Pre-spawning habitat use of Atlantic bluefin tuna (*Thunnus thynnus*) inferred from stable isotope analysis

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Abstract

Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) spawn primarily in the Gulf of Mexico and Mediterranean Sea but migrate to foraging habitats throughout the North Atlantic where they are the target of commercial and recreational fisheries. Natal origin has been characterized through otolith oxygen isotope analysis to link fish on both spawning grounds and foraging habitats to their spawning ground origins but connectivity on a shorter, seasonal timescale is still not completely understood. Nitrogen isoscapes in the North Atlantic include a distinct separation of productive, nearshore and more oligotrophic open ocean foraging habitats. We used linear discriminant analysis of bulk nitrogen isotope data to estimate the percent of ABFT that occupied shelf or open ocean foraging habitats prior to capture on eastern and western Atlantic spawning grounds. ABFT in the Gulf of Mexico were mainly classified as previous shelf foragers (91%) while ABFT associated with eastern Atlantic spawning grounds primarily had an open ocean/Mediterranean Sea classification (96% Morocco, 79% Strait of Gibraltar, 87% Balearic Sea, 100% Adriatic Sea). Amino acid nitrogen isotope data of ABFT from the Gulf of Mexico confirmed that observed bulk nitrogen isotope differences were due to baseline rather than

trophic variability and source amino acid values generally aligned most closely with literature values from shelf and slope waters rather than open ocean habitats. These data provide insight into the foraging habitats that support eastern and western Atlantic spawning assemblages.

Keywords Chemical tracers, Isoscapes, Movement, Nitrogen, North Atlantic

Introduction

Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) are highly migratory top predators that support fisheries throughout the North Atlantic and Mediterranean Sea (Mather et al. 1995; Fromentin and Powers 2005). ABFT are currently managed by the International Commission for the Conservation of Atlantic Tunas (ICCAT) as two separate (i.e., eastern and western) stocks divided at the 45ºW meridian. Stock independence has been supported by genetic (Carlsson et al. 2007) and tagging (Block et al. 2005) data. However, shelf and open ocean waters throughout the North Atlantic act as both foraging and fishing grounds for the species; furthermore, there is mounting evidence from electronic tagging and chemical tracer studies of ABFT migrations and mixing between eastern and western management areas (Walli et al. 2009; Rooker et al. 2014; Kerr et al. 2020). Electronic tagging data have revealed complex and varied migratory patterns, including movement across the stock boundary (Block et al. 2001; Walli et al. 2009; Galuardi et al. 2010; Aarestrup et al. 2022). The extent to which eastern and western stock fish occupy the feeding grounds of the other stock complicates management of this highly valuable species. Making this problem more contentious is the large difference in stock sizes with the eastern stock being estimated at an order of magnitude larger than the western stock (Anonymous 2017).

Like other tunas, 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) that collectively facilitate concentrated periods of foraging when they coincide in space and time with dense prey aggregations. ABFT undertake long migrations to access productive shelf and open ocean foraging habitats throughout the North Atlantic Ocean (Rivas 1955; Mather et al. 1995). Energy stores acquired at these productive foraging habitats in turn provide fuel for migrations to spawning grounds and reproductive output (Chapman et al. 2011).

While ABFT forage and migrate widely throughout the North Atlantic Ocean, their spawning grounds are more temporally and spatially constrained (Mather et al. 1995; Fromentin and Powers 2005; Rooker et al. 2007). Western Atlantic spawning occurs primarily from April to

June along the Loop Current front and the northern Gulf of Mexico's deep water region (Baglin 1982; Knapp et al. 2014; Domingues et al. 2016; Le-Alvarado et al. 2021). Additional spawning may occur in the Slope Sea (Richardson et al. 2016; Hernández et al. 2022), but the importance of this region is less well understood (Safina 2016; Walter III et al. 2016). Spawning in the eastern Atlantic occurs in regions of the eastern, central, and western Mediterranean Sea: the Levantine Sea, Malta and Tyrrhenian Sea, and Balearic Sea, respectively (Corriero et al. 2020). Relative to the Gulf of Mexico, ABFT spawning is delayed in the Mediterranean Sea with activity initiating in the eastern Atlantic in May and progressing in an east to west direction with Balearic Sea spawning commencing in July (Corriero et al. 2020). Age at sexual maturity for the Mediterranean Sea is estimated to be younger (3-5 years (Corriero et al. 2005)) than Gulf of Mexico spawners (8 years (Baglin 1982), although a recent reconsideration of these estimates suggests that they may be more similar (Corriero et al. 2020). The Gulf of Mexico spawning assemblage is thought to be tightly linked to western Atlantic foraging habitats based on existing catch, tagging, and chemical tracer data (Rivas 1955; Rooker et al. 2007; Dickhut et al. 2009; Wilson et al. 2015). Specifically, Gulf of Mexico spawners have documented migrations from Canadian shelf foraging habitats in the Gulf of St. Lawrence (Wilson et al. 2015) and Nova Scotia (Galuardi et al. 2010) as well as U.S. shelf waters off New England (Stokesbury et al. 2004) and the Mid-Atlantic Bight (Block et al. 2005). Recent electronic tagging data show a greater connectivity with open ocean and shelf foraging habitats in the eastern Atlantic and Mediterranean Sea for eastern Atlantic spawners (Aranda et al. 2013; Fromentin and Lopuszanski 2014; Cermeño et al. 2015; Abascal et al. 2016). ABFT tagged in Mediterranean Sea spawning habitats resided within the northwestern Mediterranean Sea and Adriatic Sea following the spawning period (Fromentin and Lopuszanski 2014; Cermeño et al. 2015). ABFT also migrated out of the Mediterranean Sea to shelf waters off the Iberian coast and Bay of Biscay and broadly across open ocean waters ranging approximately from Iceland to Ireland (Aranda et al. 2013).

The Gulf of Mexico and Mediterranean Sea also provide foraging habitat for ABFT (Karakulak et al. 2009; de la Serna et al. 2012; Battaglia et al. 2013; Butler et al. 2015). Mediterranean foraging is well documented, with diet including zooplanktivorous fishes in the western Mediterranean Sea and mesopelagic fishes and cephalopods in central and eastern Mediterranean habitats (Karakulak et al. 2009; Battaglia et al. 2013). Prolonged residence

beyond the spawning period in the Mediterranean Sea provides further evidence for this region as a foraging habitat (Cermeño et al. 2015). Arrivals months prior to spawning (Galuardi et al. 2010; Wilson et al. 2015) combined with direct evidence of foraging, elevated lipid stores, and a lack of starvation-induced ^{15}N enrichment (Butler et al. 2015) collectively suggest the Gulf of Mexico acts as both a foraging habitat and spawning ground. (Block et al. 2001; Stokesbury et al. 2004; Block et al. 2005; Teo et al. 2007a; Galuardi et al. 2010; Wilson et al. 2015). Individual ABFT residency in the Gulf of Mexico varies from one month (Block et al. 2001; Teo et al. 2007a) to more than five months (Galuardi et al. 2010) with average reported residences of 39 (Teo et al. 2007a) and 123 (Wilson et al. 2015) days. Diet during this time period includes primarily pelagic tunicates (*Pyrosoma atlanticum*) and fishes (Butler et al. 2015).

