

ORIGIN AND POPULATION CONNECTIVITY OF YELLOWFIN
TUNA (*THUNNUS ALBACARES*) IN THE ATLANTIC OCEAN

A Dissertation

by

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ABSTRACT

Understanding the stock structure and population connectivity of migratory fishes is crucial to their effective conservation and management. Yellowfin tuna is a highly migratory species that is currently managed as a single panmictic stock in the Atlantic Ocean; however, uncertainty remains regarding their population structure in this region, particularly concerning the degree of mixing among spawning populations. Analysis of naturally occurring chemical markers in otoliths provides a valuable means to reconstruct a fish's environmental history and has proven to be an effective approach for examining the population structure of marine fishes. The purpose of this research was to use otolith chemistry (trace elements and stable isotopes) to address gaps in our knowledge regarding the connectivity and mixing of yellowfin tuna populations in the Atlantic Ocean. Objectives were to develop a baseline of chemical signatures for young-of-year (YOY) yellowfin tuna from all probable nursery areas in the Atlantic Ocean, estimate the origin of adult yellowfin tuna collected from multiple regional fisheries using this baseline, and assess interannual and age-specific variability in the contribution rates of each nursery to the Gulf of Mexico fishery. Results indicated that significant regional differences in chemical signatures existed for each year class of YOY yellowfin tuna in this study, indicating that the baseline of nursery signatures created here can serve as an effective tool for assigning older yellowfin tuna to their nursery of origin. Mixed-stock analysis revealed that all adult yellowfin tuna captured in the Bahamas originated in the Gulf of Mexico, while individuals from the Caribbean Sea and Cape

Verde primarily originated in eastern Atlantic nursery areas. Additionally, significant mixing was detected among yellowfin tuna in the Gulf of Mexico, as approximately half of the adults collected each year from this region were eastern migrants while the rest were local recruits. Thus, results from this study indicate that the eastern Atlantic may be an important source of adult yellowfin tuna to several regional fisheries (Cape Verde, Martinique, Gulf of Mexico); therefore, effective management of this critical nursery area may be key to ensuring the sustainability of the yellowfin tuna stock in the Atlantic Ocean.

DEDICATION

I dedicate this to my husband Michael, for all his love, encouragement, and support along the way. I also dedicate this to my baby girl Hazel, who we can't wait to meet.

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Contributors

Part 1, faculty committee recognition

This work was supervised by a dissertation committee consisting of Professor Jay Rooker (advisor) of the Department of Wildlife and Fisheries Sciences, Professor Anja Schulze and Professor Randall Davis of the Department of Marine Biology, and Professor Karl Kaiser of the Department of Marine Sciences.

Part 2, student/collaborator contributions

Samples used in Chapters 2-4 were provided by the Louisiana Department of Wildlife and Fisheries Sciences, the French Research Institute for Exploitation of the Sea (Antilles), AZTI Tecnalia, the Ministry of Fisheries and Aquaculture Development in the Republic of Ghana, and the Oceanographic Research Center of Dakar-Thiaroye. All other work conducted for the dissertation was completed by Larissa Kitchens under the advisement of Professor Jay Rooker of the Department of Wildlife and Fisheries Sciences.

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CHAPTER I

INTRODUCTION

Effective management and conservation of highly migratory species requires a thorough understanding of how distant populations are connected (Thorrold et al. 2001, Cowen et al. 2007). This is particularly true for tunas, as these pelagic predators often travel thousands of kilometers to feed and reproduce, usually crossing several lines of jurisdiction in the process (Block et al. 2005, Galuardi et al. 2010). As a result, management of these valuable natural resources generally requires collaboration among multiple international agencies. This is especially important for stocks that are comprised of multiple geographically distinct reproductive groups, as these groups are often exposed to vastly different fishing pressures that may disproportionately affect the overall population (Armstrong et al. 2013). For instance, heavy exploitation of a species' primary spawning group can be expected to have a significantly larger negative impact on a mixed stock than exploiting less productive spawning groups. Thus, improving our understanding of population structure and mixing rates is essential, as this will allow managers to protect each component group appropriately.

Yellowfin tuna is a highly migratory species distributed in tropical and subtropical waters worldwide. This species represents the second largest tuna fishery in the world, comprising >25% of the global tuna catch with more than 1 million tons captured annually (FIGIS 2015). Catches have been declining in the Atlantic Ocean since the late 1980's (ICCAT 2011), and according to the most recent stock assessment, the Atlantic

stock is now close to being overfished (ICCAT 2016). While yellowfin tuna are currently managed as one panmictic (mixed) stock in the Atlantic Ocean, this decision was primarily based on a limited number of trans-Atlantic tag recoveries and length distribution data (NMFS 2001). Further, the single stock concept has never been proven; thus, the true nature of the stock structure and connectivity of yellowfin tuna in the Atlantic Ocean remains unknown. In fact, it is likely that the population structure of yellowfin tuna is more complex than previously thought, as multiple geographically distinct spawning areas exist throughout the Atlantic Ocean (Arocha et al. 2001, ICCAT 2011). Regardless, the relative contribution of each of these spawning areas to the overall Atlantic population remains unknown, despite the fact that this information is crucial to the effective management and conservation of this species.

One well-established approach to examining the population structure (e.g., Jónsdóttir et al. 2006, Wells et al. 2015), natal origin (e.g., Rooker et al. 2008a, 2016), and movement (e.g., Wang et al. 2009, Baumann et al. 2015) of fishes involves analyzing the chemical composition of otoliths (ear stones). Otoliths accrete layers of calcium carbonate and protein on a daily basis (Campana and Nielson 1985) and elements are often incorporated into the otolith in relation to the physical and chemical characteristics of the surrounding environment (Campana 1999). Otoliths are metabolically inert structures, so all incorporated chemical markers are preserved (Campana and Nielson, 1985); as a result, the chemistry of otolith material deposited during the early juvenile stage serves as a natural marker of the individual's nursery of origin (Thorrold et al. 2001, Rooker et al. 2008a). Previous research has shown that trace elements and stable

isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) are highly effective natural tags in otoliths (Thorrold et al. 2001, Forrester and Swearer 2002, Gao et al. 2001), and recent studies have demonstrated that these natural chemical markers can be used to reliably predict the origin of tropical and temperate tunas (Wells et al. 2012, Rooker et al. 2014, 2016).

The purpose of this dissertation was to use otolith chemistry to evaluate the natal origin, mixing rates, and trans-ocean movement of yellowfin tuna in the Atlantic Ocean. To do this, I first developed a baseline dataset that characterizes the chemical signatures in otoliths of young-of-the-year (YOY) yellowfin tuna from all probable nursery areas in the Atlantic Ocean (Gulf of Guinea, Cape Verde, Caribbean Sea, and Gulf of Mexico). I then used the baseline of nursery signatures to determine the origin of sub-adult and adult yellowfin tuna collected throughout the Atlantic Ocean (Gulf of Mexico, Martinique, Bahamas, and Cape Verde). Finally, I used an expanded sample set to assess interannual and age-specific variability in the contribution rates of each nursery to the yellowfin tuna fishery in the Gulf of Mexico.

The overall objective of this study was to examine the population connectivity of yellowfin tuna in the Atlantic Ocean using natural markers linked to ambient physicochemical conditions of the ocean. The specific objectives of each chapter are outlined below.

Chapter II objectives were to: 1) develop a baseline of chemical signatures (stable isotopes and trace elements) in otoliths of young-of-year (YOY) yellowfin tuna collected from all probable nursery areas in the Atlantic Ocean (Gulf of Guinea, Cape Verde, Caribbean Sea, and Gulf of Mexico) and 2) examine interannual variability in chemical

signatures of YOY otoliths collected over a 3-year period (2013, 2014, 2015) to determine whether nursery signatures are stable across time.

Chapter III objectives were to: 1) characterize the chemical signatures in otolith cores (corresponding to the YOY period) of sub-adult and adult yellowfin tuna collected from four regional fisheries in the Atlantic Ocean (Gulf of Mexico, Martinique, Bahamas, and Cape Verde) and 2) compare chemical signatures in otolith cores of sub-adults and adults to the baseline of nursery signatures created in Chapter II to estimate nursery origin and evaluate population connectivity and mixing rates.

Chapter IV objectives were to: 1) evaluate interannual (2012 vs. 2013) and age-specific (age-1 vs. age-2) differences in the contribution rates of each nursery area to sub-adult and adult populations in the Gulf of Mexico and 2) analyze trace elements in life history transects to determine whether the timing of trans-ocean migrations from eastern Atlantic nurseries can be estimated using otolith chemistry.

CHAPTER II

DISCRIMINATING YELLOWFIN TUNA (*THUNNUS ALBACARES*) FROM NURSERY AREAS IN THE ATLANTIC OCEAN USING OTOLITH CHEMISTRY

Introduction

Yellowfin tuna (*Thunnus albacares*) is a valuable international resource and an important predator in the open-ocean ecosystem, yet heavy fishing pressure over the last few decades has caused drastic population declines in the Atlantic Ocean. In fact, according to the most recent stock assessment, yellowfin tuna in the Atlantic Ocean are very close to being overfished (ICCAT 2016). Not only are yellowfin tuna one of the main targets of pelagic longliners throughout the Atlantic Ocean, but both juvenile and adult fish are heavily exploited by purse-seine vessels in spawning and nursery areas in the eastern Atlantic Ocean (ICCAT 2011). Currently, little is known regarding the migratory behavior and population connectivity of yellowfin tuna in the Atlantic Ocean, even though this information is critical to the development of effective management strategies.

Multiple spawning areas exist for yellowfin tuna in the Atlantic Ocean, but the primary spawning area is thought to be in the eastern Atlantic Ocean, with production centered in the Gulf of Guinea (ICCAT 2011). Commercial landing and tagging data suggest that at approximately 1 to 2 years of age, individuals spawned in this region migrate across the Atlantic Ocean into U.S. waters, with some individuals entering into the Gulf of Mexico (Hazin 1993, Fonteneau and Soubrier 1996). Migration back to the

eastern Atlantic Ocean generally occurs once these fish reach maturity at about 3 years of age, although the degree of homing to natal sites is presently unknown (Fonteneau and Soubrier 1996, Arocha et al. 2001). It is presumed that east to west trans-Atlantic migrations are for feeding purposes and that return migrations (west to east) are for spawning; however, spawning has also been documented in the western Atlantic Ocean (Lang et al. 1994, Arocha et al. 2001). In fact, it has been suggested that there are at least three other major spawning areas in the Atlantic Ocean, including the eastern Caribbean Sea, Gulf of Mexico, and Cape Verde (Arocha et al. 2001, ICCAT 2011). While differences in size and spawning frequencies suggest that multiple spawning stocks exist, genetic studies have not found any evidence of significant heterogeneity of yellowfin tuna in the Atlantic Ocean (Scoles and Graves 1993, Ward et al. 1997, Talley-Farnham et al. 2004), indicating that at least some mixing occurs among spawning populations. Thus, additional research is necessary to determine the relative importance of different spawning areas and better understand the degree of population connectivity and mixing of yellowfin tuna in the Atlantic Ocean.

