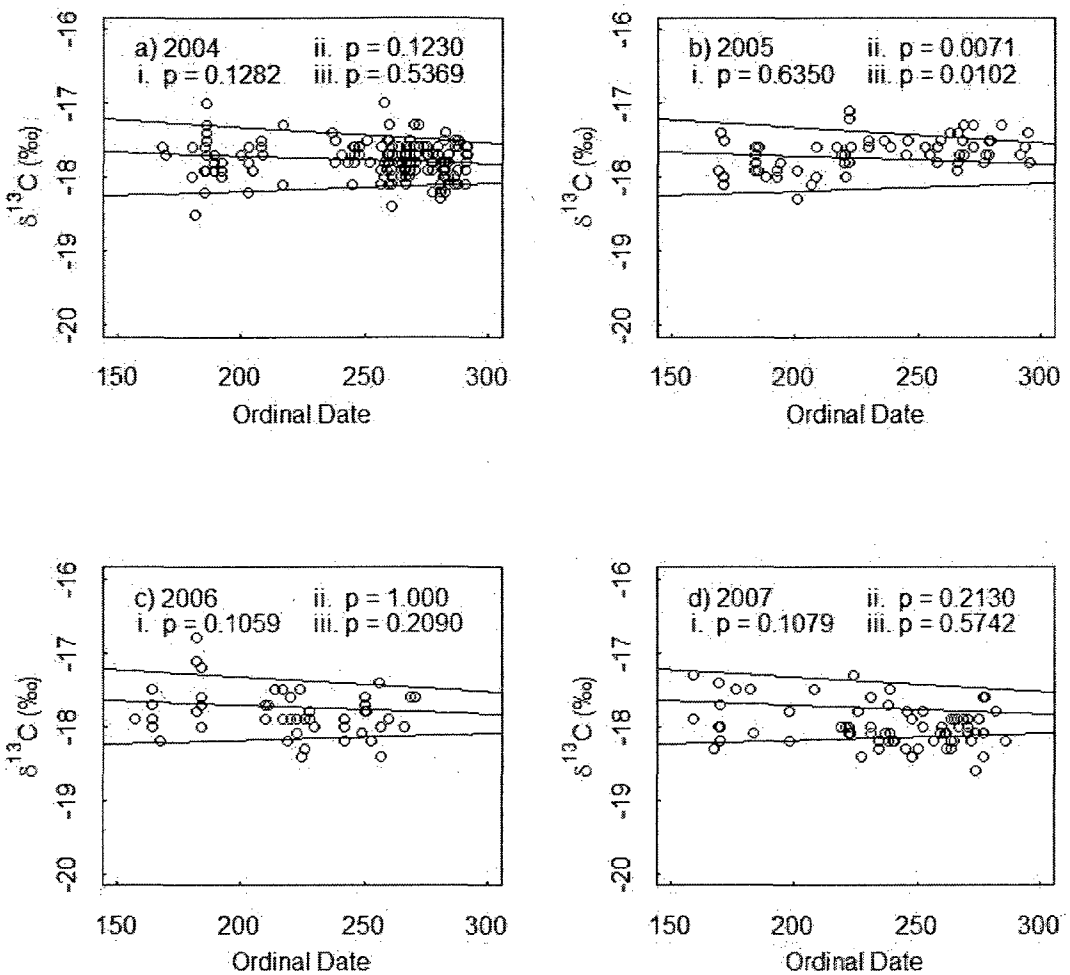

Figure 27. Quantile regressions for Atlantic bluefin tuna (*Thunnus thynnus*) muscle $\delta^{13}C$ values for a) 2004 b) 2005 c) 2006 and d) 2007. P-values refer to regressions for the i. 95th percentile, ii. 50th percentile, and iii. 5th percentile.