Bulk stable isotope analysis (BSIA) has been used as an alternative or complementary approach to tagging studies to characterize habitat use (Abrantes and Barnett 2011) and track movements of a variety of fishes and other marine taxa including top predators (Hobson 2007b; Graham et al. 2010).. Fish movements have been tracked between mangroves and coral reefs (Nakamura et al. 2008), within estuaries (McMahon et al. 2005; Suzuki et al. 2005; Haas et al. 2009), and between coastal and open ocean systems (Rodgers and Wing 2008). In the marine environment, distinct provinces show characteristic isotopic signatures (e.g., $\delta^{13}C$, $\delta^{15}N$), which allow past diet and location to be measured (Hobson 1999, 2007a, b; Rubenstein and Hobson 2004). In the North Atlantic Ocean, nitrogen isotope values show a clear spatial gradient with higher baseline values in productive shelf foraging habitats and lower baselines associated with more oligotrophic open ocean foraging habitats where nitrogen fixation is prevalent (McMahon et al. 2013). These spatial isotopic gradients, or "isoscapes" *sensu* West et al. (2010), provide a means of tracking migratory patterns for marine predators feeding in regions with different isotope values as regional isotope differences are propagated up trophic levels, all the way up to apex predators such as tunas (Graham et al. 2010; Logan et al. 2020). In the North Atlantic Ocean, isoscapes have been mapped previously for nitrogen using zooplankton values as well as biogeochemical modelling (Somes et al. 2010; McMahon et al. 2013; Schmittner and Somes 2016). Within the Gulf of Mexico, a basin-wide zooplankton-based isoscape indicated a marked north to south gradient in $\delta^{15}N$, with higher values over the northern shelf that allowed for inference of the feeding grounds of yellowfin tuna, *Thunnus albacares* (Le-Alvarado et al. 2021). An SIA approach was used to assess residency of adult ABFT on Gulf of Maine foraging

habitats (Logan et al. 2015). Similarly, nitrogen isotope gradients have been used to track arrival and residency of Pacific bluefin tuna (*Thunnus orientalis*) (Madigan et al. 2014) and yellowfin tuna (*T. albacares*) (Graham et al. 2010) on Pacific foraging habitats.

Compound specific stable isotope analysis of amino acids (AA-CSIA) is another tool that can be used to infer movements of various taxa including marine top predators (Popp et al. 2007; McMahon and Newsome 2019). AA-CSIA is a useful complement to BSIA-based movement assessments as the former can help distinguish trophic and migratory sources of variation in the latter. "Source" amino acids, which undergo minimal alteration with trophic transfer, can be subtracted from corresponding amino acids that undergo greater discrimination with metabolism (i.e., "trophic" amino acids) to separate baseline and trophic influences (McMahon and Newsome 2019). AA-CSIA of $\delta^{15}N$ has been applied to large pelagic species to infer residency and movements (Popp et al. 2007; Seminoff et al. 2012; Madigan et al. 2014).

Given that different metabolically active tissues integrate distinct feeding periods based on their rate of isotopic turnover (e.g., Hobson 1999; Heady and Moore 2012; Matley et al. 2016), it is feasible to infer feeding grounds over various time scales (Malpica-Cruz et al. 2013; Munroe et al. 2015; Le-Alvarado et al. 2021). Madigan et al. (2012) conducted a controlled feeding study on Pacific bluefin tuna 63-92 curved fork length (CFL), and estimated a nitrogen isotopic half-life of 86 and 167 days for liver and muscle tissues, respectively. Hence, while the isotopic composition of the muscle tissue of ABFT should reflect foraging habitats over the time scale of several months, that of liver tissue should reflect more local foraging, providing a means for generating an isoscape with which to infer migration.

Our goal was to improve understanding of habitat types used to fuel ABFT spawning. We created nitrogen isoscapes for ABFT shelf and open ocean foraging habitats using nitrogen isotope data from ABFT and swordfish (*Xiphias gladius*) liver based on individuals harvested from multiple regions representative of these two habitat types in the North Atlantic Ocean. We used bulk nitrogen stable isotope values of white muscle as a proxy for past foraging habitats from ABFT harvested on eastern and western Atlantic spawning grounds and migratory corridors. We then assigned ABFT sampled on the Gulf of Mexico and Mediterranean Sea spawning grounds and Atlantic migration corridors to one of these habitat types based on their muscle stable isotope values using linear discriminant analysis.

Methods

Tissue samples were collected from fisheries targeting ABFT and other large pelagic fishes in North Atlantic foraging habitats (liver) as well as Gulf of Mexico and Mediterranean Sea spawning grounds (muscle) (Fig. 1). Liver stable isotope data for all shelf and open ocean foraging habitats as well as muscle stable isotope data for spawning-related Strait of Gibraltar and Balearic Sea habitats were obtained from published datasets(Logan 2009; Varela et al. 2011, 2013, 2020, 2022; Logan and Lutcavage 2013; Logan et al. 2015; Navarro et al. 2020). White muscle tissue samples from the Gulf of Mexico (2007-2013), coast of Morocco (2010), and Adriatic Sea (2010) were collected as part of this study. Sample collection, preparation, and analysis methods for published data are described in those respective studies but all follow conventional methods for bulk stable isotope analysis. All Gulf of Mexico, coast of Morocco, and Adriatic Sea samples were stored on ice, then frozen until preparation for stable isotope analysis (SIA). Tissue samples were later thawed, rinsed in deionized water, freeze dried, pulverized, placed in sealed vials, and stored in a desiccator at -20°C until subsampling.

Bulk Stable Isotope Analysis (BSIA)

For Gulf of Mexico and Mediterranean Sea samples, an aliquot of approximately 2.5 mg of dry homogenized tissue was weighed into a tin capsule and placed in a 96-well plate. Stable nitrogen ($\delta^{15}N$) isotopes and elemental percent (%C, %N) of Gulf of Mexico ABFT muscle samples were measured at the University of New Hampshire Stable Isotope Lab on an Elementar Americas Pyrocube elemental analyzer coupled to a GeoVision isotope ratio mass spectrometer. Samples from the coast of Morocco and Adriatic Sea were analyzed at the University of A Coruña through a gas flow system using a Thermo Finnigan Flash EA1112 elemental analyzer coupled to a Thermo Finnigan Delta Plus isotope ratio mass spectrometer. The measurement uncertainty of the instrument as determined by repeated analyses of in-house QA/QC standards was approximately 0.2‰ for $\delta^{15}N$ at both laboratories. The measured ^{15}N abundance values are reported relative to atmospheric nitrogen (Air) using the following international reference materials: USGS25, USGS40, IAEA-N1, USGS42, USGS43, IAEA-N2, and USGS41. All nitrogen isotope data are reported in δ notation according to the following equation (Brand 2011):

$$\delta^{15}N_{unknown} = \frac{R(^{15}N/^{14}N)_{unknown} - R(^{15}N/^{14}N)_{standard}}{R(^{15}N/^{14}N)_{standard}}$$

Amino Acid Compound-Specific Stable Isotope Analysis (AA-CSIA)

A subset of Gulf of Mexico muscle samples classified as shelf (n=46) and open ocean (n=20) migrants (see Results) spanning a range of sizes (SFL=204-276 cm) and bulk $\delta^{15}N$ values (10.1-15.3‰) was selected for amino acid nitrogen SIA to determine if bulk $\delta^{15}N$ differences were due to baseline or trophic variation. Dried, homogenized samples were analyzed at the University of California Davis Stable Isotope Facility following acid hydrolysis using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) on a Thermo Trace GC 1310 gas chromatograph coupled to a Thermo Scientific Delta V Advantage isotope-ratio mass spectrometer via a GC IsoLink II combustion interface. Detailed methods are provided in Walsh et al. (2014) and Yarnes and Herszage (2017). CSIA generated $\delta^{15}N$ data for 15 individual amino acids: alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine.