Several approaches have been developed to examine the migration ecology and population connectivity of pelagic fishes, including molecular genetics (Ward et al. 1997, Purcell and Edmands 2001), archival tags (Block et al. 2005, Hoolihan et al. 2011), and natural markers in hard parts (Rooker et al. 2008b, Rooker et al. 2014). Examining natural chemical markers in hard parts, especially in otoliths (ear stones), is a particularly effective and widely used technique in fisheries ecology (reviewed in Campana and Thorrold 2001 and Elsdon et al. 2008). Otoliths precipitate material as a

fish grows and elements are incorporated into the calcium carbonate-protein matrix in relation to concentrations in the surrounding seawater (Campana 1999). Material is not resorbed once deposited; therefore, incorporated chemical markers are preserved (Campana and Nielson, 1985). As a result, the chemical composition of otolith material deposited during the early juvenile stage serves as a natural marker of an individual's natal origin. Previous research has shown that trace elements and stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) are highly effective natural tags in fish otoliths (Thorrold et al. 2001, Forrester and Swearer 2002), and recent studies have demonstrated that these chemical markers can be used to reliably predict the origin of tropical and temperate tunas (Wells et al. 2012, Rooker et al. 2014, 2016).

The purpose of this study is to determine whether young-of-the-year (YOY) yellowfin tuna from different nursery areas in the Atlantic Ocean have distinct chemical signatures in their otoliths. If so, otolith chemistry could be used to retrace the origin of adult fish and determine the degree of stock mixing by yellowfin tuna from different production zones in the Atlantic Ocean. In this study, I create a comprehensive database of chemical signatures (trace elements and stable isotopes) for all putative nursery areas in the Atlantic Ocean (Gulf of Guinea, Cape Verde, Caribbean Sea, and Gulf of Mexico). Further, I evaluate the interannual variability in nursery-specific chemical signatures of YOY yellowfin tuna collected over a 3-year period (2013, 2014, 2015) to determine whether nursery signatures are stable across time. Ultimately, this information will help to determine natal origin and trans-oceanic migration patterns as well as population connectivity and mixing rates of yellowfin tuna in the Atlantic Ocean.

Methods

Sample collections

Young-of-the-year (YOY) yellowfin tuna were collected from 4 geographically distinct nursery areas in the Atlantic Ocean: 1) Gulf of Mexico, 2) Eastern Caribbean Sea (Martinique and Saint Lucia, hereafter referred to as Caribbean Sea), 3) Gulf of Guinea, and 4) Cape Verde (Fig. 1). YOY were collected over a 3-year period (2013-2015), and all specimens were captured either by hook and line or purse seine. Samples were collected across multiple years in each region to investigate interannual variability in nursery signatures. Further, within each nursery area, samples were collected on multiple dates and/or from multiple locations each year to account for natural variability in region-specific chemical signatures. For all samples collected, fork length (FL), capture date, and capture location were recorded. An effort was made to collect only the smallest juveniles available (<45 cm FL) to minimize the possibility that any large-scale movement occurred prior to sampling. Thus, specimens were considered to have been collected in the same region as their place of origin. While specimens of this size were not always available, the majority of yellowfin tuna collected (80%) were approximately 5 to 9 months of age and all fish were less than 1 year old (ca. <55 cm FL; based on the growth curve developed by Shuford et al. 2007).

Otolith preparation

Sagittal otoliths from fresh or frozen yellowfin tuna were extracted from the cranial cavity, cleansed of adhering tissue, rinsed with deionized water (DIH₂O), and stored dry in plastic vials. One otolith from each specimen was embedded in EpoFix

resin (Struers A/S) and sectioned using a low-speed ISOMET saw (Beuhler) to obtain a 1.5 mm section of the core of the otolith, following protocols described by Rooker et al. (2008a). Thin sections were mounted onto a glass slide using Crystalbond thermoplastic glue (SPI Supplies/Structure Probe Inc.) and polished using 0.3 mm MicroPolish Alumina Powder and 600-1200 grit silicone-carbide paper (Buehler). All otoliths were polished until the antistrostrum became transparent, which indicated that the core was exposed.

Trace element analysis

Trace element concentrations were measured using a laser ablation inductively coupled plasma mass spectrometer (LA-ICP-MS) at Texas A&M University (Galveston Campus). The system consists of an ultraviolet laser ablation unit (NWR 213, New Wave Research) connected to a XSeries II Thermo Scientific ICP-MS. Eight elements were measured in all otoliths: ^7Li , ^{24}Mg , ^{55}Mn , ^{59}Co , ^{65}Cu , ^{88}Sr , ^{137}Ba , ^{66}Zn . The element ^{44}Ca was also measured and was used as an internal standard to correct for variations in ablation yield among samples (Rooker et al. 2001); this element was assumed to be evenly distributed in otoliths at a concentration of 38%. Ablation occurred inside a sealed chamber, and ablated material was carried by helium gas (800 mL/min flow rate) to the ICP-MS where it was mixed with argon gas. Prior to ablation, the chamber was purged for 10 minutes to remove any gas or particle contamination. The National Institute of Standards and Technology (NIST) 614 standard was used to create calibration curves for each sample and monitor instrument drift (measured every 2 samples). Mean counts of a background reading taken before each ablation point was

used as the blank and was subtracted from the raw ion counts for each element. The laser was operated with a repetition rate of 10 Hz with a scan speed of 5 mm per second for all analyses. In order to remove any surface contamination, each sample spot was pre-ablated for approximately 10 seconds prior to analysis. Five replicate spots were ablated in the core region of the otolith, which contains material accreted within the first 3 months of life (Fig. 2A). Ablation spots were 50 μm in diameter and each spot was ablated by the laser for approximately 12 seconds. The first ablation spot was always placed at the otolith core (narrowest part of the section), followed by two spots on each side of the core. Trace element data from the five replicate ablation sites was averaged to create a composite signature for each individual yellowfin tuna. Samples from multiple capture locations were analyzed in the same runs to prevent any bias resulting from instrument drift (Hamer et al. 2003). The limit of detection (LOD) for each element was calculated as 3X the standard deviation (SD) of the blank signal. Trace element concentrations (E, ppb) were converted to element:Ca ratios ($\mu\text{mol/mol}$) based on the molar mass of each element (M, g/mol) standardized to ^{44}Ca concentrations:

$$element:Ca = \frac{E}{1000} \left(M \frac{0.38}{44} \right)^{-1}$$

Stable isotope analysis

A high-resolution mill (New Wave MicroMill System) was used to isolate material from the otolith cores of YOY yellowfin tuna for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis. After trace element analysis, otoliths were polished lightly (removing $\sim 30\text{-}50 \mu\text{m}$) until all ablation pits completely disappeared and the antirostrum was no longer visible, which allowed for all chemical analyses to be conducted on a single otolith. Similar to Wells et al.

(2012), a drill path was developed from otolith measurements of the 5 smallest YOY yellowfin tuna in our set of samples (24-30 cm fork length). This drill path covers the area of the otolith corresponding to the first 5-6 months of life (Fig. 2B). Otoliths were milled to a depth of ~770 μm (14 passes, 55 μm depth) using a 350 μm diameter carbide bit (Brasseler U.S.A.). Powdered core material was collected in weigh paper and sent to the Environmental Isotope Laboratory at the University of Arizona for stable isotope analysis, where otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was quantified using a gas-ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific) equipped with an automated carbonate preparation device (KIEL-III, Thermo Fisher Scientific). Isotopic ratio measurements were calibrated based on repeated measurements of NBS-18 and NBS-19 (National Bureau of Standards). Otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (‰) are expressed in standard delta (δ) notation as $^{13/12}\text{C}$ and $^{18/16}\text{O}$ ratios (R) relative to the Pee Dee Belemnite (PDB) scale after comparison with an in-house laboratory standard calibrated to PDB:

$$\delta_{\text{sample}} = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 10^3$$

Data analysis

Individual ages were calculated based on a published age-length curve for yellowfin tuna in the Atlantic Ocean (Shuford et al. 2007), and spawning dates were back-calculated from the date of capture. Individuals were assigned to one of three year classes based on their spawning dates. Those spawned from mid (June) 2012 to early (May) 2013 were assigned to the 2012 year class (hereafter “2012”). Similarly,

individuals spawned from mid-2013 to early-2014 were assigned to the 2013 year class (“2013”) and those spawned from mid-2014 to early-2015 were assigned to the 2014 year class (“2014”).

To determine whether the otolith chemistry of YOY yellowfin tuna varied spatially, multivariate analysis of variance (MANOVA) was used to test for differences in element:Ca ratios and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values among regions and year classes. Statistical significance was determined based on Pillai’s trace statistic because it is the most robust to violations of homogeneity of variance (Wilkinson et al. 1996). Univariate tests were conducted for each stable isotope and trace element using analysis of variance (ANOVA) and *a posteriori* differences among means were detected using Tukey’s honestly significant difference (HSD) test. Additionally, quadratic discriminant function analysis (QDFA) was performed to test the ability of trace element and stable isotope signatures to discriminate among the four nursery areas. QDFA is the preferred classification method because it does not require multivariate normality or assume homogeneity of the covariance matrices (McGarigal et al. 2000). Jackknifed cross-validation classification accuracies were calculated to estimate the success of classification to the regions in which the samples were collected. QDFAs were performed for each year class as well as a combined dataset including all year classes. The constituents most important in distinguishing yellowfin tuna from different nursery areas were identified through stepwise discrimination procedures for each QDFA and only significant variables were retained in the final model. Canonical variate coefficients were plotted to visualize the separation in chemical signatures among nursery areas. All

statistical analyses were performed using MYSTAT (SYSTAT Software) and JMP 13 (SAS Institute) and significance was determined at an α -level of 0.05.

Results

In total, otoliths from 238 YOY yellowfin tuna collected from 4 nursery regions were analyzed for trace elements and stable isotopes (Table 1). Otoliths were collected from all 4 nursery areas each year except in 2014, for which no samples could be obtained from the Gulf of Guinea. Sizes were similar across regions and years and the overall mean fork length of YOY yellowfin tuna used in this study was 38.6 cm (± 7.4 cm). Spawning dates ranged widely throughout the year for YOY from the Gulf of Guinea, Cape Verde, and Caribbean Sea, but the majority of YOY captured in the Gulf of Mexico were spawned during spring months (March-May). Concentrations of six trace elements examined (Li, Mn, Mg, Zn, Sr, Ba) were consistently above detection limits for all samples, and these elements (in addition to $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) were used in all subsequent analyses.

Several geographic trends in otolith element:Ca ratios were observed; however, region-specific chemical signatures also varied significantly among years (MANOVA, $p < .001$). For instance, otolith Li:Ca values showed significant regional differences in each year of this study (ANOVA, $p < 0.05$), but trends were not consistent across time. Eastern Atlantic Ocean samples were enriched in lithium relative to western Atlantic Ocean samples in 2013, while an opposite pattern was observed in 2014 (Fig. 3). Mg:Ca was significantly different among nursery areas for two out of the three years in the baseline (ANOVA, $p < 0.01$), with the highest Mg:Ca values observed in Caribbean Sea

samples in 2012 and 2014 ($484 \mu\text{mol mol}^{-1}$ and $463 \mu\text{mol mol}^{-1}$, respectively). Mn:Ca values of eastern Atlantic Ocean samples were nearly double those of other regions in 2013 (Gulf of Guinea: $6.2 \mu\text{mol mol}^{-1}$) and 2014 (Cape Verde: $6.0 \mu\text{mol mol}^{-1}$), though no significant regional differences were detected in 2012. Zn:Ca values in Gulf of Guinea samples were more than an order of magnitude higher than in all other regions investigated in 2013 (Gulf of Guinea: $119 \mu\text{mol mol}^{-1}$, other regions: $2.4\text{-}3.9 \mu\text{mol mol}^{-1}$); however, in 2012, Gulf of Guinea samples were much lower ($3.6 \mu\text{mol mol}^{-1}$) and were statistically similar to values in other areas. Sr:Ca also showed significant regional differences, with Gulf of Mexico Sr:Ca values distinct from Caribbean Sea samples in each year of this study (Tukey's HSD, $p < 0.05$). Ba:Ca varied regionally, though significant differences were only detected in 2012 and 2013 (ANOVA, $p < 0.01$). As with other elements, Ba:Ca patterns were not consistent across time, as Gulf of Mexico samples were significantly enriched in otolith Ba:Ca in 2012 ($1.0 \mu\text{mol mol}^{-1}$, Tukey's HSD, $p < 0.001$), while Gulf of Guinea samples exhibited the most enriched Ba:Ca values in 2013 ($0.9 \mu\text{mol mol}^{-1}$). In general, all but one or two element:Ca ratios were distinct among nursery areas each year (ANOVA, $p < 0.05$), and each element showed significant regional differences in at least one year of the baseline.