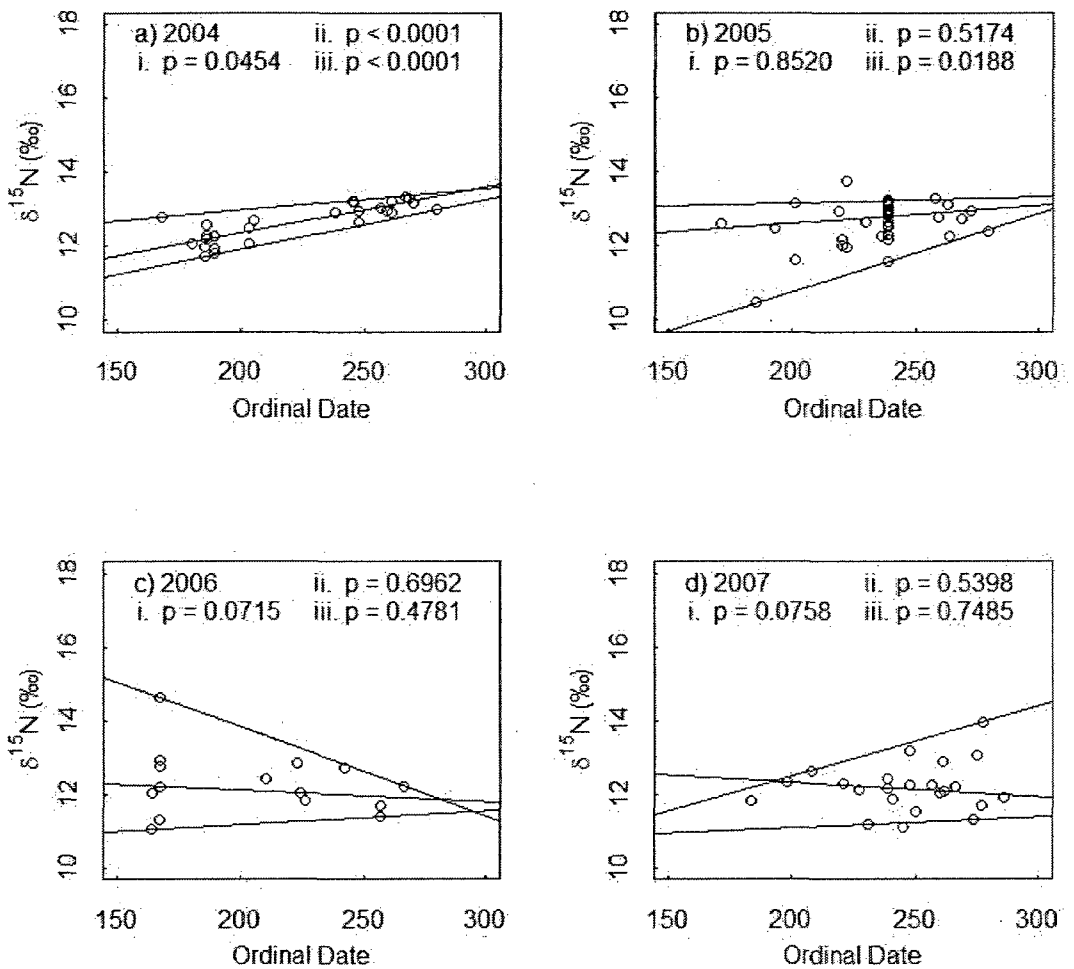

Figure 28. Quantile regressions for Atlantic bluefin tuna (*Thunnus thynnus*) liver $\delta^{15}N$ values for a) 2004 b) 2005 c) 2006 and d) 2007. P-values refer to regressions for the i. 95th percentile, ii. 50th percentile, and iii. 5th percentile.