Trophic position (TP) was estimated using the weighted averages of a suite of three representative "source" (glycine, lysine, and phenylalanine) and "trophic" (alanine, glutamic acid, and leucine) amino acids following methods described by Choy et al. (2015) and Bradley et al. (2015). A recent meta-analysis demonstrated that this approach of combining multiple amino acids increases the precision of TP estimates (Nielsen et al. 2015). TP is calculated based on the weighted average source and trophic $\delta^{15}N$ values, estimates of the difference in the weighted $\delta^{15}N$ averages of the source and trophic amino acids at the base of the marine food web (β=3.6‰), as well as the trophic discrimination factor (TDF=5.7‰) separating these two classes of amino acids with each trophic transfer. Values for β and TDF were based on published data for marine teleosts (Bradley et al. 2015).

$$eq.1 \qquad TP = 1 + \frac{\delta^{15}N_{Trophic} - \delta^{15}N_{Source} - \beta}{TDF}$$

Propagation of error was calculated for these AA-CSIA-based TP estimates. Analytical error was estimated as 0.5‰, while error for $\beta_{Tr\text{-}Src}$ and $TDF_{Tr\text{-}Src}$ was estimated as 0.5 and 0.3 ‰, respectively, following Choy et al. (2015). Propagation of analytical and methodological error (SD_{a+m}) was completed using the propagate package (Spiess 2014) in R (R Core Team 2022). Propagated analytical and methodological error (SD_{a+m}) was then combined with individual error (SD_{ind}) to generate a total propagated error using the following equation:

$$SD_{total} = \sqrt{((\text{average}(SD_{a+m}))^2 + (SD_{ind})^2)}$$

Calculated TP estimates were compared to bulk $\delta^{15}N$ values and migratory classifications to determine if bulk $\delta^{15}N$ differences were being driven primarily by baseline (i.e., migratory) rather than trophic variation. We tested for any size-based (straight fork length) correlation with TP for the open ocean and shelf classification groups using linear regression.

To further explore the spatial identity of general shelf and open ocean classifications, representative source amino acid $\delta^{15}N$ values (Lys, Phe, and Met) from Gulf of Mexico ABFT were compared to values reported in the literature from other marine fauna in representative shelf and open ocean ABFT foraging habitats (McClelland et al. 2003; Choy et al. 2012; Mompeán et al. 2016; Uriarte et al. 2016 Unpubl. Data; Varela et al. 2018a; Laiz-Carrión et al. 2019; Varela et al. 2019; Phillips et al. 2020; Le-Alvarado et al. 2021; Austin 2022; Logan Unpubl. Data; Sherwood Unpubl. Data; Herzka Unpubl. Data). These source amino acids were selected since they are currently considered the most reliable baseline indicators due to minimal isotopic discrimination upon trophic transfer (McMahon and Newsome 2018). To account for expected differences among fauna due to different TPs, all literature values were normalized based on their estimated TP relative to our shelf migrant ABFT group and estimated diet tissue discrimination factors. TP estimates were based on amino acid data calculated in the source literature with the exception of soft corals from the NW Atlantic for which TP was based on bulk consumer and baseline $\delta^{15}N$ values (Sherwood et al. 2005). Literature values were normalized to our Gulf of Mexico shelf migrant dataset based on estimated TP and isotopic discrimination using the following equation:

$$eq.2 \;\; \Delta\delta^{15}N = \delta^{15}N_{ABFT\ Shelf} - (\delta^{15}N_{Literature} + TDF * (TP_{ABFT\ Shelf} - TP_{Literature}))$$

where $\delta^{15}N_{ABFT\ Shelf}$ is the average source amino acid $\delta^{15}N$ value for Gulf of Mexico shelf migrant ABFT, $\delta^{15}N_{Literature}$ is the average source amino acid $\delta^{15}N$ value for a given literature taxa, and TDF is the estimated trophic discrimination factor for the specific source amino acid used in the calculation. TDFs used for the three selected source amino acids were 0.1‰ (Phe), 0.4‰ (Met), and 0.8‰ (Lys) based on results of a meta-analysis reported in McMahon and McCarthy (2016).

$TP_{ABFT\ Shelf}$ and $TP_{Literature}$ were the estimated respective trophic positions based on source and trophic amino acid data (eq. 1).

Data Analysis

A nitrogen isoscape was generated using liver tissue samples of ABFT collected from shelf and open ocean foraging habitats (Fig. 1). Liver data were used to classify foraging areas since isotopic turnover is more rapid in this tissue relative to white muscle (Madigan et al. 2012; Thomas and Crowther 2015) and generally reflects a more recent diet rather than the past migratory history (MacNeil et al. 2005; Le-Alvarado et al. 2021). Liver $\delta^{15}N$ values were adjusted for the estimated isotopic offset between white muscle and liver of +0.8 ‰ (Madigan et al. 2012). Liver $\delta^{15}N$ values from ABFT harvested on shelf foraging habitats were enriched in ^{15}N relative to open ocean foraging habitats with an approximate median separation between regional datasets from each group of 3-4 ‰ (Fig. 2). Liver $\delta^{15}N$ values were compared between shelf and open ocean groups using a Wilcoxon rank sum test. Median values significantly differed between groups ($p<0.001$) with a large effect size ($r=0.86$) which supports the use of liver isotope ratios in the nitrogen isoscape.

Shelf vs. open ocean foraging history based on bulk isotope values was determined with linear discriminant analysis (LDA) using the MASS package in R (R Core Team 2022). A training and test dataset was created using liver $\delta^{15}N$ values from representative Atlantic shelf (Gulf of St. Lawrence, Nova Scotia, Gulf of Maine, Mid-Atlantic Bight) and open ocean (eastern and western central Atlantic, Canary Islands, and Mediterranean Sea) foraging habitats (Figs. 1 and 2). Liver data were not available for ABFT or comparable fish predators from the eastern Atlantic shelf region, but similar prey $\delta^{15}N$ values provide support for selected western shelf habitats also being representative of eastern shelf habitats. For example, average squid (*Illex* spp.), euphausiid, and Atlantic herring (*Clupea harengus*) $\delta^{15}N$ values differ by less than 0.7 ‰ for the two shelf regions (Table S1; Fry 1988; MacNeil et al. 2005; Lavoie et al. 2010; Logan et al. 2011, 2015; Chouvelon et al. 2012; Ryan et al. 2014; Malek et al. 2016). Eastern Atlantic shelf prey also have higher $\delta^{15}N$ values than conspecifics from the Mediterranean Sea with average $\delta^{15}N$ offsets of 1.7 ‰ (anchovy), 1.9 ‰ (euphausiid), 2.1 ‰ (*Illex* spp.), and 3.0 ‰ (sardine) among common prey (Table S1; Bode et al. 2007; Cardona et al. 2012; Chouvelon et al. 2012, 2014; Costalago et al. 2012; Barría et al. 2015; Martínez-Baena et al. 2016; Rumolo et

al. 2016, 2018; Mellon-Duval et al. 2017; Zorica et al. 2021). The persistence of this $\delta^{15}N$ gradient between Atlantic shelf and Mediterranean waters even for prey sampled in Mediterranean Sea shelf waters (e.g., sardines and anchovies from the Gulf of Lions (Table S1; Costalago et al. 2012)) indicates that the open ocean classification group could include shelf foragers within the Mediterranean Sea. The dataset was randomly sub-divided with 60% used for training and 40% for testing. All liver data were derived from commercially landed ABFT with the exception of the western central Atlantic and Mediterranean Sea, which were represented by Atlantic swordfish (Logan and Lutcavage 2013; Navarro et al. 2020). Swordfish were used as a proxy due to a lack of adult ABFT data in these regions and similar offshore TP and diet between species (Matthews et al. 1977; Logan et al. 2013; Varela et al. 2018b; Navarro et al. 2020). Accuracy was estimated using resubstitution error. LDA was then applied to muscle tissue from the mixed stocks consisting of the Gulf of Mexico (western Atlantic spawning ground), coast of Morocco and Strait of Gibraltar (eastern Atlantic spawning migration corridors), and Balearic and Adriatic Sea (eastern Atlantic spawning grounds) to classify individual fish according to past foraging habitat (shelf or open ocean). For samples from the eastern Atlantic, only individuals > 130 cm SFL, the estimated minimum length of 100% maturity for Mediterranean spawners (Corriero et al. 2005), were included in migration classification analyses since the Mediterranean Sea functions as both spawning and foraging habitat (Sara and Sara 2007; Corriero et al. 2020).