Regional variability was also observed in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of YOY yellowfin tuna otoliths (Fig. 4). Otolith $\delta^{13}\text{C}$ values were distinct among nursery areas for all three year classes (ANOVA, $p < 0.05$). In 2013 and 2014, YOY otoliths from Cape Verde and the Gulf of Mexico were depleted in $\delta^{13}\text{C}$ by more than 0.34‰ relative to Caribbean Sea and Gulf of Guinea; however, samples from the Gulf of Guinea were

significantly enriched relative to the Caribbean Sea in 2012 (Tukey's HSD, $p < 0.05$). A temporal effect was detected in $\delta^{13}\text{C}$ signatures, with all regions showing significant interannual variability (ANOVA, $p < 0.01$) except the Gulf of Mexico. Otolith $\delta^{13}\text{C}$ values of Cape Verde samples were depleted in 2013 (-10.22‰) relative to 2012 (-9.88‰) and 2014 (-9.70‰). In the Caribbean Sea, otolith $\delta^{13}\text{C}$ values became more enriched each year, with 2014 samples enriched by 0.64‰ compared to 2012. Geographic variability in $\delta^{18}\text{O}$ values were only observed in 2012, when Caribbean Sea samples were depleted by more than 0.25‰ relative to other nursery areas. No significant differences in otolith $\delta^{18}\text{O}$ were detected in 2013 and 2014, with regional mean differences less than 0.08‰. Interannual variability in $\delta^{18}\text{O}$ was detected in samples from two regions: Caribbean Sea (ANOVA, $p < 0.001$) and Gulf of Guinea (ANOVA, $p < 0.01$). In 2012, $\delta^{18}\text{O}$ values were enriched in the Gulf of Guinea (-1.55‰) relative to 2013 (1.75‰), while $\delta^{18}\text{O}$ values were depleted in the Caribbean Sea (-1.97‰) compared to 2013 (-1.66‰) and 2014 (-1.69‰). Despite the observed interannual variability, regional differences in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were maintained when 2012, 2013, and 2014 year classes were pooled (ANOVA, $\delta^{13}\text{C}$: $p < 0.001$, $\delta^{18}\text{O}$: $p < 0.01$), with samples from the Gulf of Guinea and Caribbean Sea generally exhibiting more enriched $\delta^{13}\text{C}$ values relative to Cape Verde and the Gulf of Mexico (Tukey's HSD, $p < 0.05$) and with Caribbean Sea samples showing more depleted $\delta^{18}\text{O}$ signatures relative to nurseries in the eastern Atlantic Ocean (Tukey's HSD, $p < 0.05$).

The elemental composition of YOY yellowfin tuna otoliths differed significantly among nursery areas in each year of this study (2012-2014: MANOVA, $p < .001$).

QDFAs parameterized with otolith element:Ca (Li, Mg, Mn, Zn, Sr, Ba) and stable isotope ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) values from 2012 YOY indicated an overall classification success (jackknifed) of 64% to the four nurseries (Fig. 5), with an expected classification success of 25% based on random assignment. Particularly high classification success was observed for samples from the Caribbean Sea (78%), Gulf of Mexico (75%), and Cape Verde (74%) in 2012. Some overlap was observed between Cape Verde and Gulf of Guinea signatures; thus, an additional QDFA was performed using otolith chemistry data from both of these areas combined. By using the combined dataset, classification success increased from 64% to 79%, with 87% of samples correctly assigned to the eastern Atlantic Ocean (Cape Verde + Gulf of Guinea). In 2013, the overall classification success to the four nursery areas was 78%, with 100% of samples correctly assigned to the Gulf of Guinea and 80% correctly assigned to Cape Verde. Although samples were not available from one region (Gulf of Guinea) in 2014, the overall classification success was high that year (observed: 85%, expected: 33%), with 90% and 86% correctly assigned to the Caribbean Sea and Cape Verde, respectively. When data from 2012, 2013, and 2014 year classes were pooled, regional differences in signatures were still observed (MANOVA: $p < 0.001$, QDFA classification success: 66%); however, region-specific classification success was relatively low for the Gulf of Guinea (44%) and the Gulf of Mexico (43%). An additional QDFA was performed to determine whether chemical signatures in otoliths were different for yellowfin tuna from the eastern Atlantic Ocean (Cape Verde + Gulf of Guinea) and western Atlantic Ocean (Gulf of Mexico + Caribbean Sea); overall jackknifed classification success based on eastern and

western nursery areas was consistently high, with 78%, 84% and 89% of samples successfully classified in 2012, 2013, and 2014, respectively.

In 2012, $\delta^{18}\text{O}$, Ba:Ca, and Mg:Ca were the three most significant variables in the QDFA ($p < 0.001$), providing clear separation among Cape Verde/Gulf of Guinea, Gulf of Mexico, and Caribbean Sea samples, respectively (Fig. 5). Regional discrimination in 2013 was primarily influenced by Sr:Ca, Ba:Ca, and $\delta^{13}\text{C}$. The QDFA plot indicates that Sr:Ca played an important role in distinguishing Gulf of Guinea samples in 2013, which is expected given the high Sr:Ca values in the Gulf of Guinea (mean: $3442 \mu\text{mol mol}^{-1}$) relative to other areas investigated that year (Cape Verde: $2306 \mu\text{mol mol}^{-1}$, Gulf of Mexico: $2398 \mu\text{mol mol}^{-1}$, Caribbean Sea: $2143 \mu\text{mol mol}^{-1}$). In 2014, the strongest regional differences were observed in otolith Mn:Ca, Sr:Ca, and Li:Ca ($p < 0.001$), and these three elements alone provided clear separation among nursery areas. Thus, otolith Sr:Ca was a significant variable in QDFAs for each year class ($p < 0.001$) and was the only element to be included in all three models. Li:Ca and Ba:Ca were significant in two out of the three models, and all other elements ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Mg:Ca, Mn:Ca, Zn:Ca) were only retained in QDFAs for one year class.

Additional QDFAs were performed to determine whether stable isotopes or trace elements alone would be effective in discriminating yellowfin tuna from nursery areas in the Atlantic Ocean. QDFAs based on stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) yielded low overall classification success rates, ranging from 47% (2012) to 52% (2013). Stable isotopes were most successful in distinguishing Caribbean Sea samples, with classification success rates of 65% (2014) to 74% (2012); however, these isotopes were not useful for

classifying Gulf of Mexico samples (2012: 0%, 2013: 25%, 2014: 0%). Trace elements provided higher classification success rates than stable isotopes alone (2012: 57%, 2013: 78%, 2014: 85%). In fact, overall classification success rates for trace elements alone were identical to QDFAs including the full dataset in 2013 and 2014. However, classification success improved with the addition of stable isotope data in 2012 (57% vs 64%) and $\delta^{13}\text{C}$ was a significant factor in the 2013 QDFA, suggesting that combining both chemical markers is a more effective method for discriminating yellowfin tuna from different nursery areas in the Atlantic Ocean.

Discussion

Regional differences in otolith chemistry were detected for YOY yellowfin tuna collected from four nursery areas in the Atlantic Ocean using multiple tracers. Element:Ca ratios provided the highest discriminatory power among nursery areas and usually matched expected patterns based on local environmental conditions. In particular, otolith Sr:Ca showed significant variability among regions and was an influential variable in QDFAs for each year class of YOY yellowfin tuna. Several studies have shown that strontium concentrations in otoliths generally exhibit a positive relationship with ambient salinity (Limburg 1995, Secor and Rooker 2000, Zimmerman 2005). Mean sea surface salinities (calculated from cumulative sea surface salinity data from HYCOM+NCODA Global 1/12 Degree Analysis) were relatively homogenous across regions, with Cape Verde and the Gulf of Mexico exhibiting only slightly higher salinities (35.7 ± 0.3 and 35.5 ± 0.5 , respectively) than sampling locations in the Caribbean Sea (35.2 ± 0.2) and Gulf of Guinea (35.0 ± 0.3). Thus, variability in otolith

Sr:Ca appears to have been influenced more by local climate events rather than large-scale regional differences. For example, countries bordering the Gulf of Guinea experienced much higher than average air temperatures during the spring and summer of 2014 (NOAA 2015); increased salinities were also observed in the Gulf of Guinea during this time period relative to previous years (2012, 2013), likely as a result of increased evaporation rates due to higher temperatures. This would have affected the 2013 year class (the majority of which were spawned from Nov 2013-Jan 2014), as these individuals would have been less than 6 months old at the time of capture, thus explaining the significantly higher Sr:Ca values observed in Gulf of Guinea samples that year. Otolith Li:Ca, which also tends to exhibit a positive relationship with salinity (Hicks et al. 2010, Sturrock et al. 2014), followed similar patterns as Sr:Ca, with peak Li:Ca and Sr:Ca values both occurring in the same regions each year (2012: Caribbean Sea, 2013: Gulf of Guinea, 2014: Caribbean Sea). Barium exhibits a nutrient-type distribution in seawater and is typically found in higher concentrations in coastal regions or in areas of riverine input (Coffey et al. 1997, Elsdon and Gillanders 2002). In late 2012 and early 2013, discharge from the Mississippi River into the Gulf of Mexico was 20% higher than in other years examined (USACE 2016). Most of the YOY yellowfin tuna collected from this region were captured in the vicinity of the Mississippi River plume and would therefore have been impacted by variability in river runoff; thus, increased freshwater discharge during that time period may be responsible for the significantly enriched otolith Ba:Ca values observed in Gulf of Mexico samples in the 2012 year class relative to other regions/years.