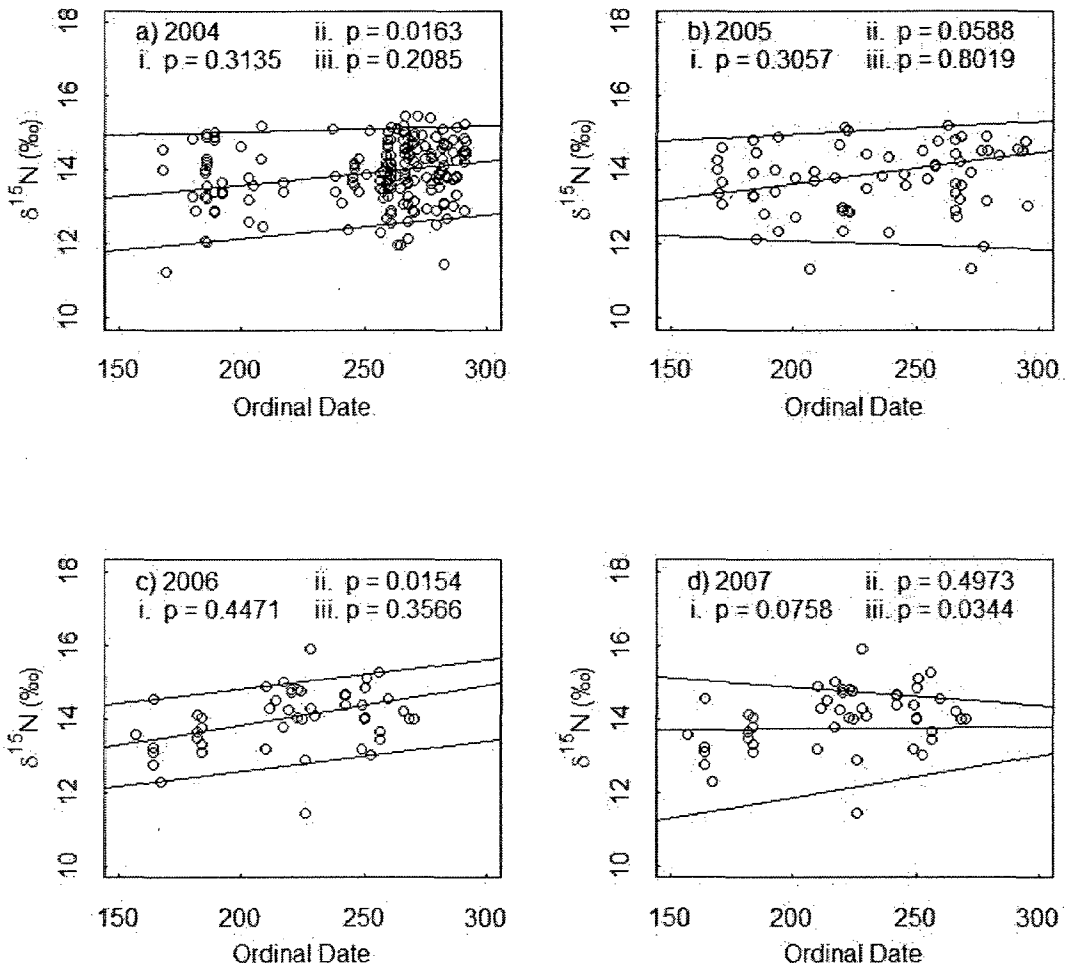

Figure 29. Quantile regressions for Atlantic bluefin tuna (*Thunnus thynnus*) muscle $\delta^{15}N$ values for a) 2004 b) 2005 c) 2006 and d) 2007. P-values refer to regressions for the i. 95th percentile, ii. 50th percentile, and iii. 5th percentile.

Isotope values varied among season and condition groups for liver and muscle (Table 34). Liver $\delta^{15}N$ values from ELL fish were lower than LHL fish, with no differences in $\delta^{13}C$. Liver samples were only available from two LLL fish, so statistical analyses were not performed for this group, but these fish had similar $\delta^{15}N$ values (12.8 ± 0.2 ‰) relative to LHL fish (12.6 ± 0.7 ‰) and similar $\delta^{13}C$ values (-18.0 ± 0.2 ‰) to

both groups. Late season high C:N muscle samples from LHL fish had significantly lower $\delta^{13}C$ and higher $\delta^{15}N$ than ELL and LLL groups, with no differences detected for either isotope between low C:N groups. Isotope differences between liver and muscle were significantly different for $\delta^{13}C$ but not $\delta^{15}N$ between ELL and LHL groups.