Results

Classification accuracy of training and testing data was 97.9% and 97.5%, respectively, with the majority of western and eastern Atlantic samples classified as shelf and open ocean foragers, respectively (Table 1). Over 90% of Gulf of Mexico spawners were classified as shelf migrants while eastern Atlantic regions had greater open ocean/Mediterranean Sea representation ranging from 79% for the Strait of Gibraltar to 100% for the Adriatic Sea. For regions with multi-year datasets, the relative percent of shelf and open ocean/Mediterranean Sea migrant classifications varied across years with 2012 having the highest open ocean/Mediterranean Sea representation for the Gulf of Mexico (22%) and highest shelf representation for the Strait of Gibraltar (58%) (Table 1; Fig. 3).

Gulf of Mexico shelf and open ocean/Mediterranean Sea migrant groups had similar TP estimates based on AA-CSIA data of ($\overline{x}$ ± SD: 4.4±0.6) and ($\overline{x}$ ± SD: 4.1±0.9) for shelf and open ocean/Mediterranean Sea groups, respectively, while average bulk $\delta^{15}N$ values for the two groups were 13.3 and 11.5‰. Assuming a $\delta^{15}N$ diet-tissue discrimination factor for tuna white muscle of 1.9 ‰ (Madigan et al. 2012), the ΔTP for the two groups based on bulk $\delta^{15}N$ would be ~ 1 TP. TP was not significantly correlated with fork length for either open ocean (r^2=0.06, $F_{1,17}$=0.05, p=0.83) or shelf ($r^2 < 0.01$, $F_{1,43}$=1.19, p=0.28) groups.

After accounting for differences in TP, source amino acid $\delta^{15}N$ values for Gulf of Mexico ABFT matched most closely with marine fauna from Northwest and Northeast Atlantic shelf and slope waters (Fig. 4, S1; Table S2). ABFT source values aligned most closely with TP-corrected values for loggerhead sea turtles (*Caretta caretta*) from the western Atlantic shelf as well as ocean sunfish (*Mola mola*) from both the eastern and western Atlantic shelves with all values falling within 3.6 ‰ following TP adjustment (Table S2). Similar TP-adjusted source amino acid $\delta^{15}N$ values ($\Delta\delta^{15}N$ <4 ‰; Table S2) were also observed for some fauna from open ocean

habitats (e.g., Lys for ocean sunfish from the Mediterranean Sea and yellowfin tuna from the Gulf of Mexico). Literature source amino acid values from the Tropical North Atlantic and Mid Atlantic Ridge differed most from Gulf of Mexico ABFT with TP-adjusted differences of ~ ≥10 ‰ for most source amino acids and literature datasets (Table S2).

Discussion

Atlantic bluefin tuna (ABFT) occupying eastern and western spawning grounds showed different prior foraging habitat preferences with western Atlantic spawners relying mainly on shelf waters and eastern Atlantic spawners instead primarily using open ocean and/or Mediterranean Sea foraging habitats. While percentages varied annually and among sampling regions, ABFT from eastern and western spawning grounds had a consistent primary reliance on the respective dominant past foraging habitat of approximately 80-100% of the sampled population. These findings are consistent with habitat use characterized by past tagging and chemical tracer studies (Dickhut et al. 2009; Walli et al. 2009; Galuardi et al. 2010; Aranda et al. 2013; Wilson et al. 2015).

Bulk stable isotope data alone cannot distinguish eastern and western shelf foraging habitats (e.g., Table S1), but past catch, tagging, and chemical tracer data have shown a high level of connectivity between western shelf foraging and subsequent Gulf of Mexico occupancy (Rivas 1955; Mather et al. 1995; Stokesbury et al. 2004; Dickhut et al. 2009; Galuardi et al. 2010; Wilson et al. 2015). Analysis of catch records in the 1950's suggested a connectivity between seasonal foraging habitats in the Northwest Atlantic and Gulf of Mexico spawning grounds (Rivas 1955), and conventional tagging studies in the 1970's provided direct evidence of this migratory pathway (Mather et al. 1995). Conventional and electronic tagging results have shown movements of 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. 2010; Wilson et al. 2015), that were included in our shelf isoscape (Fig. 1), to the Gulf of Mexico. Organochlorine contaminant ratios in ABFT (n=16) from the Gulf of Mexico were all similar to values from western North Atlantic ABFT, providing evidence of recent energy acquisition from western shelf habitats (Dickhut et al. 2009). Most ABFT tagged during fall months in the Gulf of St. Lawrence foraging habitat (n=49; 74%) traveled to the Gulf of Mexico (Wilson et al. 2015).

While ABFT use the Gulf of Mexico as foraging habitat (Butler et al. 2015) and tagging data suggest it may act as a winter foraging habitat prior to spring spawning (Galuardi et al. 2010; Wilson et al. 2015), high muscle bulk $\delta^{15}N$ values suggest that their primary energy source prior to spawning comes from productive shelf waters rather than the open ocean waters that they occupy in the Gulf of Mexico (Teo et al. 2007b). Foraging hotspots occur on shelf and slope waters from Nova Scotia to the Mid-Atlantic Bight (Walli et al. 2009) where ABFT feed on seasonal aggregations of lipid-rich schooling fish prey including Atlantic herring, Atlantic mackerel (*Scomber scombrus*), sand lance (*Ammodytes* spp.), and menhaden (*Brevoortia tyrannus*) (Chase 2002; Butler et al. 2010; Pleizier et al. 2012; Logan et al. 2015; Varela et al. 2020). Muscle $\delta^{15}N$ values appear to reflect summer and autumn periods of increased consumption of these high caloric prey (Chase 2002; Butler et al. 2010; Pleizier et al. 2012; Logan et al. 2015; Varela et al. 2020) rather than winter and spring Gulf of Mexico forage species of lower energy density (e.g., gelatinous prey) (Butler et al. 2015). Bulk $\delta^{15}N$ values of higher trophic level Gulf of Mexico fish prey (e.g., lancetfish, x = 9.3 ‰, (Keller et al. 2016)) are lower than western Atlantic shelf forage (e.g., Atlantic herring, $\bar{x}$ = 11.7 ‰, (Fry 1988; MacNeil et al. 2005; Logan et al. 2015; Malek et al. 2016)). The primary documented Gulf of Mexico

prey, the pelagic tunicate *P. atlanticum*, is a filter feeder (Conley et al. 2018) that would presumably have an even lower bulk $\delta^{15}N$ value based on its TP and data for pyrosomes from other pelagic habitats (e.g., Décima et al. 2019; Schram et al. 2020). Source amino acid $\delta^{15}N$ values also match western shelf and slope fauna more closely than Gulf of Mexico fauna overall (but see yellowfin tuna; Table S2), further suggesting ABFT muscle $\delta^{15}N$ values reflect prior foraging habitats rather than local waters where they were harvested.

The prevalence of open ocean foraging classification among eastern Atlantic ABFT is consistent with previous tagging and chemical tracer studies showing a high degree of residence in the Mediterranean Sea prior to spawning as well as movements from foraging habitats in the eastern Central Atlantic Ocean (Dickhut et al. 2009; Aranda et al. 2013; Fromentin and Lopuszanski 2014; Cermeño et al. 2015). Organochlorine contaminant ratios in ABFT muscle samples from the Mediterranean Sea largely reflected past foraging within the Mediterranean Sea (n=33; 87%) with a smaller percentage of Atlantic migrants (n=5; 13%) (Dickhut et al. 2009). Mediterranean Sea residency was observed among ABFT electronically tagged in the western Mediterranean and Adriatic Sea regions (Fromentin and Lopuszanski 2014; Cermeño et al. 2015). Indeed, our Adriatic Sea dataset was composed entirely of open ocean/Mediterranean Sea "migrants", consistent with local foraging in Mediterranean Sea waters. While foraging within the Gulf of Mexico did not appear to be of sufficient duration and intensity to influence ABFT muscle $\delta^{15}N$ values, the high percent of open ocean/Mediterranean Sea classifications for Mediterranean Sea ABFT is consistent with prolonged foraging within the Mediterranean Sea. Relatedly, open ocean/Mediterranean Sea classifications were higher among fish sampled within the Mediterranean Sea than the group sampled at the Strait of Gibraltar, presumably in transit

from the Atlantic to the Mediterranean Sea (Table 1). This could be due to Mediterranean Sea residency for some of the fish sampled in the Balearic and Adriatic Sea regions.