Significant regional differences were also detected in manganese, magnesium, and zinc concentrations in YOY yellowfin tuna otoliths. Atmospheric dust is a major source of manganese in seawater (Statham and Chester 1988, Guieu et al. 1994), and peak manganese concentrations in the Atlantic Ocean typically occur off the west coast of Africa near 5-20°N where dust deposition from the Sahara Desert is greatest (Statham et al. 1998, Chester 1990). As expected, the highest mean otolith Mn:Ca values were observed in samples from the Gulf of Guinea (2013: $6.2 \mu\text{mol mol}^{-1}$) and Cape Verde (2014: $6.0 \mu\text{mol mol}^{-1}$). Increased dust deposition in 2013 in the Gulf of Guinea would likely have increased primary productivity in these regions (due to increased nutrient input), which correlates well with the increased Ba:Ca values observed in these regions. While the relationship between otolith Mg:Ca and environmental conditions remains unclear, it is thought that magnesium uptake rates increase in warmer waters, potentially as a function of increased otolith precipitation and somatic growth (Martin and Thorrold, 2005). In support of this, enriched otolith Mg:Ca values were observed in Caribbean Sea samples each year, which was the region with the warmest mean sea surface temperature ($27.8^\circ \pm 0.2$, based on sea surface temperature data from HYCOM+NCODA Global 1/12 Degree Analysis) relative to other regions examined (Cape Verde: $23.9^\circ \pm 1.6$, Gulf of Guinea: $26.8^\circ \pm 0.9$, Gulf of Mexico: $25.4^\circ \pm 0.8$). Zinc, a physiologically active metal, is typically bound to protein and otolith Zn:Ca values do not necessarily reflect zinc concentrations in the surrounding seawater (Campana 1999, Miller et al. 2006). Instead, dietary uptake is thought to be the primary route through which zinc accumulates in otoliths (Ranaldi and Gagnon, 2008). Therefore, the highly enriched Zn:Ca values

observed in Gulf of Guinea samples in 2013 are most likely due to a shift in the zinc concentrations of prey items rather than any change in seawater concentrations.

Although zinc may not be a reliable indicator of water mass residency, it can nonetheless be a useful discriminator of populations that have unique dietary histories.

Otolith $\delta^{13}\text{C}$ values also differed significantly among nursery regions for all three year classes. Previous research has shown that otolith $\delta^{13}\text{C}$ values can be influenced by $\delta^{13}\text{C}$ in dissolved inorganic carbon (DIC) in seawater (Thorrold et al. 1997, Solomon et al. 2006). In equatorial upwelling zones, DIC in surface waters tends to become enriched in $\delta^{13}\text{C}$ due to air-sea gas exchange (Lynch-Stieglitz et al. 1995). The Gulf of Guinea (in the equatorial Atlantic Ocean) is characterized by an extensive seasonal upwelling system (Bakun 1978), and intense upwelling generally occurs from July to September (Roy 1995), which is when most of YOY yellowfin tuna collected from this region were approximately 1-6 months of age. Therefore, regional upwelling is likely responsible for the significantly enriched otolith $\delta^{13}\text{C}$ values observed in Gulf of Guinea samples.

Otolith $\delta^{13}\text{C}$ matched patterns of global seawater $\delta^{13}\text{C}_{\text{DIC}}$, with the Gulf of Guinea exhibiting the most enriched $\delta^{13}\text{C}$ values relative to other nursery areas in the Atlantic Ocean (McMahon et al. 2013). Equatorial upwelling also occurs in the Caribbean Sea along the northern coast of Venezuela, though this upwelling system is not as extensive as in the Gulf of Guinea (Muller-Karger et al. 2004). Thus, seasonal equatorial upwelling may also be the reason that otolith $\delta^{13}\text{C}$ values in Caribbean samples were enriched compared to northern nursery areas (Cape Verde and the Gulf of Mexico) in 2013 and 2014. Interannual variability in otolith $\delta^{13}\text{C}$ values was observed for most

regions surveyed, potentially due to variability in the intensity and/or timing of seasonal upwelling. However, temporal differences in otolith $\delta^{13}\text{C}$ values could be due to changes in diet or metabolism, as these factors are also known to influence otolith $\delta^{13}\text{C}$ (Høie et al. 2003). Regardless, regional differences in otolith $\delta^{13}\text{C}$ were still detected when 2012, 2013, and 2014 year classes were pooled, indicating that geographic variability was stronger than temporal variability in otolith $\delta^{13}\text{C}$ values.

Regional variation in otolith $\delta^{18}\text{O}$ was observed for YOY yellowfin tuna, though significant differences were only detected in 2012. $\delta^{18}\text{O}$ values of both seawater and carbonates are predictably linked to salinity and sea surface temperature, becoming more depleted as temperature increases (Thorrold et al. 1997, Høie et al. 2004) and as salinity decreases (Elsdon and Gillanders 2002, Kerr et al. 2007). Warmest sea surface temperatures and lowest salinities were observed in the Caribbean Sea sampling location, and as expected, otolith $\delta^{18}\text{O}$ values were significantly depleted in this region in 2012 relative to all other regions. However, temporal variability in otolith $\delta^{18}\text{O}$ was observed in the Caribbean Sea sample; sea surface temperatures decreased and salinities increased in 2013 (35.5°C, 27.8) and 2014 (35.2°C, 27.7) relative to 2012 (35°C, 29), which resulted in enriched otolith $\delta^{18}\text{O}$ values and less regional discrimination for these year classes (2013, 2014: ANOVA, $p > 0.05$). Interannual variability was also observed in Gulf of Guinea otolith $\delta^{18}\text{O}$ values, with significantly depleted otolith $\delta^{18}\text{O}$ values observed in the Gulf of Guinea in 2013 relative to 2012. As previously discussed, this region experienced much higher than normal temperatures in the spring/summer of 2014 (affecting the signatures of the 2013 year class), which likely explains the depleted

otolith $\delta^{18}\text{O}$ values observed for that year class. Despite this, regional differences were still observed when data from the three year classes were pooled, indicating that interannual variability may not be strong enough to outweigh geographical differences in otolith $\delta^{18}\text{O}$. Similar to $\delta^{13}\text{C}$, otolith $\delta^{18}\text{O}$ values followed the same pattern observed in isoscapes developed from global seawater $\delta^{18}\text{O}$ values, with lowest $\delta^{18}\text{O}$ values occurring in the Caribbean Sea relative to other nursery areas in the Atlantic Ocean (Schmidt et al. 1999, McMahon et al. 2013).

Regional variability in otolith chemistry resulted in the successful classification of YOY yellowfin tuna to four nursery areas in the Atlantic Ocean, with classification accuracies ranging from 64-85%. Classification accuracies in this study were similar to those reported for yellowfin tuna in the Pacific Ocean (Wells et al. 2012, Rooker et al. 2016). Classification success was lowest in 2012 (64%) due to overlap between Gulf of Guinea and Cape Verde signatures, which resulted in a greater number of misclassified individuals; however, classification success improved significantly (79%) when Gulf of Guinea and Cape Verde signatures were combined, with strong separation observed among Gulf of Mexico, Caribbean Sea, and eastern Atlantic Ocean nurseries. Success rates remained high when both eastern Atlantic (Cape Verde and Gulf of Guinea) and western Atlantic (Gulf of Mexico and Caribbean Sea) regions were pooled, suggesting that these chemical signatures are effective for detecting trans-Atlantic migrations of yellowfin tuna. Combining the four nursery signatures from all three years resulted in modest overall classification success (66%); however, region-specific classification success was less than 50% for certain regions (Gulf of Mexico and Gulf of Guinea),

highlighting the need to age-class match adult yellowfin tuna to the appropriate baseline year when predicting nursery origin.

The majority of studies involving nursery discrimination of tunas and other pelagic fishes have primarily utilized stable isotope ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) signatures in otoliths (Wells et al. 2010, Wells et al. 2012, Rooker et al. 2014) rather than trace elements combined with stable isotopes. To test the effectiveness of using stable isotopes alone in this study, QDFAs were run for each year class using only $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures. Results showed that classification success decreased significantly (by up to 34%) when only stable isotopes were included in the model; this was particularly true for Gulf of Mexico samples, for which classification successes were no higher than predicted success based on chance alone. Conversely, QDFAs using only trace elements provided success rates similar to (but slightly lower than) models using both classes of tracers. A recent study tested the effectiveness of using otolith trace elements vs. stable isotopes to discriminate among nursery areas of yellowfin tuna in the Pacific Ocean, and contrary to findings in the present study, adding trace element data to the baseline of stable isotope signatures did not significantly improve classification success (Rooker et al. 2016). Thus, while stable isotopes alone may be sufficient for nursery discrimination of yellowfin tuna in the Pacific Ocean, trace elements proved to be significantly more effective for discriminating among nursery areas in the Atlantic Ocean. It is possible that differences in classification success between the two types of tracers could be due to the fact that a smaller area of the otolith was sampled for trace elements than stable isotopes. While the portion of the otolith analyzed for trace elements corresponds to ~3 months,

stable isotope signatures encompassed the first 5-6 months of life. Thus, decreased resolution using stable isotopes alone could be due to increased movement of individuals after 3 months of age. Regardless, all tracers indicated separation among the four nursery regions for at least one year class, and strongest discriminatory power was obtained by combining trace element and stable isotope data.

In summary, otolith chemistry proved to be useful for discriminating YOY yellowfin tuna from the four major nursery areas in the Atlantic Ocean. Chemical signatures (otolith element:Ca, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of YOY yellowfin tuna varied significantly among regions and classification success was high, indicating that these tracers can be used in future studies to determine the natal origin of sub-adult and adult yellowfin tuna in the Atlantic Ocean. However, interannual variability in otolith trace elements and stable isotopes was also detected, highlighting the importance of age-class matching when sourcing adults using the baseline of nursery signatures. Ultimately, baseline signatures for YOY yellowfin tuna developed in this study can be used to elucidate trans-oceanic migration patterns and evaluate population connectivity and mixing rates of this species. As a result, fundamental questions regarding the stock structure of yellowfin tuna in the Atlantic Ocean may soon be resolved in future otolith-based studies.

CHAPTER III

ORIGIN AND POPULATION CONNECTIVITY OF YELLOWFIN TUNA IN THE ATLANTIC OCEAN REVEALED BY OTOLITH CHEMISTRY

Introduction

Understanding connectivity or exchange among geographically separated groups is pivotal to the effective management of highly migratory species because connectivity patterns play a major role in determining population replenishment and persistence (Thorrold et al. 2001, Cowen et al. 2007). Management of migratory species such as tunas is complicated by the fact that populations often have widespread distributions that cross several lines of jurisdiction and, as a result, successful management generally requires collaboration among multiple international agencies (Rooker et al. 2008b). Further, mixing among populations can bias stock assessments and impede management efforts, particularly if populations differ greatly in abundance (Kerr et al. 2016a). Harvest of mixed stocks also creates the potential for overexploiting less productive spawning components while more productive spawning groups remain underexploited (Kerr et al. 2016b). Thus, the relative contribution of different production zones to mixed stock fisheries needs to be assessed, as this will allow resource managers to identify and protect the key nursery areas that are most important in supporting these populations.

Yellowfin tuna (*Thunnus albacares*) is a highly migratory species found throughout tropical and subtropical regions of the world's oceans. This economically

valuable species supports commercial and recreational fisheries worldwide and constitutes the second largest tuna fishery in the world (FIGIS, 2015). Extensive fisheries for yellowfin tuna exist throughout the Atlantic Ocean, including in the Gulf of Mexico, Caribbean Sea, mid-Atlantic Bight, and eastern tropical Atlantic Ocean (Cape Verde, Gulf of Guinea). However, the degree of mixing and relative value of different nursery areas to the Atlantic stock remains unknown. According to the most recent stock assessment, the Atlantic yellowfin tuna stock is now very close to being overfished (ICCAT 2016); thus, a better understanding of natal origin and connectivity is urgently needed in order to protect the main nursery areas that support these important fisheries and ensure that yellowfin tuna in the Atlantic Ocean are sustainably managed.