Discussion

Bluefin tuna had variable early and late season isotope values, indicative of different diets or residency times in the Gulf of Maine. Since the range of observed isotope values was similar for both seasons, residency cannot be assessed at a fine scale using multiple tissues as a migratory clock (Fry et al. 2003; Fry 2006). If early season fish had similar stable isotope values characteristic of past movements, comparisons of fast (i.e., liver) and slow (i.e., muscle) isotope values to these two end members could provide individual residency estimates (Fry et al. 2003). Since the range of isotope values measured for early season fish contained values characteristic of local Gulf of Maine food webs, isotopes alone cannot distinguish between long term local residency and recent immigration from regions with similar isotope values. However, general patterns can still be assessed since longer term residents in the Gulf of Maine would likely have less variable isotope values than recent migrants from different geographic regions. Variability in muscle isotope values for early and late season fish indicate that they had similar movement and feeding patterns.

While C:N values increased across seasons, individual ABFT with low lipid liver and muscle tissues were observed during all months. The presence of poor condition fish early in the season is not surprising, since they may have migrated long distances or recently spawned (Rivas 1955; Mather et al. 1995; Goldstein et al. 2007). In a historic

study, the weight of large bluefin tuna harvested in mid-summer varied by more than 45 kg (Crane 1936). More recently, variability in muscle condition and $\delta^{15}N$ values has been observed for fish sampled from early to mid-summer (Estrada et al. 2005). The presence of fish with limited lipid stores later in the season in September and October is less expected, as these fish are presumed to have spent the past several months foraging in productive local regions. Early and late season fish with poor somatic condition share common isotope values that differ from late season fish with elevated lipid stores, due to either differences in diet or local residency.

While diet segregation cannot be ruled out, stomach content analyses do not demonstrate individual variability in diet that would produce such isotope differences (Chase 2002). Dietary specialization for a generalist predator like the ABFT (Dragovich 1969; Dragovich 1970) would not be expected over the timescale (months) incorporated in muscle isotope values. Diet can vary spatially within the Gulf of Maine (Chase 2002), but ABFT traverse regional forage grounds on a daily basis (Lutcavage et al. 2000) and would not be expected to feed exclusively at a single local forage ground over the time scale of months. Also, while ABFT with elevated lipid stores had higher $\delta^{15}N$ values, their $\delta^{13}C$ values did not demonstrate a similar stepwise increase, but were instead slightly lower than poor condition fish. While increases in muscle $\delta^{15}N$ across trophic levels are greater than changes in $\delta^{13}C$ (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Minagawa and Wada 1984), isotope changes caused by a local shift to higher trophic level prey should still cause increases in both isotopes in a consumer's tissue (Figure 18). A decoupling of tissue isotope values, in which $\delta^{15}N$ increases but $\delta^{13}C$ decreases, would be more consistent with spatial baseline shifts linked to previous

movements (Hobson 1999). This lack of agreement between isotopes suggests that isotopic differences between groups are not caused by trophic separation within the Gulf of Maine.

Observed isotope differences could instead be explained by variable arrival times to the Gulf of Maine, with the poor condition ABFT as recent migrants and individuals with elevated lipid stores being longer term residents. Given that ABFT are known to migrate throughout the north Atlantic across regions with differing isotope baselines (Graham et al. 2009), observed scatter in early season bluefin tuna is not surprising. This variability is likely due to fish arriving to the Gulf of Maine from regions with different isotope baselines (Estrada et al. 2005). Similar scatter in late season fish is less expected as it suggests that the assemblage of ABFT occupying the Gulf of Maine in September and October includes a similar proportion of recent migrants found in June and July.

Some amount of individual isotope variability is to be expected, as factors like feeding rate can affect the magnitude of tissue isotope discrimination (Barnes et al. 2007), and individual fish of the same species consuming the same diet still have a range of $\delta^{15}N$ values of ~ 1.4 ‰ (Barnes et al. 2008). The larger range of values observed for ABFT cannot be explained simply by inherent variability, but instead is likely due to variability in equilibration with local prey values. The ~ 4 ‰ range of $\delta^{15}N$ values is similar to the baseline shift observed across ocean basins between regions dominated by N_2 fixation and denitrification (Wallace et al. 2006), and matches observed spatial shifts in tuna isotope values between coastal and open ocean forage ground (Table 28).