For ABFT sampled within the Mediterranean Sea, open ocean classification could also reflect shelf foraging habitats within the Mediterranean Sea given similarities in prey baseline $\delta^{15}N$ values between Mediterranean shelf and open ocean habitats (Table S1). Tagging data identified bluefin in presumed foraging grounds between the Gulf of Lions and Balearic Sea (Cermeño et al. 2015). Foraging on sardines and anchovies in shelf waters of the Gulf of Lions, for example, would presumably create ABFT tissue $\delta^{15}N$ values classifiable as open ocean foraging habitat. Tagging data have shown that ABFT in the Mediterranean Sea primarily use the basin regions rather than nearshore waters (Cermeño et al. 2015). Shelf habitat is fairly constrained across the Mediterranean Sea with only limited areas < 200 m in depth, which are found primarily in the north Adriatic, the Tunisian shelf, Gulf of Lions, the shelf between Sicily and Malta, and the shelf containing Sardinia and Corsica (Leanza 1993). This uncertainty applies to ABFT sampled in the Gulf of Mexico and Mediterranean Sea classified as open ocean migrants but would not impact classifications for ABFT intercepted prior to Mediterranean Sea entry (i.e., Morocco and Strait of Gibraltar samples).

For these fish sampled from traps off Morocco and the Strait of Gibraltar that were intercepted prior to entry into the Mediterranean Sea, the high open ocean classification rate is consistent with extensive occupancy of offshore waters in the eastern Central Atlantic Ocean observed following departure from the Balearic Sea (Aranda et al. 2013). ABFT forage on ommastrephid squids, barracudinas and other mesopelagic prey in the central Northeast Atlantic (Olafsdottir et al. 2016). Mesopelagic fauna are also among the dominant prey in open ocean foraging habitats in the Mediterranean Sea, where myctophids and ommastrephid squids are

among the primary ABFT prey (Karakulak et al. 2009; Battaglia et al. 2013). Many of these prey groups are found in dense aggregations (Collins et al. 2008; Gartner et al. 2008) and have high caloric value (Ackman et al. 1972; Saito and Murata 1998; Glaser 2010; Goetsch et al. 2018; Logan et al. 2021); thus, open ocean foraging habitats also provide energy resources to fuel spawning activity.

The Mediterranean Sea-associated ABFT in our study also included shelf migrants, particularly among the Strait of Gibraltar dataset, reflecting a diversity of foraging habitats among the overall eastern Atlantic spawning assemblage. Some ABFT tagged on Balearic Sea spawning grounds traveled to eastern Atlantic shelf and slope waters in the Bay of Biscay (Aranda et al. 2013), an important foraging habitat for ABFT with lipid-rich schooling fishes like anchovies (*Engraulis encrasicholus*) (Uriarte et al. 1996; Logan et al. 2011) that provide similar forage bases to western shelf habitats (Chase 2002; Butler et al. 2010; Pleizier et al. 2012; Logan et al. 2015; Varela et al. 2020). Two ABFT tagged on Gulf of St. Lawrence foraging habitats in the autumn traveled to the Mediterranean Sea the following spring, providing some direct evidence of linkages between western shelf foraging habitats and Mediterranean Sea spawners (Wilson et al. 2015). A minority of ABFT sampled at the Strait of Gibraltar from 2008 to 2010 had fatty acid profiles consistent with prior foraging in high latitude North Atlantic habitats, although the majority had profiles reflective of past foraging in temperate and subtropical waters (Mourente et al. 2015).

While our Mediterranean Sea stable isotope dataset mainly contained values representative of past open ocean or Mediterranean Sea foraging, a previous dataset of ABFT muscle from the western Mediterranean Sea (southern Tyrrhenian Sea) included a large size class (>178 kg) with elevated $\delta^{15}N$ values ($\bar{x}$ ± SD: 13.1 ± 0.2 ‰) that would be classified as

shelf migrants (Sara and Sara 2007). The authors attributed these high $\delta^{15}N$ values to past migration from Atlantic waters where isotopic baselines are higher than Mediterranean Sea pelagic habitats (Sara and Sara 2007). These values closely match our Gulf of Mexico shelf dataset ($\bar{x}$ ± SD: 13.5 ± 0.6 ‰) and exceed the mean values for shelf migrant groups from our eastern Atlantic dataset ($\bar{x}$: 12.1-12.5 ‰; Table 1). This Tyrrhenian Sea region was not included in our dataset, so differences between studies could reflect differences in past movements among Mediterranean spawning grounds, although our Strait of Gibraltar dataset should reflect the diversity of ABFT spawning assemblages traveling from the Atlantic. Differences could potentially also reflect temporal variation in relative reliance on shelf and open ocean foraging habitats.

For regions for which we had multiple sampling years (Gulf of Mexico and Strait of Gibraltar), relative shelf and open ocean prevalence varied annually with greatest divergence observed for both regions in 2012 (Table 1). Sea surface temperatures (SSTs) were elevated globally in 2012, particularly in the Northwest Atlantic (Mills et al. 2013; Xue et al. 2013). Muscle isotope values from ABFT sampled in spring to summer 2012 would mainly reflect foraging habitats in the prior fall (2011) and winter (2011/2012) rather than the summer 2012 period when the anomaly was most pronounced (Mills et al. 2013), but warming in the Northwest Atlantic occurred throughout winter 2012 (Mills et al. 2013) and so could have influenced prey distributions within the timescale reflected in muscle isotope data (Madigan et al. 2012). We did not have 2013 data from the eastern Atlantic to examine for potential differences following the 2012 heat wave, but 2013 Gulf of Mexico data maintained the shelf dominance seen across the overall dataset. Temperature anomalies could have affected the spatial and/or temporal distribution of prey resources available to ABFT in the months prior to

spawning in 2012, although simultaneous anomalies in habitat use in 2012 could also be spurious results.

Compound-specific stable isotope data offered further insight into past foraging habitats relative to the general shelf and open ocean classifications produced from bulk nitrogen isotope data. For the Gulf of Mexico dataset, the relative similarity of source amino acid $\delta^{15}N$ values of both classification groups to fauna from shelf and slope waters of the eastern and western Atlantic supports the primary shelf classification and suggests that even the minority open ocean group is likely associated with slope waters rather than more offshore waters of the Gulf of Mexico, Mediterranean Sea, or central Atlantic Ocean. ABFT source amino acid values most closely aligned with other mobile fauna (e.g., loggerhead sea turtles, ocean sunfish, other tuna species; Table S2), which could indicate a similar integration of baseline values in muscle tissue among species foraging across multiple areas (e.g., shelf and slope waters).

Caveats

The amino acid literature comparison is limited by several caveats. For example, source amino acid trophic fractionation dynamics are still not fully understood (McMahon and Newsome 2019), all TP estimates used to normalize literature values have a degree of uncertainty, and other highly migratory fauna (e.g., yellowfin tuna) included in our literature dataset may reflect source isotope values from past migratory habitats rather than the area of collection. Uncertainty in trophic fractionation will have the greatest bias for low trophic level fauna that are several TPs below ABFT (e.g., zooplankton) and may partly explain the lack of agreement between Gulf of Mexico ABFT and literature zooplankton values. Isotopic differences between Gulf of Mexico ABFT and literature fauna had intra-region variability among species (Table S2; Fig. S1), likely due to the previously mentioned caveats. While Gulf of Mexico ABFT

source amino acid values were most similar to Atlantic shelf and slope fauna, individual taxa from the Mediterranean Sea and Gulf of Mexico were also closely aligned with ABFT values (Table S2). These discrepancies suggest that, much like bulk $\delta^{15}N$ values, source amino acid $\delta^{15}N$ values may not be distinct for all habitats and geographic regions. Further exploration of source amino acid $\delta^{15}N$ isoscapes is needed to better determine the efficacy of amino acid $\delta^{15}N$ in distinguishing past movements and habitat use of mobile marine predators.