It is well known that tunas are capable of travelling long distances, and tagging and length frequency data from the Atlantic Ocean has shown that yellowfin tuna regularly make massive trans-ocean migrations (Bard 1993, Ortiz 2001). Ortiz (2001) found that the majority of yellowfin tuna tagged in the western Atlantic Ocean (Gulf of Mexico, mid-Atlantic Bight) were later recaptured in the eastern Atlantic Ocean (Cape Verde, Gulf of Guinea). Further, genetic studies have failed to find any significant heterogeneity among yellowfin tuna populations, suggesting that some mixing occurs in the Atlantic Ocean (Scoles and Graves 1993, Ward et al. 1997, Talley-Farnham et al. 2004). This has led resource managers to assume that all yellowfin tuna in the Atlantic Ocean are part of a single ocean-wide stock (NMFS 2001). However, the existence of multiple geographically distinct spawning areas throughout the Atlantic Ocean indicates that the population structure of yellowfin tuna is likely much more complex than

previously assumed, with subpopulations potentially exhibiting different migratory behaviors.

In this study, the stock structure, mixing, and trans-ocean movement of yellowfin tuna in the Atlantic Ocean is characterized using natural chemical markers in otoliths (ear stones). Chemical analysis of otoliths has proven to be a reliable method for tracing movement of temperate (Rooker et al. 2014, Baumann et al. 2015) and tropical (Wells et al. 2012, Rooker et al. 2016) tunas. These metabolically inert structures continuously accrete layers of calcium carbonate and protein throughout an individual's life, and chemical markers (trace elements and stable isotopes) become incorporated into the aragonite matrix in relation to concentrations in the surrounding seawater (Campana 1999). Thus, otolith material deposited during the juvenile stage (i.e., the "core" of the otolith) serves as a natural marker of the individual's place of origin (Thorrold et al. 2001, Rooker et al. 2008a). In a previous study, a baseline of chemical signatures was created from otoliths of juvenile yellowfin tuna from all of the major nursery areas in the Atlantic Ocean (Chapter II). The present study uses this baseline to identify the nursery origin of sub-adult and adult yellowfin tuna collected from four regional fisheries in the Atlantic Ocean (Gulf of Mexico, Cape Verde, Martinique, and Bahamas).

Methods

Sample collections and otolith preparation

Sub-adult and adult yellowfin tuna (age-1 to age-3) were collected from 2014-2016 from 4 geographically distinct regions in the Atlantic Ocean: 1) Gulf of Mexico (Venice, Louisiana), 2) Cape Verde, 3) Martinique, and 4) Bahamas. Yellowfin tuna

from the Gulf of Mexico and Bahamas were captured via hook and line by recreational fishermen, while samples from Cape Verde and Martinique were collected by observers on commercial fishing vessels. Fork length (FL), capture date, and capture location were recorded for all samples collected (Table 2). Individual ages were calculated based on published age-length curves for yellowfin tuna in the Atlantic Ocean (Shuford et al. 2007, Driggers et al. 1999), and spawning dates were back-calculated from the date of capture.

Sagittal otoliths were extracted from the brain cavity of fresh or frozen fish and cleansed of adhering tissue in deionized water. After drying, one sagittal otolith from each fish was embedded in Struers EpoFix resin. Embedded otoliths were cut into 1.5 mm sections using a low-speed ISOMET saw (Buehler) following protocols described by Rooker et al. (2008a); each section included the core of the otolith, which represents the early life period. Sections were then attached to glass slides using Crystalbond thermoplastic glue (SPI Supplies/Structure Probe Inc.) and polished to the core using 0.3 mm MicroPolish Alumina Powder and 600-1200 grit silicone-carbide paper (Buehler).

Chemical analysis

Otolith cores were analyzed to determine trace element and stable isotope concentrations following methods described in Chapter II. Elemental chemistry was determined using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Specifically, otolith cores were analyzed for six elements (${}^7\text{Li}$, ${}^{24}\text{Mg}$, ${}^{55}\text{Mn}$, ${}^{88}\text{Sr}$, ${}^{137}\text{Ba}$, and ${}^{66}\text{Zn}$) at Texas A&M University (Galveston Campus) using an ultraviolet laser ablation unit (NWR 213, New Wave Research) connected to an XSeries II Thermo

Scientific ICP-MS. Ablation diameters were 50 μm , and 5 spots were ablated near the core for each sample. The first spot was placed at the core (narrowest part of the otolith) and two equally spaced spots were placed on each side of the core (Figure 6A). The portion of the otolith analyzed for trace elements included material deposited within approximately the first 3 months of life, and the mean of the 5 ablation spots was used for all statistical analyses. Ablation occurred inside a sealed chamber and ablated material was carried by helium gas (800mL/min flow rate) to the ICP-MS where it was mixed with argon gas. All spots were pre-ablated for 10 seconds prior to analysis to remove any surface contamination and then ablated for 12 seconds during quantification. The National Institute of Standards and Technology (NIST) 614 standard was used to create calibration curves for each sample and monitor instrument drift (measured every 2 samples), and ^{44}Ca (measured for each ablation spot) was used as an internal standard to correct for variations in ablation yield. Blank-corrected ion counts were converted to element:Ca ratios ($\mu\text{mol/mol}$) based on the molar mass of each element (g/mol) standardized to ^{44}Ca concentrations.

Otoliths were lightly polished after trace element analysis to remove all ablation spots, and stable isotope concentrations ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) were then measured in the cores of the same otoliths. Otolith cores were isolated and powdered using a high-resolution mill (New Wave MicroMill System). A drill path encompassing material accreted within the first 5-6 months of life (described in Chapter II) was used for each sample (Figure 6B), and otolith material was powdered by running a 350 μm carbide bit (Brasseler) over series of drill passes until a depth of approximately 770 μm was reached. Powdered

material was collected in weigh paper and sent to the Environmental Isotope Laboratory at the University of Arizona, where $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was measured using a gas-ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific) equipped with an automated carbonate preparation device (KIEL-III, Thermo Fisher Scientific). Isotope ratios were calibrated based on repeated measurements of NBS-18 and NBS-19 (National Bureau of Standards), and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are reported relative to the Pee Dee Belemnite (PDB) scale after comparison with an in-house laboratory standard calibrated to PDB.

Data analysis

Nursery origin of sub-adult and adult yellowfin tuna from each of the four regions was estimated by comparing core element:Ca and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values with a baseline of chemical signatures in the otoliths of young-of-year (YOY) yellowfin tuna from putative nursery areas in the Atlantic Ocean (described in Chapter II). Core signatures of sub-adults and adults from each region were age-class matched with the appropriate year class in the YOY baseline to eliminate any effects of interannual variability in environmental chemistry on estimates of nursery origin. Region-specific nursery assignments were determined using direct maximum-likelihood estimation (MLE) and maximum classification likelihood (MCL) within HISEA, a mixed-stock analysis program (Millar, 1990). HISEA was run under bootstrap mode with 500 simulations to provide a measure of variability around the estimated proportions.

Results

A total of 135 sub-adult and adult yellowfin tuna otoliths were analyzed for stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) and trace elements (element:Ca). Individual ages ranged from 1.7-4.2 years (based on fork lengths, Figure 7) with mean ages being similar (2.1-3.4 years) across regions, though individuals from Martinique were slightly older (Table 2). Otolith chemistry data from YOY yellowfin tuna collected from the four major nursery areas in the Atlantic Ocean were used as the baseline for mixed-stock predictions (described in Chapter II). Individuals from Cape Verde, Martinique, and the Gulf of Mexico were age-class matched to the 2012 baseline of nursery signatures; however, some individuals from Martinique ($n=19$) did not match with any of the baseline years and were therefore not included in mixed-stock analyses. All yellowfin tuna from the Bahamas were age-class matched with the 2014 baseline. While four nursery areas (Gulf of Mexico, Caribbean Sea, Gulf of Guinea, and Cape Verde) are included in the 2012 baseline, considerable overlap existed between Gulf of Guinea and Cape Verde signatures that year (Chapter II). However, regional discrimination improved significantly when Gulf of Guinea and Cape Verde signatures were combined into one “eastern Atlantic” signature (quadratic discriminant function analysis: 79% classification success). Thus, in this study individuals were sourced to one of three nursery areas: Gulf of Mexico, Caribbean Sea, or eastern Atlantic Ocean.

Otolith $\delta^{18}\text{O}$, Mg:Ca, Zn:Ca, Sr:Ca, and Ba:Ca were the variables proven to be most effective in discriminating among nursery regions in 2012 (Chapter II); thus, these tracers were used to source all sub-adult and adult yellowfin tuna from Cape Verde.

MLE estimates indicated that the majority ($84.9 \pm 14.2\%$) of individuals from Cape Verde originated from eastern Atlantic nurseries (Table 3), suggesting that local production is the main source of recruits to this region with only limited contributions from other nursery areas. HISEA results indicate that a small portion of individuals originated from the Caribbean Sea ($13.9 \pm 13.0\%$) with negligible contribution from the Gulf of Mexico ($1.2 \pm 3.0\%$). However, standard deviations around estimated proportions were high for both of these regions, possibly limiting the significance of these minor contributions to the Cape Verde population. MCL estimates were nearly identical to MLE results, with $84.2\% (\pm 12.5)$, $13.6\% (\pm 11.1)$, and $2.3\% (\pm 4.1)$ of individuals originating from eastern Atlantic Ocean, Caribbean Sea, and Gulf of Mexico nurseries, respectively.

For yellowfin tuna collected in Martinique, HISEA results indicated that nearly all (96.5%) of the individuals in this sample originated from the eastern Atlantic Ocean (based on MLE), with a small contribution from the Gulf of Mexico ($3.5 \pm 6.8\%$) and no contribution ($0.0\% \pm 0.0\%$) from the Caribbean Sea. The proportion of Gulf of Mexico migrants was slightly more pronounced using MCL estimation, with $6.0 \pm 8.6\%$ of individuals originating from this region; however, the error term for this estimate was relatively high. Similar to results from MLE, MCL also indicated that there was no local contribution ($0.0 \pm 0.0\%$) for yellowfin tuna captured in Martinique. Thus, results of both models suggest that trans-Atlantic migration of yellowfin tuna is prevalent, with nearly all individuals in the Martinique sample originating from eastern Atlantic Ocean nurseries.

Mixed-stock analysis revealed that yellowfin tuna populations in the Gulf of Mexico and adjacent waters (Bahamas) were linked to local production in the Gulf of Mexico. While results indicated that all yellowfin tuna collected from the Bahamas originated in the Gulf of Mexico, individuals in the Gulf of Mexico sample were comprised of a mixture of eastern migrants and local recruits. For the Gulf of Mexico sample, predicted contribution (based on MLE) of eastern Atlantic nurseries was $53.9 \pm 10.1\%$, with the remainder derived from local production ($46.1 \pm 10.1\%$). The classification-based model yielded similar results, with $48.5 \pm 11.5\%$ and $51.5 \pm 11.5\%$ of individuals originating from the eastern Atlantic and Gulf of Mexico, respectively, and no migrants detected from the Caribbean Sea. Thus, results from both MLE and MCL indicate that significant mixing of yellowfin tuna from eastern (Cape Verde + Gulf of Guinea) and western (Gulf of Mexico) Atlantic nursery areas occurred in the Gulf of Mexico. This provides further evidence that yellowfin tuna undertake trans-Atlantic migrations, particularly in the east to west direction. Yellowfin tuna captured in adjacent waters in the Bahamas were all age-class matched with the 2014 baseline, and mixed-stock analysis indicated that all these fish originated entirely from the Gulf of Mexico (MLE and MCL: 100%). However, it should be noted that the significance of this finding is unclear because of the small sample size obtained from this region.