Electronic tagging studies of ABFT have shown a diversity of movement patterns, with individual differences in migratory routes and arrival times to forage grounds (Block

et al. 2001; Wilson et al. 2005; Galuardi et al. submitted). A metapopulation structure has been proposed as a possible description of ABFT population dynamics, with the north Atlantic population characterized as a collection of sub-populations with unique migratory patterns (Fromentin and Powers 2005). The presence of groups of ABFT in the Gulf of Maine in late season with distinct somatic conditions and isotope values provides evidence for multiple migration schedules for fish occupying this northern forage ground. The presence of poor condition ABFT in late season is likely due to late arrival to Gulf of Maine forage grounds. The causes and implications of these different migration strategies cannot be determined from isotope data alone, but are discussed in the following paragraphs as testable hypotheses for future studies.

Past studies have shown highly variable reproductive states among individuals (Goldstein et al. 2007) and declines in somatic condition (Golet et al. 2007) in recent decades in the Gulf of Maine. These studies have suggested skipped spawning, both temporal and spatial asynchrony in spawning, and migrations from distant regions (e.g., Mediterranean Sea) as possible explanations of observed patterns in condition and reproductive status (Goldstein et al. 2007; Golet et al. 2007). Tunas in open ocean forage grounds in the eastern central Atlantic and western central Atlantic along the edge of the Gulf Stream and Sargasso Sea, as well as the Mediterranean Sea, have lower baseline $\delta^{15}N$ values than coastal forage grounds like the Gulf of Maine (Figure 19; Sara and Sara 2007). Recent migration from any of these regions to the Gulf of Maine would likely produce the lower $\delta^{15}N$ values observed in muscle samples from poor condition fish. The corresponding slight increase in $\delta^{13}C$ for poor condition fish also agrees with patterns of $\delta^{13}C$ elevation for tunas and billfishes in offshore regions of the central north Atlantic

near the Gulf Stream edge and Sargasso Sea (Figure 19). ABFT occupy offshore regions of the central north Atlantic near the edge of the Gulf Stream (Lutcavage et al. 1999), which could possibly represent an additional spawning ground (Mather et al. 1995). The Mediterranean Sea is the main spawning ground for ABFT in the eastern Atlantic (Mather et al. 1995), and late season poor condition fish could be crossing the Atlantic from this region.

ABFT harvested in the Gulf of Maine in poor condition as late as October would likely leave this region without the necessary lipid stores to fuel spawning and long distance migrations (Schaefer 2001). These ABFT may skip spawning during the following year (Lutcavage et al. 1999; Wilson et al. 2005; Goldstein et al. 2007). Many ABFT that occupy Gulf of Maine forage grounds in the fall travel to winter forage grounds in the Mid-Atlantic Bight (Block et al. 2001; Wilson et al. 2005) where they feed on aggregations of menhaden (*Brevoortia tyrannus*) and portunid crabs (Butler 2007). Fish leaving the Gulf of Maine in poor condition could undergo the relatively short migration to shelf forage grounds in the Mid-Atlantic Bight and restore lipid reserves by exploiting this prey base. ABFT occupy this winter forage ground from mid-October through May, with peak activity from December to March (Walli et al. 2009). This residency period is of a similar duration to Gulf of Maine residency (Mather et al. 1995; Wilson et al. 2005; Walli et al. 2009), and some ABFT may rely more heavily on the Mid-Atlantic Bight as a feeding area with its dense aggregations of lipid-rich prey (Deegan 2006; Butler 2007).

Combined analysis of condition and isotope data provides quantitative support for historical observations of individual variability in ABFT condition in the Gulf of Maine

(Crane 1936). Commercial fishermen have documented variable condition in bluefin tuna landings (Golet et al. 2007), but causes of this observed variability had not previously been tested. Catch records, conventional and electronic tagging studies, and aerial surveys (Lutcavage and Kraus 1995; Mather et al. 1995; Wilson et al. 2005) have documented the residency of ABFT in the Gulf of Maine, and analysis of landings data has shown a declining trend in the condition of this Gulf of Maine assemblage (Golet et al. 2007). This study contributes to previous information by beginning to test for causality in observed condition trends. Using information reflecting both somatic condition (C:N) and arrival times (isotopes), results from this study support the theory that variable lipid content is related to differences in duration of residency in the Gulf of Maine.