Bulk SIA is a powerful tool for assessing general habitat use of highly migratory species, but when applied without complementary methods, these data can result in false interpretations (Fry 2006). While liver has a more rapid turnover rate than muscle in tunas (Graham 2008; Madigan et al. 2012), if ABFT or swordfish used in our training dataset were recent migrants, their liver tissue may not have been in steady state with the local baseline (Logan et al. 2021). Several outliers in our open ocean/Mediterranean Sea training dataset had high nitrogen isotope values that overlapped with our shelf dataset (Fig. 2), which could have been from recent shelf migrants. Many of the eastern Atlantic ABFT in our dataset with past shelf foraging assignments had $\delta^{15}N$ values that were similar to the higher end of the open ocean assignment range (~12.2 ‰). These fish may have actually been open ocean migrants or fish that foraged in both open ocean and shelf waters prior to sampling. This error could be further compounded by any annual variability in $\delta^{15}N$ baselines among sampling years. Amino acid $\delta^{15}N$ CSIA confirmed that bulk $\delta^{15}N$ variation among individual ABFT was mainly driven by spatial (i.e., habitat) influences rather than trophic variability, but trophic influences cannot be ruled out for the eastern Atlantic dataset. Our isoscape dataset was also not comprehensive of all of the foraging habitats encountered by ABFT in the North Atlantic Ocean, although ocean-scale isoscapes (McMahon et al. 2013) suggest that our general open ocean and shelf classification $\delta^{15}N$ groups are robust.

Conclusions

Bulk and compound specific stable isotope analyses provide insight into general past foraging habitat use of ABFT on spawning grounds. These data reveal the habitats that fuel spawning migrations and activity and reflect clear differences in the relative reliance on shelf and open ocean/Mediterranean Sea foraging habitatss for western and eastern Atlantic spawning assemblages. Gulf of Mexico spawners primarily rely on shelf and slope-associated food webs while Mediterranean Sea spawners have a greater connectivity to open ocean and Mediterranean Sea food webs. While bulk SIA lacks the resolution to identify the geographic locations of these respective habitat types, prior tagging and chemical tracer data suggest that most Gulf of Mexico spawners traveled from western Atlantic shelf and slope foraging habitats (Dickhut et al. 2009; Galuardi et al. 2010; Wilson et al. 2015) while Mediterranean Sea spawners primarily used either local foraging habitats or similar habitats in the eastern Central Atlantic Ocean (Aranda et al. 2013; Fromentin and Lopuszanski 2014; Cermeño et al. 2015). Bulk SIA provides a cost-effective tool to infer large scale movements and habitat use of ABFT and other highly migratory marine predators. ABFT migration patterns and habitat use vary temporally (e.g., recent return to Nordic foraging habitats (Aarestrup et al. 2022)); a bulk SIA monitoring approach offers a way of tracking potential changes in habitat use over time. Future studies would benefit from the inclusion of additional chemical tracers and electronic tagging to complement bulk and compound-specific SIA.

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Authors contribution Conception and design were performed by JL, AW, and AR. Material preparation was performed by AR and JLV. Sample collection was performed by JLV. Data analysis was performed by JL. The manuscript was drafted by JL and edited by AW, JLV, and AR. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors have not disclosed any competing interests

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Data availability The datasets used in this manuscript are available from the corresponding author on request.