Discussion

In this study, the nursery origin of sub-adult and adult (age-1 to age-3) yellowfin tuna from four regions in the Atlantic Ocean was determined by comparing trace elements and stable isotopes in otolith cores to a baseline of nursery signatures (Chapter

II). Results suggest that local production is the main source of yellowfin tuna in the eastern Atlantic Ocean, while fisheries in the western Atlantic Ocean are supported by both eastern migrants and locally produced fish. While there is evidence that several individuals migrated long distances (>4,500 km) from their place of origin in the eastern Atlantic Ocean, the movement of western migrants into Cape Verde appears to be negligible. It is important to note that the yellowfin tuna population in the eastern Atlantic is significantly larger than the western Atlantic population (ICCAT 2016). Thus, west to east movement may occur, but western migrants would represent a much smaller fraction of the overall population in the eastern Atlantic Ocean, making it more difficult to detect western nursery contributions in this region. Regardless, results from this study are consistent with the presently accepted migration model for yellowfin tuna, which states that sub-adults (60-80 cm) generally migrate from eastern Atlantic nursery areas towards the western Atlantic Ocean, with the majority returning to the eastern Atlantic Ocean later to spawn (Fonteneau and Soubrier 1996, ICATT 2002).

Estimates of nursery origin for yellowfin tuna collected in Cape Verde indicate that these individuals were predominantly derived from local production, highlighting the importance of the eastern Atlantic nursery area to the Cape Verde fishery. Given that the principal spawning ground for yellowfin tuna is assumed to be in the Gulf of Guinea, it is not unexpected that this productive nursery area would support the local population. The presence of western migrants was limited in Cape Verde, though I did detect a small number of potential migrants from the Caribbean Sea (MLE and MCL: 14%). However, the contribution of Caribbean migrants is possibly even less significant given the fact

that standard deviation around estimated proportions ranged from 11% (MCL) to 13% (MLE). Using conventional tags, Hallier (2005) investigated the movement patterns of yellowfin tuna in the eastern Atlantic Ocean. Restricted movement was observed among yellowfin tuna tagged in Cape Verde and the Gulf of Guinea, with individuals generally staying in the same region in which they were tagged (i.e., within 250 km of the release location). My results are in agreement with tagging data, with both providing evidence for regional fidelity in the eastern Atlantic Ocean. Upwelling near the western coast of Africa is thought to produce rich feeding grounds for yellowfin tuna (Beardsley 1969), which may explain the enhanced residency and limited movement observed in this region.

In contrast, yellowfin tuna from Martinique were mostly comprised of eastern migrants. Despite being several thousand kilometers away, results indicated that 94-97% of individuals in the Martinique sample (based on MCL and MLE, respectively) originated from nursery areas in the eastern Atlantic Ocean. A small number of potential migrants from the Gulf of Mexico were detected (MLE: $4 \pm 7\%$, MCL: $6 \pm 9\%$), though again the error term of these estimates were relatively high. Interestingly, I observed negligible contribution from local production to the Martinique fishery. Little research has been conducted on the Caribbean Sea as a nursery area for yellowfin tuna, but it is believed that production is less significant in this region relative to the Gulf of Guinea nursery (ICATT 2011). This study provides the first piece of evidence that a yellowfin tuna fishery in the Caribbean is comprised of eastern Atlantic migrants, as no fish have previously been tagged or recaptured in this region. Strong east to west equatorial

currents exist in the intertropical convergence zone (ITCZ) of the Atlantic Ocean, and increased catches of yellowfin tuna are closely linked with the position of the ITCZ (Zagaglia et al. 2004). Therefore, it is likely that yellowfin tuna take advantage of this current system during their trans-ocean migration from the eastern Atlantic Ocean to the Caribbean Sea (Figure 8). Although the Caribbean Sea nursery did not contribute to the local adult population sampled in my study, it is believed that this area contributes to fisheries in waters near Brazil and the Guyanas (Gaertner and Medina-Gartner 1994, Arocha et al. 2001). Thus, even though Arocha et al. (2001) previously concluded that the Caribbean Sea nursery contributes significantly to the local fishery based on the presence of spawning-ready females in the area, my research instead indicates that the majority of individuals spawned in the Caribbean Sea migrate away from this region, potentially contributing to other fisheries in the equatorial Atlantic Ocean. It should be noted that sampling was conducted over a fairly restricted time period in this region (May-July) and it is possible that contribution rates vary seasonally in these waters.

The yellowfin tuna sample from the Bahamas consisted entirely of migrants from the Gulf of Mexico. Considering regional oceanographic patterns, it is likely that these individuals followed the Loop Current and Florida Current during their migration to the Bahamas, as these high-speed surface currents transport water from the Gulf of Mexico to the Atlantic Ocean through the Straits of Florida (Figure 8). No spawning has been documented in the Bahamas, and occurrence of sub-adult and adult yellowfin tuna is highly seasonal in this region. Thus, it is possible that the Bahamas may be a stopping point along a larger migratory route. For instance, individuals in the Bahamas may

continue following the Florida Current (which turns into the Gulf Stream) northward along the east coast of the United States towards North Carolina, where a major fishery for yellowfin tuna occurs. In fact, Ortiz (2001) observed that yellowfin tuna tagged near the Bahamas were later recaptured in waters off North Carolina. While only a few individuals were tagged near the Bahamas in the study by Ortiz (2001), these results provide evidence that migration does occur between these regions. Therefore, it is possible that migrants from the Gulf of Mexico—passing through the Bahamas—may make up a component of the yellowfin tuna fishery off of North Carolina.

Mixed-stock analysis revealed that the Gulf of Mexico is an important mixing zone for yellowfin tuna originating from the eastern (Cape Verde + Gulf of Guinea) and western (Gulf of Mexico) Atlantic Ocean. Both models detected significant contributions from these regions, with nearly equal numbers of individuals originating from the eastern Atlantic Ocean and the Gulf of Mexico, and neither model detected any migrants from the Caribbean Sea. Previous tagging studies support my finding that some local retention occurs in the Gulf of Mexico. Ortiz (2001) investigated the movement patterns of yellowfin tuna in the Atlantic Ocean using conventional tags and found that several individuals tagged in the Gulf of Mexico remained in the Gulf of Mexico, despite the fact that some were recaptured more than two years later. Additionally, a recent archival tagging study conducted in the Gulf of Mexico revealed limited movement of yellowfin tuna in this region (Hoolihan et al. 2014). On the other hand, individuals tagged in the Gulf of Mexico have been recaptured near the Gulf of Guinea (Ortiz 2001), further supporting the premise of population connectivity between these two regions. The

northern Gulf of Mexico is a highly productive region, with nutrient input from the Mississippi River stimulating enhanced primary and secondary productivity in the area (Lohrenz et al. 1997, Dagg and Breed 2003). Tunas are known to migrate large distances to reach suitable feeding grounds (Block et al. 2001, Gunn et al. 2001, Fonteneau et al. 2005) and it is possible that eastern migrants move into this region to take advantage of the high quality/quantity of prey resources in the Gulf of Mexico during weak upwelling periods in the eastern Atlantic Ocean. Further, abundant prey availability may also explain the enhanced residency of locally produced yellowfin tuna in this region. Regardless of the reason, my results reveal that the yellowfin tuna population in the Gulf of Mexico is comprised of large numbers of trans-Atlantic migrants, indicating that the U.S. fishery for yellowfin in the Gulf of Mexico is likely subsidized by migrants from nursery areas in the eastern Atlantic Ocean.

This study highlights the value of the eastern Atlantic as a critical nursery area for yellowfin tuna and indicates that this region may be an important source of sub-adult and adult yellowfin tuna to several fisheries throughout the Atlantic Ocean (Cape Verde, Martinique, Gulf of Mexico). Results from this study confirm that a strong east to west migration route occurs for age-1 to age-3 yellowfin tuna, which until now has only been assumed based on size-frequency distributions of regional catches (ICCAT 2002, Shuford 2005). Considering that the eastern Atlantic Ocean appears to contribute substantially to fisheries in both eastern and western regions, this nursery area likely plays an important role in supporting the Atlantic yellowfin tuna stock. Currently, yellowfin tuna catches are dominated by a largely unregulated purse seine fleet in the

eastern Atlantic Ocean (ICCAT 2016). These purse seine vessels are highly efficient at catching young (30-90 cm) yellowfin tuna (Wild, 1994); thus, proper management of the eastern Atlantic fishery is essential in order to allow individuals in this critical spawning area to reach reproductive maturity. Future work should focus on determining the nursery origin of individuals from multiple cohorts, as the relative contribution rates of different nursery areas to regional fisheries likely varies across time. Additionally, extending this work to other regions in the Atlantic Ocean will help clarify the population structure and migratory pathways for yellowfin tuna, further improving our ability to sustainably manage this species throughout its range.

CHAPTER IV
IDENTIFYING THE NURSERY ORIGIN OF YELLOWFIN TUNA IN THE GULF
OF MEXICO

Introduction

Effective management of highly migratory fishes (e.g. billfishes, tunas) that inhabit open-ocean ecosystems relies on understanding the source of production for harvested stocks. Sources (i.e., spawning and/or nursery areas; hereafter denoted as nursery areas) need be protected in order to replenish adult populations, both within and outside of these regions, as individuals often egress from nursery areas to utilize multiple habitats during their life cycle (Svedäng et al. 2007, Rooker et al. 2014). As a result, managers must not only consider the abundance of adults in harvested regions when assessing population trends but also the sustainability of the nursery areas that supply recruits to fished stocks. Nursery areas are of crucial importance to sustaining marine fish stocks (Beck et al. 2001, Sundblad et al. 2013) and certain nurseries may contribute disproportionately to adult populations (Tanner et al. 2013, Rooker et al. 2016). Therefore, it is important to understand the role that each nursery area plays in supporting adult populations, as this will allow managers to focus their efforts on conserving the most productive regions. This is particularly important for highly migratory species such as tunas, as these species often have multiple geographically distinct nursery areas and thus require extensive multinational management plans to ensure their sustainability.

In order to understand the contribution of different nursery areas to adult populations of pelagic fishes, we must have a robust means of determining an individual's place of origin. One of the most effective methods of determining natal origin involves examining natural chemical markers in calcified structures (Campana and Thorrold 2001). Otoliths (ear stones) are particularly effective, as the chemical signature of material deposited onto the otolith often reflects the chemistry of the seawater inhabited by the fish (Campana 1999). Thus, material deposited during the early life period may serve as a natural tag of an individual's place of origin (Thorrold et al. 2001, Rooker et al. 2008b). Both trace elements and stable isotopes have been successfully used to trace movement and discriminate fishes from different geographic locations (Thorrold et al. 2001, Forrester and Swearer 2002). Additionally, these chemical markers have been used to determine nursery origin for many pelagic species, including tunas in the Atlantic Ocean (Rooker et al. 2008b, 2014) and Pacific Ocean (Wells et al. 2012, Rooker et al. 2016).