Isotope and C:N data can provide information on an individual's condition at the time of sampling and some estimate of its past migrations, but to better understand relationships between ABFT movements and condition, electronic tagging should be combined with C:N and isotope analyses (Cunjak et al. 2005). Electronic tags and isotopes could provide information on ABFT movements pre- and post-arrival to Gulf of Maine forage grounds while C:N values could provide an index of somatic condition. Muscle tissue for both C:N and isotope analysis could easily be obtained via a non-lethal biopsy in the dorsal musculature during tagging. By gathering all of these data from individual fish, linkages among body condition and arrival times to the Gulf of Maine and subsequent movements could be better defined.

CONCLUSIONS

Tunas are "energy speculators" (Brill 1996) that gamble by expending energy on maintaining a high metabolism and migrating long distances with the expectation of eventual discovery and efficient exploitation of dense prey assemblages. Atlantic bluefin tuna (*Thunnus thynnus*) represent an extreme among large pelagic fishes in that they migrate throughout the north Atlantic (Mather et al. 1995) and warm their brain and eyes (Linthicum and Carey 1972), stomach (Carey et al. 1984), and swimming muscles (Carey and Teal 1966) to maximize feeding efficiency in cold, north Atlantic forage grounds. Predictable concentrations of energy-rich prey, such as Atlantic herring and sand lance in the Gulf of Maine and menhaden in the Mid-Atlantic Bight, provide vital fuel for migrations across more oliotrophic regions. Bluefin tuna migrate to regions of high productivity (Walli et al. 2009) to feed on these temporally and spatially ephemeral concentrations of prey.

Atlantic bluefin tuna are commonly described as top predators in the literature, but this classification is inaccurate given that they feed primarily on small schooling fishes and cephalopods. While stomach contents data reveal that many of these predators do consume higher trophic level prey (e.g., bluefish and spiny dogfish), their primary predatory influence is on mid-trophic level species. For both juvenile and adult bluefin tuna on shelf forage grounds, diet consists mainly of zooplanktivorous fishes with juveniles also consuming high proportions of crustaceans.

Across regions, stable isotope results reflect a lower trophic position (TP) than stomach content data. This consistent discrepancy could be due to biases associated with stomach content data, including differential prey digestion rates and inadequate sampling due to rapid gut evacuation rates. If isotope values are not in equilibrium with local prey sources, observed differences in trophic position could instead be biased by dietary information from past forage grounds. In the NW Atlantic, stomach content results show herring and menhaden (Butler 2007) as the major prey species in the Gulf of Maine and Mid-Atlantic Bight, respectively, while isotope results indicate large dietary contributions from sand lance (Gulf of Maine) and swimming crabs (Mid-Atlantic Bight). Stomach content data for juvenile bluefin tuna in the Mid-Atlantic Bight and Bay of Biscay showed a prevalence of schooling fishes while isotopes also reflect contributions from crustacean prey. Both isotopes and stomach content data demonstrate a primary reliance on mid-trophic level prey. Isotope results suggest that any supplements to this mid-trophic level diet are provided by lower rather than higher TP prey.

Across forage grounds, bluefin tuna consistently target small prey that tend to form dense aggregations (e.g., herring, euphausiids, cephalopods). Tuna prey are generally small relative to their body sizes compared with many piscivorous predators (Scharf et al. 2000; Ménard et al. 2006). This disparity is evident in nitrogen isotope patterns among co-occuring species. In relative isotope comparisons among bluefin tuna and their higher TP prey, a surprising similarity emerges with select prey species feeding at a slightly higher average TP than their predators. Bluefish (Gulf of Maine) and cephalopods (Bay of Biscay) are among the largest prey species observed in bluefin tuna stomach contents and have higher $\delta^{15}N$ values than their predator. These smaller species

feed on large prey items by breaking them down into smaller pieces prior to ingestion (Nixon 1987; Scharf et al. 1997), thus increasing foraging efficiency relative to piscivores that must swallow prey whole (Scharf et al. 1997; Scharf et al. 2009). For tunas, a diet consisting of larger numbers of small prey found in dense aggregations may be more energetically favorable than selecting large individual prey items. Smaller prey are locally more abundant and require less handling time (Scharf et al. 1998). These prey aggregations may allow for relatively efficient ram feeding by tunas. These combined features would make a diet based on lower trophic level prey more energetically favorable for tunas.