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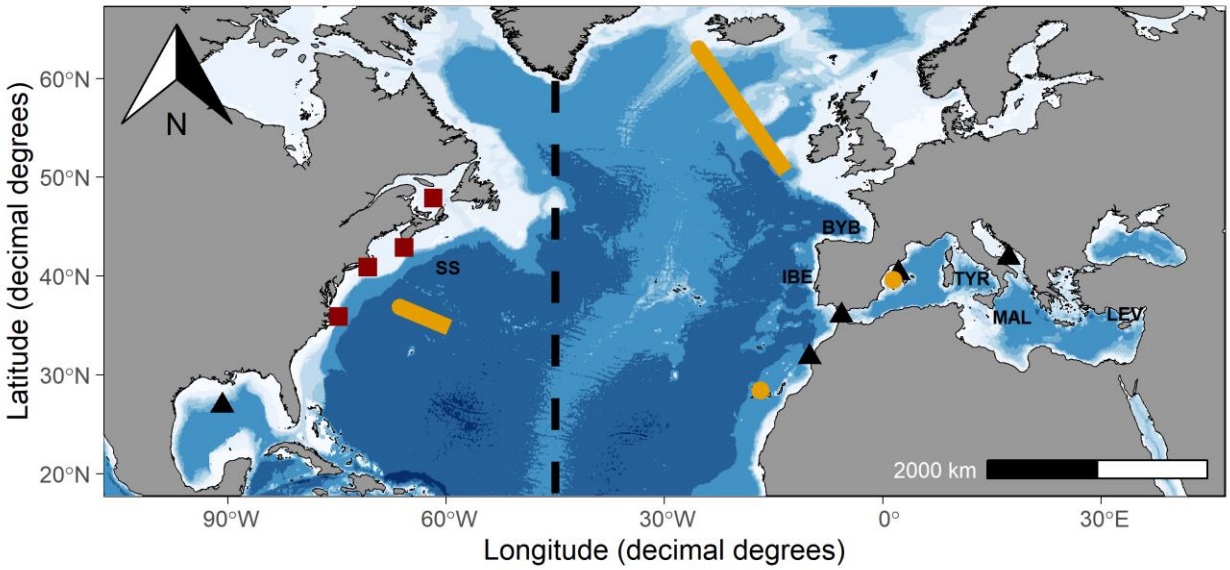
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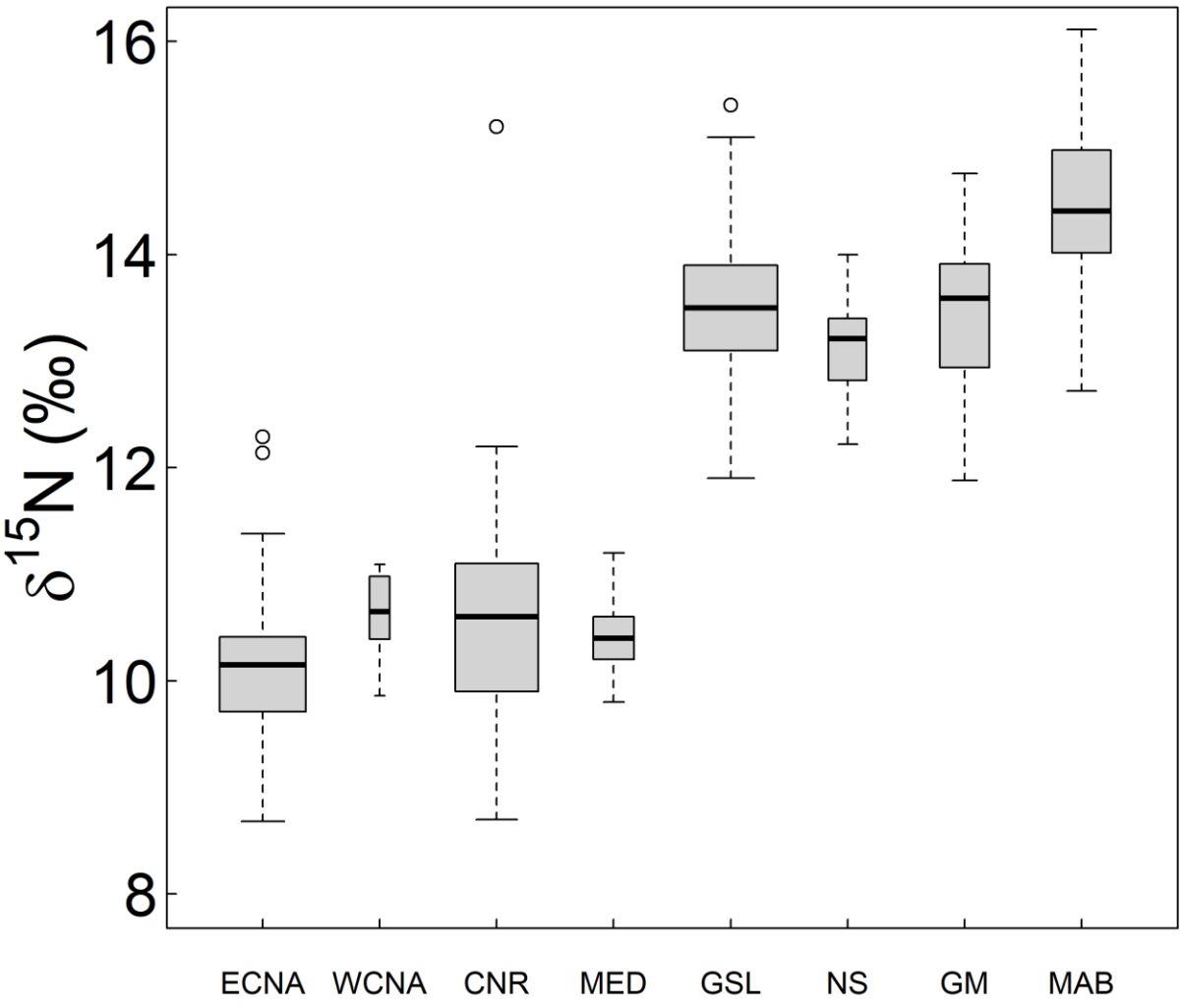
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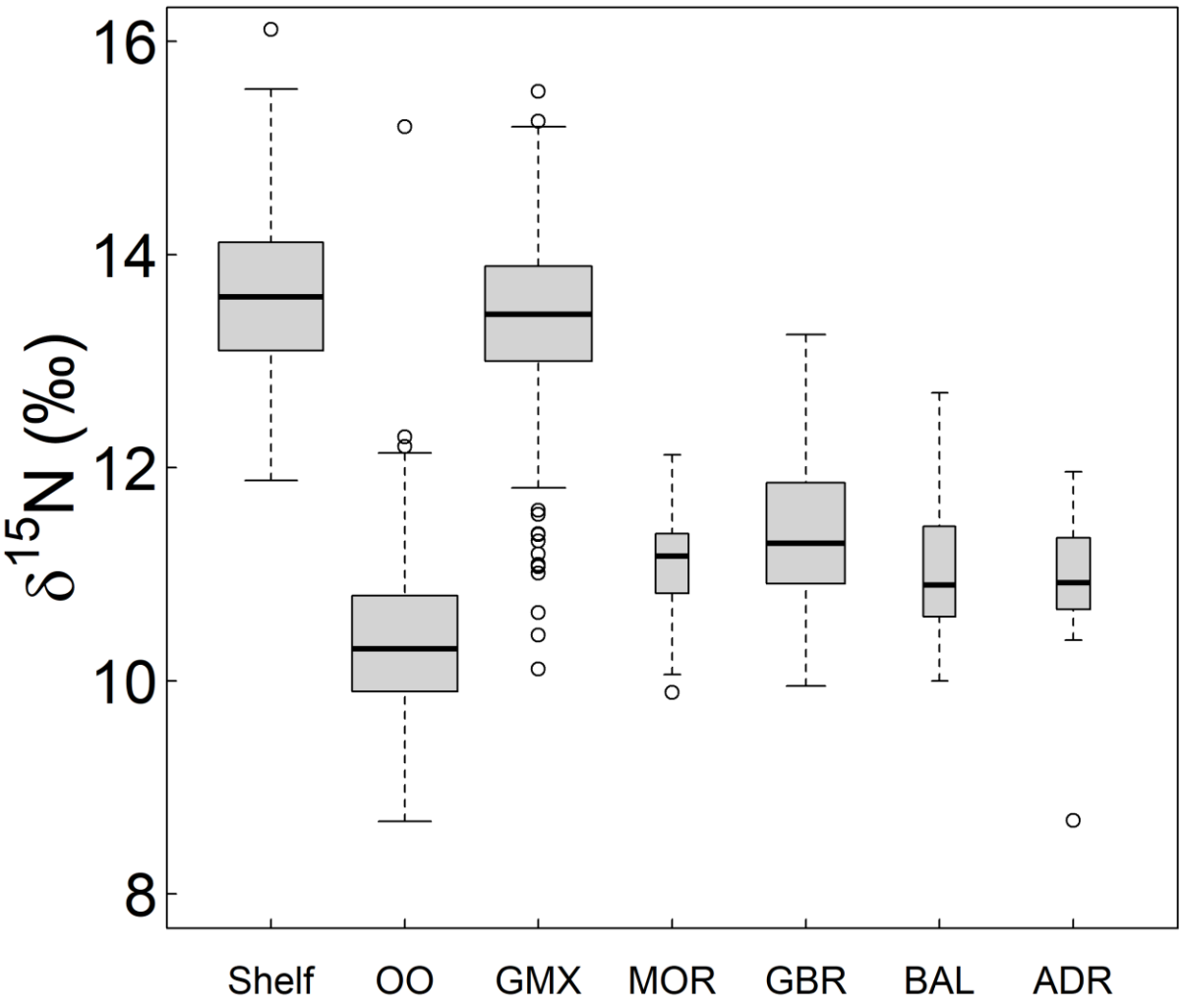
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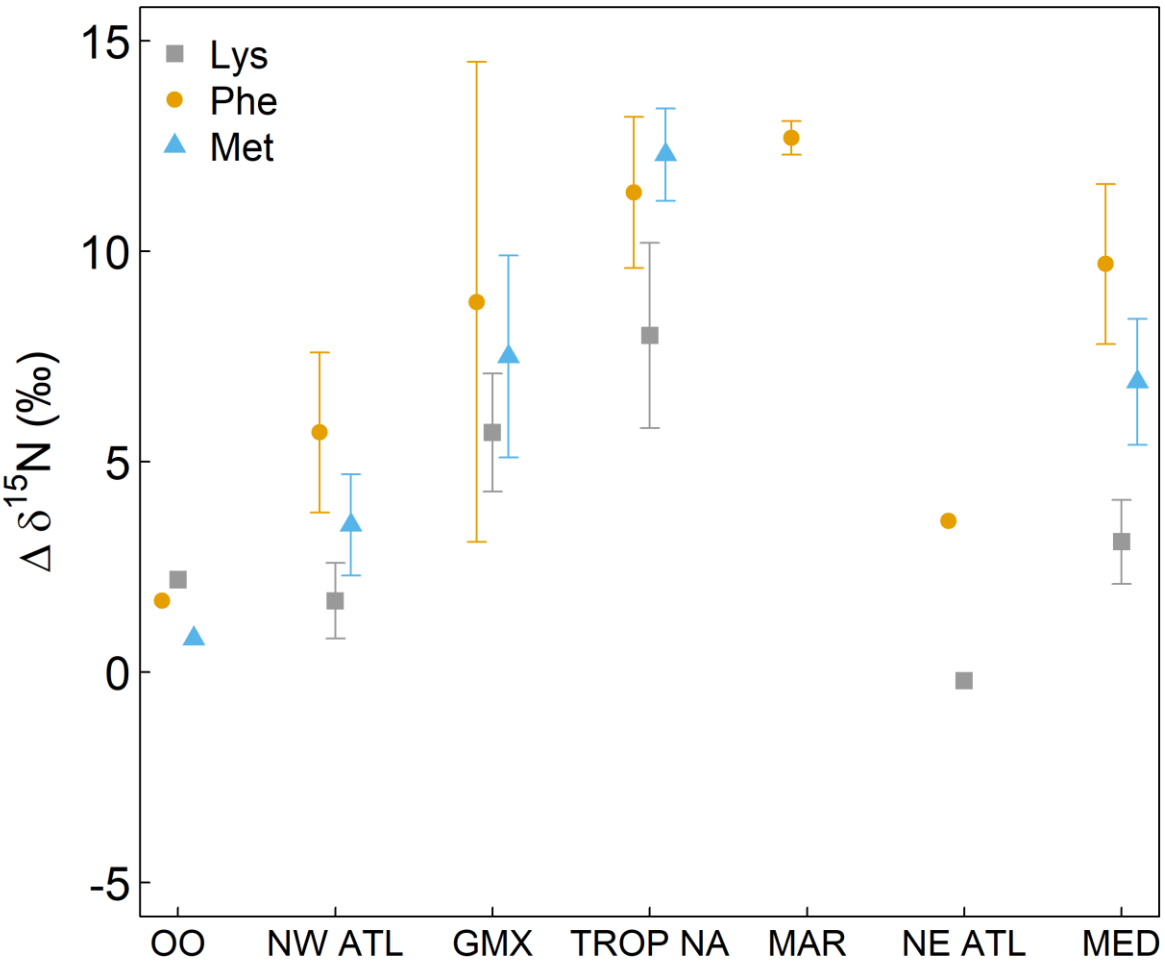