Yellowfin tuna (*Thunnus albacares*) represent one of the most important highly migratory species contributing to commercial and recreational fisheries in the Atlantic Ocean (Beerkircher et al. 2009, Levesque 2011). Despite its value, very little information exists regarding the movement and origin of yellowfin tuna in this region. Using conventional tagging data, Ortiz (2001) reported that some yellowfin tuna undertake trans-ocean migrations, as several fish tagged in the western Atlantic Ocean (Gulf of Mexico and Mid-Atlantic Bight) were later recaptured in the eastern Atlantic Ocean (Gulf of Guinea and Cape Verde). Even though trans-ocean migrations are well

documented for yellowfin tuna, other tagging studies suggest that yellowfin tuna may exhibit some degree of residency in certain regions. Archival tagging studies conducted in the Gulf of Mexico showed that movement was more limited than expected, with individuals often remaining close to release locations for several months (Edwards and Sulak 2006, Hoolihan et al. 2014). Therefore, the degree of movement and mixing by yellowfin tuna from different production zones in the Atlantic Ocean is unresolved and warrants further attention.

While the Gulf of Guinea is considered to be the primary spawning area for yellowfin tuna in the Atlantic Ocean, spawning has also been documented in the Gulf of Mexico, signifying that this region could potentially serve as another important production zone (Lang et al. 1994, ICCAT 2011). However, additional research needs to be conducted in order to fully assess the relative value of this nursery area to the Atlantic population. Currently, yellowfin tuna in the Atlantic Ocean are managed as one panmictic (ocean-wide) stock, though this decision was largely based on data from a limited number of trans-Atlantic tag recoveries and length distribution data (NMFS 2001). Increasing exploitation rates during the last few decades have caused a steady decline in yellowfin tuna biomass, and as a result, the yellowfin tuna stock in the Atlantic Ocean is now close to being overfished (ICCAT 2016). Therefore, an improved understanding of population structure and natal origin is urgently needed in order to ensure that yellowfin tuna in the Gulf of Mexico and throughout the Atlantic Ocean are sustainably managed.

In the present study, I examined the origin of sub-adult and adult yellowfin tuna in the Gulf of Mexico using natural chemical markers in otoliths. Specifically, I measured stable isotopes and trace elements in otolith cores of sub-adult and adult yellowfin tuna, which reflects their early juvenile stage or the interval that corresponds to the nursery period. Otolith core signatures were then compared to a baseline of nursery signatures developed from young-of-year (YOY) yellowfin tuna (Chapter II) and mixed-stock analysis was performed to predict the natal origin of sub-adult and adult yellowfin tuna captured in the Gulf of Mexico. This study builds upon previous work (Chapter III) by using an expanded sample set to assess interannual (2012 vs. 2013 year classes) and size-specific (age-1 vs. age-2) differences in contribution rates of yellowfin tuna collected in Gulf of Mexico. Additionally, trace elements were analyzed in later life stages to determine whether otolith chemistry can be used to estimate the timing of trans-Atlantic migration from eastern Atlantic nurseries.

Methods

Sample collections

Sub-adult and adult yellowfin tuna of unknown nursery origin were collected dockside in the northern Gulf of Mexico (Venice, Louisiana) in 2014 and 2015. All yellowfin tuna were captured via hook and line by recreational fishermen. Collections occurred throughout the year (January-August) in order to obtain a representative sample from this region, which would include specimens from multiple schools, contingents, or sub-populations. Additionally, samples were collected across multiple years to assess interannual variability in mixing rates among spawning populations. Fork length (FL)

was measured to the nearest cm prior to otolith extraction for all samples collected, and lengths were used to calculate ages for each individual based on published growth curves for yellowfin tuna in the Atlantic Ocean (Driggers et al. 1999, Shuford et al. 2007). Spawning date (i.e., birth year) of each individual was back-calculated using estimated age and date of capture; this was then used to age-class match individuals to the correct baseline or reference chemical dataset created from YOY yellowfin tuna assigned to 2012 and 2013 year classes (Chapter II).

Otolith chemical analyses

Sagittal otoliths of yellowfin tuna were extracted from the brain cavity, cleansed of biological tissue in DI water, and stored dry. After cleaning, one sagittal otolith from each fish was embedded in Struers EpoFix resin and sectioned using a low-speed ISOMET saw (Buehler) to isolate 1.5 mm transverse sections of the core of the otolith following the procedure described in Chapter II. The transverse sections were then affixed to clean glass slides using Crystalbond thermoplastic glue (SPI Supplies/Structure Probe Inc.) and polished to expose the otolith core using 600-1200 grit silicone-carbide paper (Buehler) and 0.3 mm MicroPolish Alumina Powder. Sections were further polished with a microcloth to ensure a smooth surface and then given a final rinse with DI water prior to any chemical analyses.

Each otolith section was analyzed to determine core trace element and stable isotope concentrations. All chemical analysis procedures followed those described in Chapter II. Briefly, trace element concentrations (${}^7\text{Li}$, ${}^{24}\text{Mg}$, ${}^{55}\text{Mn}$, ${}^{88}\text{Sr}$, ${}^{137}\text{Ba}$, ${}^{66}\text{Zn}$) were measured in otolith cores using a laser ablation inductively coupled plasma mass

spectrometer (LA-ICP-MS) at Texas A&M University (Galveston Campus). The system consists of an ultraviolet laser ablation unit (NWR 213, New Wave Research) and an XSeries II Thermo Scientific ICP-MS. All elements were analyzed at five different ablation spots (ablation diameter: 50 μm) near the core. The first spot was set at the core (narrowest part of the otolith) and two equally spaced spots were placed on either side of the core in an effort to capture any natural variability in otolith chemical signatures. These 5 ablation spots covered a portion of the otolith consisting of material deposited within the first 3 months of life. Surface contamination was avoided by pre-ablating all spots for 10 seconds before measurements began; each spot was then ablated by the laser for approximately 12 seconds during quantification. A standard reference material (NIST 614) was analyzed every two samples for instrument calibration and ^{44}Ca (measured each ablation spot) was used as an internal standard to correct for variations in ablation yield. Raw ion counts were blank-corrected and converted to element:Ca ratios ($\mu\text{mol mol}^{-1}$).

After trace element analysis, otoliths were lightly polished until ablation pits were no longer visible. Stable isotope concentrations ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) were then measured in the cores of the same otoliths. Core material was isolated using a high-resolution mill (New Wave MicroMill System). The drill path used for each sample (described in Chapter II) covered a portion of the otolith corresponding to the first 5-6 months of life. Otolith material was powdered by running a 350 μm diameter carbide bit (Brasseler) over the programmed drill path until a depth of approximately 770 μm was reached. Powdered otolith material was then collected in weigh paper and analyzed for

$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ at the Environmental Isotope Laboratory at the University of Arizona using a gas-ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific) equipped with an automated carbonate preparation device (KIEL-III, Thermo Fisher Scientific). Isotopic ratio measurements were calibrated based on repeated measurements of NBS-18 and NBS-19 (National Bureau of Standards). Otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (‰) are expressed in standard delta (δ) notation as $^{13/12}\text{C}$ and $^{18/16}\text{O}$ ratios relative to the Pee Dee Belemnite (PDB) scale after comparison with an in-house laboratory standard calibrated to PDB.

Trace elements were also analyzed in life history transects along the ventral arm of six yellowfin tuna otoliths (Figure 9). Samples were chosen based on results of mixed-stock analysis to allow for comparisons between local residents from the Gulf of Mexico (n=3) and migrants from the eastern Atlantic Ocean (n=3). Transects consisted of evenly spaced ablation spots (50 μm diameter) positioned along a straight line ~ 200 μm from the ventro-distal edge of the otolith section. The first ablation spot was always placed immediately adjacent to the YOY region previously milled out for stable isotopes, with the transect line extending from this point to the terminal edge of the otolith. All life history transects were analyzed by LA-ICP-MS following the same methods as described for otolith core analysis. Otolith microstructure analysis was used to estimate the ages associated with each ablation spot in the life history transects. To create age estimates, transverse otolith sections from four yellowfin tuna (59-103 cm FL) in the Gulf of Mexico sample were first polished to a thickness of ~ 50 -100 μm . Daily growth increments in these otoliths were then counted using an Olympus microscope

(400x magnification) equipped with an image analysis system (Image-Pro Plus), and a regression was created based on the distances between daily increments and otolith cores. Otolith measurements were also taken for all life history transects, and the regression was used to estimate the ages associated with each ablation spot based on its distance from the core.

Data analysis

Nursery origin of sub-adult and adult yellowfin tuna was predicted using HISEA, a maximum-likelihood based mixed-stock analysis program (Millar, 1990). Otolith core chemistry (element:Ca, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of sub-adults and adults from each region was compared with the baseline dataset of nursery signatures constructed from otoliths of YOY yellowfin tuna (Chapter II). All yellowfin tuna samples were age-class matched to the YOY baseline. Nursery assignments were determined using direct maximum likelihood estimation (MLE) and maximum classification likelihood (MCL) within the HISEA program. MLE typically performs better than classification-based methods such as MCL; however, MCL is generally more robust than MLE to anomalies in baseline data (Millar 1987, 1990). Therefore, similar to Rooker et al. (2014), proportions estimated using both methods are included for the purpose of comparing predictions. Standard deviations were calculated by bootstrapping with 500 resamplings of the baseline dataset to determine the variability of estimated proportions.

Results

A total of 177 yellowfin tuna collected from the Gulf of Mexico were analyzed for otolith core chemistry to determine their nursery of origin. Otolith core element:Ca

(Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, Zn:Ca) and stable isotope ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) values of sub-adults and adults were compared with YOY baseline samples collected from major nursery areas in the Atlantic Ocean (described in Chapter II) for mixed-stock predictions. All samples were age-class matched with the appropriate baseline year, with 130 individuals age-class matched to the 2012 baseline and 47 matched with the 2013 baseline. Mean estimated ages of these individuals were 2.4 ± 0.4 years and 1.7 ± 0.2 years for 2012 and 2013 year classes, respectively (Table 4). Individuals from multiple age classes were collected each year, with age-1 (n=20) and age-2 (n=49) fish captured in 2014 and age-1, age-2, and age-3 fish (n=42, 64, and 2, respectively) captured in 2015.

Baselines used for mixed-stock predictions included otolith chemistry data for YOY collected from 4 nursery regions: 1) Gulf of Mexico, 2) Caribbean Sea, 3) Cape Verde, and 4) Gulf of Guinea. Significant overlap was detected between Cape Verde and Gulf of Guinea nursery signatures in the 2012 baseline (Chapter II), but nursery discrimination significantly improved when these two neighboring regions were grouped (64% vs. 79% classification success using quadratic discriminant function analysis). Thus, Cape Verde and Gulf of Guinea signatures were combined into one “eastern Atlantic Ocean” nursery for all mixed-stock analyses of yellowfin tuna age-class matched to 2012 baseline. However, regional discrimination was high for the four nursery regions in 2013 (78% classification success), with strong separation observed among Cape Verde and Gulf of Guinea signatures that year; as a result, mixed-stock analysis for individuals age-class matched to 2013 baseline was based on signatures

from all four nurseries. Optimal discrimination for the 2012 and 2013 baselines were attained using otolith $\delta^{18}\text{O}$, Mg:Ca, Zn:Ca, Sr:Ca, and Ba:Ca and otolith $\delta^{13}\text{C}$, Li:Ca, Sr:Ca, and Ba:Ca respectively, so these markers were used to determine the nursery of origin of sub-adults and adults matched with each of these baselines.