Because tunas exploit seasonal aggregations of mid-trophic level prey, their evolutionary strategies have necessarily developed in such a way that allows tunas to exploit this resource most efficiently. Diet analyses reflect a stability to these prey aggregations in the NW Atlantic. Possible future changes in the availability of these resources, including spatial or temporal shifts of existing prey assemblages or regime shifts to other prey species, as a result of fishery removals, climate change, or other factors, could significantly impact these large pelagic species. Catastrophic changes in prey distribution could result in an ineffective gamble by bluefin tuna as they travel to historically rich forage grounds that no longer support high-energy prey aggregations. An example of a shift in feeding strategies as a result of changes in prey availability is observed in juvenile bluefin tuna in the Bay of Biscay. These bluefin tuna have altered their diet from anchovies to euphausiids, apparently shifting diet based on local prey abundance. In this case, movements do not appear to have been affected by ecosystem shifts, although possible energetic effects cannot be quantified without a better

understanding of the relative energetic value of these two prey resources. Future studies should examine the energy density of potential prey species (e.g., Lawson et al. 1998) and the relationship between predator and prey distributions (e.g., Schick and Lutcavage 2009). These additional data would allow the energetic consequences of prey species shifts to be quantified.

To predict effects of ecosystem perturbations on bluefin tuna, a better understanding of the relationship between tuna movement patterns and seasonal availability of prey resources is needed. Recent tagging results showing adult bluefin tuna returning to coastal forage grounds off Nova Scotia (Galuardi et al. submitted) lend support for the theory that individual fish have site fidelity to specific forage grounds. Over the same time period, many bluefin tuna migrate past adjacent Gulf of Maine forage grounds (Galuardi et al. submitted), despite the availability of a stable prey base of herring and sand lance in this location. This apparent discrepancy could in fact lend further support for learned movements, as this assemblage of fish may simply be following a schedule linked to Nova Scotia rather than Gulf of Maine forage grounds. If individual fish are in fact continually reliant on ephemeral prey patches in given regions and seasons, disturbances to such resources could have drastic effects on the condition, reproductive output, and potential survival of these individuals. If they are able to adapt and seek out prey resources in other regions, these individuals may then permanently abandon disrupted forage grounds, even if prey resources later return to such regions. The former scenario could have negative impacts for the tuna population while the latter scenario would affect regional ecosystem structure and supported fisheries.

For bluefin tuna, movement patterns among forage grounds actually appear to be even more complex. Adult bluefin tuna have high inter-individual variability in somatic condition for all sampled locations and dates (Figure 30), indicating variable usage of seasonal forage grounds. While most fish occupying Gulf of Mexico spawning grounds previously used food resources from shelf forage grounds, some individuals instead occupied offshore regions. More detailed comparisons in Gulf of Maine forage grounds suggest that variable condition is related to different migration schedules rather than differences in local diet. Bluefin tuna isotope values have a general periodicity, with $\delta^{15}N$ values increasing during summer and fall as tissues equilibrate with shelf forage grounds, followed by a decline during winter and spring residency in offshore regions. Based on this pattern, late season fish with lower $\delta^{15}N$ and C:N values would appear to be late arrivals to the Gulf of Maine. Further questions then remain regarding the status of these fish arriving late to shelf forage grounds. They could arguably have gambled poorly and missed most of the seasonally available prey resources of this region. Rather, they may simply have a completely different migration schedule from fish that arrive earlier in the season instead relying on other seasonal prey aggregations to sustain their migrations. Resolving these differential uses of regional forage grounds will help in understanding the complex interplay between movement, diet, and body condition for this highly migratory species.