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Figure 1. Map showing collection locations of Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) used in the migration classification analysis. Green squares show locations of ABFT liver samples used to define previous shelf foraging (Gulf of St. Lawrence, Nova Scotia, Gulf of Maine, Mid-Atlantic Bight). Orange circles and lines show locations of ABFT liver samples used to define previous open ocean foraging (western Central Atlantic Ocean*, eastern Central Atlantic Ocean, Canary Islands, Mediterranean Sea*). Black triangles show spawning locations and migratory corridors to spawning locations where ABFT muscle samples were sampled to infer past movements using the liver training dataset (Gulf of Mexico, Morocco coast, Strait of Gibraltar, Balearic Sea, Adriatic Sea). *Swordfish (*Xiphias gladius*) were used as a proxy for ABFT in the western Central Atlantic Ocean and Mediterranean Sea.

Figure 2. Boxplots of bulk nitrogen stable isotope ratios ($\delta^{15}N$) of Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) liver tissue from open ocean (Eastern Central North Atlantic (ECNA), Western Central North Atlantic (WCNA), Canary Islands (CNR), and Mediterranean Sea (MED)) and western shelf (Gulf of St. Lawrence (GSL), Nova Scotia (NS), Gulf of Maine (GM) and Mid-Atlantic Bight (MAB)) foraging grounds. All samples collected from adult ABFT except WCNA and MED, which consist of adult swordfish (*Xiphias gladius*). Values were adjusted to reflect predicted equivalent white muscle $\delta^{15}N$ values based on an estimated offset of +0.8‰. The dark line of the boxed data represents the median and the box represents the interquartile range (IQR). Whiskers capture 1.5 IQR, and outliers are shown as circles. Variable box widths are proportional to sample size for each group.

Figure 3. Boxplots of bulk nitrogen stable isotope ratios ($\delta^{15}N$) of Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) liver tissue from shelf (Shelf) and open ocean (OO) foraging grounds and muscle tissue from the Gulf of Mexico (GMX), Morocco coast (MOR), Strait of Gibraltar (GBR), Balearic Sea (BAL), and Adriatic Sea (ADR). All samples collected from adult ABFT except open ocean samples from the western Central Atlantic Ocean and Mediterranean Sea, which consist of adult swordfish (*Xiphias gladius*). Liver values were adjusted to reflect predicted equivalent white muscle $\delta^{15}N$ values based on an estimated offset of +0.8‰. The dark line of the boxed data represents the median and the box represents the interquartile range (IQR). Whiskers capture 1.5 IQR, and outliers are shown as circles. Variable box widths are proportional to sample size for each group.

Figure 4. Mean ± SD Δ $\delta^{15}N$ (‰) offset of source amino acids for marine fauna from representative shelf and open ocean Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) foraging grounds in the Gulf of Mexico, Mediterranean Sea, and North Atlantic Ocean. All values were normalized to our Gulf of Mexico shelf migrant dataset based on estimates of trophic position for this ABFT group (TP=4.4) and each comparative faunal group with estimated diet-tissue discrimination factors of 0.1‰ (Phe), 0.4‰ (Met), and 0.8‰ (Lys).

Supplementary Figure S1. $\Delta\delta^{15}N$ (‰) offset of source amino acids for individual marine fauna from representative shelf and open ocean Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) foraging grounds in the Gulf of Mexico, Mediterranean Sea, and North Atlantic Ocean. All values were normalized to our Gulf of Mexico shelf migrant dataset based on estimates of trophic position for this ABFT group (TP=4.4) and each comparative faunal group with estimated diet-tissue discrimination factors of 0.1‰ (Phe), 0.4‰ (Met), and 0.8‰ (Lys). Individual datasets corresponding to each region are detailed in Supplementary Table 2.

Table 1. Summary of classification estimates and associated stable isotope ($\delta^{15}N$; ‰), carbon to nitrogen ratio (C:N), and length (straight fork length (SFL: cm) data for Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) on or approaching spawning habitat. Values are reported as means with standard deviations provided in parentheses.

Region	Year	Classification	Percent (%)	N	$\delta^{15}N$ (‰)	C:N	SFL (cm)
Gulf of Mexico	2007	Shelf	95.8	23	13.6 (0.6)	4.7 (2.0)	243 (24)
		Open Ocean	4.2	1	11.8	3.5	240
	2008	Shelf	91	31	13.6 (0.6)	4.4 (1.6)	247 (20)
		Open Ocean	9	3	11.0 (0.8)	16.5 (22.7)	252 (8)
	2009	Shelf	92.5	74	13.5 (0.7)	4.4 (1.6)	252 (18)
		Open Ocean	7.5	6	11.7 (0.4)	3.7 (0.3)	247 (11)
	2010	Shelf	89	33	13.5 (0.7)	4.0 (0.6)	247 (19)
		Open Ocean	11	4	11.0 (0.7)	6.7 (3.9)	237 (10)
	2011	Shelf	100	6	13.7 (0.6)	4.0 (0.6)	247 (16)

		Open Ocean	0	0	NA	NA	NA
	2012	Shelf	78	14	13.7	3.7	247
					(0.6)	(0.4)	(14)
		Open Ocean	22	4	11.7	3.7	244
					(0.2)	(0.8)	(19)
	2013	Shelf	89	25	13.6	4.9	237
					(0.7)	(1.8)	(17)
		Open Ocean	11	3	11.5	3.4	240
					(0.5)	(0.2)	(14)
	All	Shelf	91	233	13.5	4.6	245
					(0.6)	(1.9)	(20)
		Open Ocean	9	23	11.4	6.1	242
					(0.5)	(8.2)	(12)
Morocco	2010	Shelf	4	1	12.1	5.1	151
		Open Ocean	96	23	11.1	4.5	188
					(0.4)	(0.8)	(49)
Strait of Gibraltar	2009	Shelf	24	11	12.5	3.1	203
					(0.3)	(0.0)	(29)
		Open Ocean	76	34	11.5	3.2	205
					(0.3)	(0.1)	(19)
	2010	Shelf	6	3	12.3	3.9	198
					(0.4)	(0.6)	(9)
		Open Ocean	94	44	10.9	4.7	206

					(0.4)	(1.5)	(16)
	2011	Shelf	5	1	12.4	3.6	180
		Open Ocean	95	20	10.9	3.6	204
					(0.4)	(0.5)	(25)
	2012	Shelf	58	14	12.4	4.4	208
					(0.3)	(0.9)	(26)
		Open Ocean	42	10	11.5	4.7	220
					(0.4)	(1.1)	(23)
	All	Shelf	21	29	12.4	3.8	204
					(0.3)	(0.9)	(22)
		Open Ocean	79	108	11.1	4.0	207
					(0.5)	(1.2)	(22)
Balearic	2009	Shelf	9	2	12.5	3.9	171
Sea					(0.3)	(1.1)	(3)
		Open Ocean	91	21	10.9	3.8	189
					(0.5)	(0.8)	(28)
Adriatic	2010	Shelf	0	0	-	-	-
Sea		Open Ocean	100	25	10.8	3.9	194
					(0.6)	(1.0)	(32)