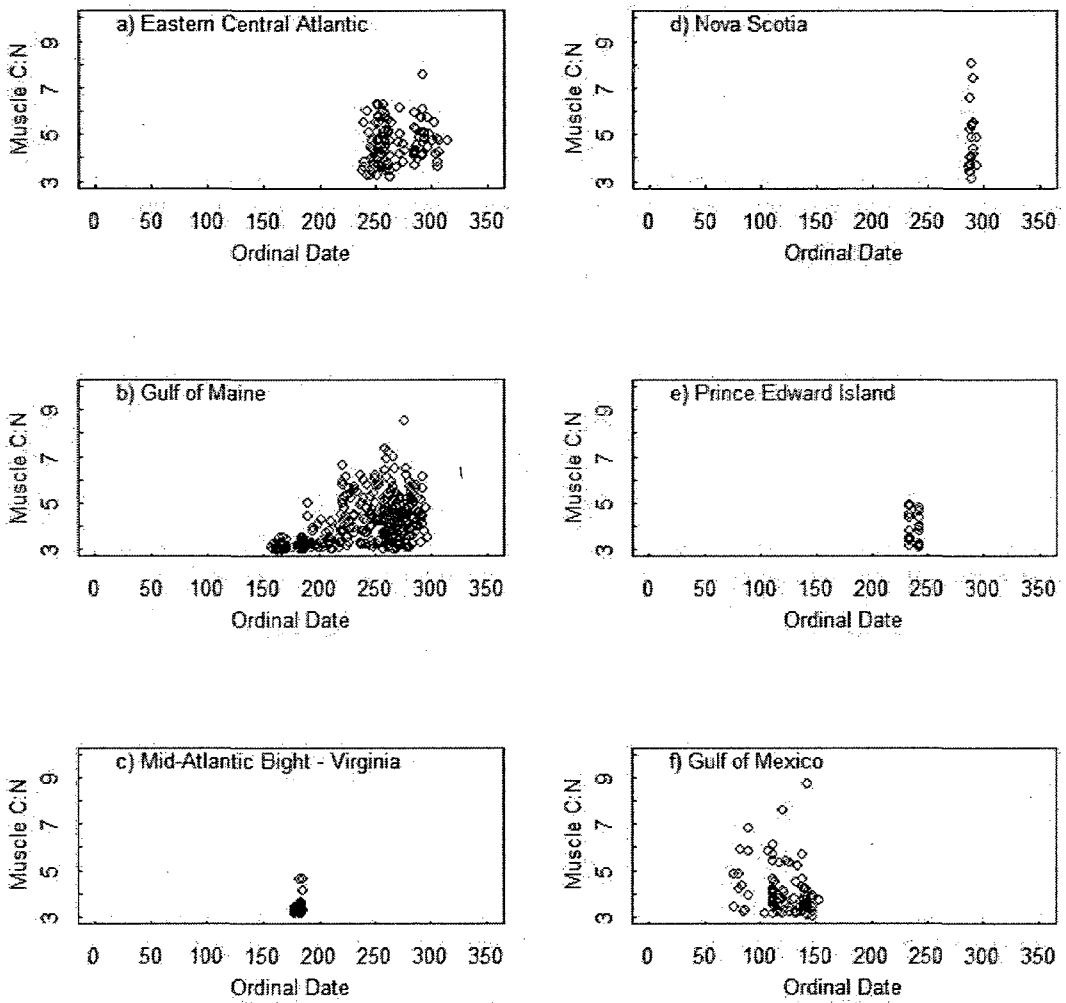

Figure 30. Muscle C:N values across sampling dates for Atlantic bluefin tuna (*Thunnus thynnus*) sampled from a) the eastern central Atlantic b) Gulf of Maine c) Mid-Atlantic Bight d) Nova Scotia e) Prince Edward Island and f) Gulf of Mexico.

These complex patterns of movement and residency (Block et al. 2001; Wilson et al. 2005; Galuardi et al. submitted) could potentially be resolved with a joint chemical and electronic tagging approach. Stable isotopes are generally most useful as complements to other techniques (Peterson 1999; Fry 2006). Inclusion of additional chemical tracers (e.g., fatty acids and organochlorines (Iverson et al. 2004; Dickhut et al.

2009)), could further clarify feeding and movement history. During tagging operations, non-lethal samples of white muscle and possibly rapid-turnover mucus material (Church et al. 2009) could be collected to provide both recent and past information on diet and migrations through SIA. Analysis of muscle C:N would provide information on body condition at time of tagging, with electronic tagging data revealing subsequent movements, allowing aspects of diet, condition, and movement to be assessed for individual fish. This type of integrated approach would allow for better predictions of how tunas will respond to future perturbations to their environment and prey base.

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