Mixed-stock predictions indicated that approximately half of the yellowfin tuna sample from the Gulf of Mexico was from local production while the rest originated from the eastern Atlantic Ocean, with little interannual variability detected in contribution rates. MLE estimates indicated that $51 \pm 6\%$ and $49 \pm 6\%$ (mean \pm SD) of yellowfin tuna in the 2012 year class originated from the Gulf of Mexico and eastern Atlantic Ocean, respectively (Figure 10). The classification-based model detected a similar but slightly higher contribution of local recruits from the Gulf of Mexico ($57 \pm 7\%$), with the remainder ($43 \pm 7\%$) originating from the eastern Atlantic Ocean. No contribution from the Caribbean Sea was detected by either model. Estimates of nursery origin for the 2013 year class were similar, with $41 \pm 8\%$ (MLE) to $44 \pm 8\%$ (MCL) of individuals produced locally. The majority of remaining individuals in the 2013 year class were predicted to have originated from the Gulf of Guinea (MLE: $53 \pm 8\%$, MCL: $52 \pm 8\%$), with negligible contribution detected from Cape Verde (MLE: $1 \pm 2\%$, MCL: $3 \pm 3\%$) and the Caribbean Sea (MLE: $5 \pm 3\%$, MCL: $2 \pm 3\%$).

Size-specific differences in contribution rates were examined by sourcing yellowfin tuna of different age classes (age-1, age-2). In general, no consistent trends were observed among nursery assignments for the different age classes examined. Mixed-stock analysis indicated that the majority of age-1 individuals in the 2012 year class

were of eastern Atlantic origin (67-69%, Table 5), while the majority of individuals in the sample of age-2 fish were from the Gulf of Mexico (58-64%). Only two age-3 fish were in the sample and both were migrants from the eastern Atlantic Ocean. The 2013 year class consisted of a nearly equal proportion of age-1 individuals originating in the Gulf of Mexico (45-48%) and Gulf of Guinea (48-49%), and unlike the 2012 year class, age-2 fish in this year class were primarily migrants from the eastern Atlantic Ocean (68-69%); however, there was a limited number of age-2 individuals in the 2013 year class (n=6) and standard deviations around estimated proportions were high for these estimates (14-21%). Overall, results show that eastern migrants of both age classes (age-1 to age-2) were detected in the Gulf of Mexico, suggesting that yellowfin tuna may remain in this region for an extended time period after migrating from the eastern Atlantic Ocean.

Trace elements were measured in life history transects of six yellowfin tuna collected from the Gulf of Mexico. According to mixed-stock analysis of core chemistry data, three of these fish were of local origin and three were migrants from the eastern Atlantic Ocean. Regional differences were observed in otolith cores and early in the life history transects, which represents the time period in which fish were presumably still near their nursery of origin. This was particularly evident in Mn:Ca and Ba:Ca values, for which significant differences were detected between yellowfin tuna from the Gulf of Mexico and eastern Atlantic Ocean in both the core region (ANOVA $p < 0.01$) and first 10 ablation spots of the transect (ANOVA $p < 0.05$, Figure 11). Individuals from the eastern Atlantic Ocean exhibited higher Mn:Ca and lower Ba:Ca values (mean: $5.7 \mu\text{mol mol}^{-1}$

and $1.3 \mu\text{mol mol}^{-1}$, respectively) than individuals from the Gulf of Mexico (mean: $4.1 \mu\text{mol mol}^{-1}$ and $1.8 \mu\text{mol mol}^{-1}$) in the first 10 ablation spots, with similar trends observed in core values. In contrast, no significant differences in element:Ca ratios were observed between migrants and residents for the remainder of the life history transect (beyond the 10th ablation spot or > 300 days in age). When taking estimated age into account, these results suggest that migrants may have remained in the eastern Atlantic during the first ~10 months (300 days) of life. Otolith Mn:Ca and Ba:Ca values for the two groups appear to merge shortly afterwards near ablation spots 10 to 17 (ANOVA, $p > 0.05$), after which eastern migrants begin to show signatures similar to Gulf of Mexico residents. This indicates that trans-ocean migration (east to west) likely occurred for these individuals between approximately 300 to 600 days of age or before they reach the age of 2.

Discussion

This study compared chemical signatures in otolith cores of yellowfin tuna to a baseline of nursery signatures (Chapter II) to determine the origin of sub-adults and adults captured in the Gulf of Mexico. Similar to results from Chapter III, findings presented here suggest that the Gulf of Mexico represents a significant mixing zone for yellowfin tuna originating from the eastern (Gulf of Guinea, Cape Verde) and western (Gulf of Mexico) Atlantic Ocean. In fact, mixed-stock analysis results indicate that the Gulf of Mexico population consists of nearly equal proportions of eastern migrants and local recruits. Therefore, while local production does appear to be important to sustaining the Gulf of Mexico population, overall findings from this study suggest that

nursery areas in the eastern Atlantic Ocean likely play an important role in supporting the U.S. yellowfin tuna fishery in the Gulf of Mexico.

Contribution rates of Gulf of Mexico and eastern Atlantic nursery areas were similar for both year classes of yellowfin tuna collected in the Gulf of Mexico. Significant proportions of eastern migrants were detected in each year class, with eastern Atlantic Ocean contribution (based on MLE) accounting for approximately 50% of the yellowfin tuna sample from the Gulf of Mexico. The classification-based model also indicated that the presence of eastern migrants accounted for approximately half of the sample, but estimated proportions were slightly lower than what was predicted based on MLE; regardless, substantial contributions from eastern Atlantic Ocean nurseries were detected each year using both types of mixed-stock approaches (Figure 10). Thus, results indicate that local production in the Gulf of Mexico is subsidized with individuals from the eastern Atlantic Ocean. Although Cape Verde and Gulf of Guinea nursery signatures had to be combined in the 2012 baseline due to significant regional overlap (Chapter II), individuals in the 2013 year class were sourced separately to each of these regions. Mixed-stock analysis revealed that nearly all (95-98%) of the eastern migrants detected in the 2013 year class originated from the Gulf of Guinea, which is not surprising given that this region is considered to be the main production zone for yellowfin tuna in the Atlantic Ocean (ICCAT 2011). Therefore, it is possible that the majority of individuals sourced to the combined eastern Atlantic nursery region (Cape Verde + Gulf of Guinea) in the 2012 year class were also produced in this region. Only a trivial number of Caribbean Sea migrants were detected in 2013, though this contribution is possibly

insignificant given the standard deviation around these estimates (MLE: $5\pm 4\%$, MCL: $2\pm 3\%$), and no contribution was detected from this region in the 2012 year class.

Therefore, the Caribbean Sea nursery does not appear to support the U.S. yellowfin tuna fishery in the Gulf of Mexico, despite its proximity to this region. While significant interannual variability in stock mixing rates ($>30\%$) have been reported for bluefin tuna in the Atlantic Ocean (Rooker et al. 2014), regional contribution estimates for yellowfin tuna only varied by 5-12% each year, indicating that the composition of the yellowfin tuna fishery in the Gulf of Mexico may be relatively stable across time, albeit additional sampling years are needed to confirm this finding.

Age-1 and age-2 yellowfin tuna collected in the Gulf of Mexico were compared to assess age-specific differences in nursery origin estimates. Eastern migrants of both age classes were detected each year in the Gulf of Mexico, comprising $>40\%$ of all age-1 and age-2 yellowfin tuna in this study. Results indicated that age-specific nursery contributions varied between the 2012 and 2013 year classes. In the 2012 year class, the majority of age-1 fish originated in the eastern Atlantic Ocean, while age-2 fish were dominated by local recruits from the Gulf of Mexico (Table 5). In contrast, contributions from the eastern and western Atlantic Ocean for the 2013 year class were nearly equal for age-1 fish, while age-2 fish were primarily of eastern origin. Thus, no consistent age-specific trends in nursery origin were observed, and results indicated that eastern migrants of all age classes (age-1 to age-2) were present in the Gulf of Mexico. The presently accepted migration model for yellowfin tuna states that after moving into the western Atlantic Ocean, return migration to the eastern Atlantic Ocean occurs once

individuals reach ~110 cm FL (ICCAT 2002). However, the majority of individuals >110 cm FL in this study were migrants from the eastern Atlantic (61-66%). In fact, the largest individuals in our sample (n = 13, 130-141 cm) were primarily of eastern Atlantic origin, suggesting that following east to west trans-Atlantic migration, individuals may remain in the Gulf of Mexico for a longer time period than previously thought.

Considering that yellowfin tuna in the Atlantic Ocean attain sexual maturity by ~140 cm (Hazin 1993, ICCAT 2016), it is possible that many migrants remain in productive waters of the Gulf of Mexico until returning to their natal sites to spawn. Nonetheless, additional sampling of older age classes (age-3+) is required to fully assess the timing of return migrations to the eastern Atlantic Ocean.

Trace elements were analyzed in later life stages of six yellowfin tuna to test whether the timing of trans-ocean migrations from eastern Atlantic nurseries could be estimated using otolith chemistry. Of the six trace elements examined, otolith Mn:Ca and Ba:Ca values showed the most significant differences between migrants and local recruits. Otolith Ba:Ca has been successfully used to trace the movement of tunas across different water masses (Wang et al. 2009, Baumaunn et al. 2015), and significant regional differences were observed in YOY baselines for both of these elements, particularly in otolith Mn:Ca (Chapter II). Consequently, these elements appear to be suitable tracers for detecting the migration of yellowfin tuna in the Atlantic Ocean. Otolith Mn:Ca and Ba:Ca transects showed significant differences between individuals originating in Gulf of Mexico and eastern Atlantic Ocean in the core region and early in the transects (Figure 11), indicating that these fish inhabited distinct bodies of water during the early

life period. However, otolith Ba:Ca and Mn:Ca ratios of these fish began to merge with the signatures of local recruits from the Gulf of Mexico near the end of the YOY period. Age estimates for individual ablation spots, derived by combining otolith microstructure and trace element analysis, suggest that individuals in this sample remained in the eastern Atlantic Ocean until approximately 10 months of age, with trans-Atlantic migration towards the Gulf of Mexico potentially occurring near the end of the YOY period or well into the second year of life. It is currently believed that yellowfin tuna undertake trans-Atlantic migrations once they reach 60-80 cm FL (Fonteneau and Soubrier 1996, ICATT 2002), which corresponds to about 10 to 20 months of age based on published age-length curves for yellowfin tuna in the Atlantic Ocean (Shuford et al. 2007, Driggers et al. 1999). Results from this study closely align with the current migration model, with both indicating that east to west migrations occur during the second year of life. However, only three individuals from the eastern Atlantic were analyzed to estimate migration timing in this study, so further research with additional samples is required to confirm my findings. Regardless, results indicate that age-resolved chemical analysis of otoliths shows considerable promise as a way to estimate the timing of trans-Atlantic migration from nursery areas.

Results from this study have important implications for the management of yellowfin tuna in the Atlantic Ocean. Findings indicate that a large portion of the Gulf of Mexico population may be comprised of eastern migrants; thus, it is crucial for the welfare of the U.S. fishery that the yellowfin tuna population in the eastern Atlantic Ocean is properly managed. This is particularly important for the Gulf of Guinea nursery area, as it appears

to be the main source of migrants in the Gulf of Mexico. Landings in the Gulf of Guinea are dominated by a highly efficient purse-seine fishery (ICCAT 2016), and purse-seine vessels are known to capture large quantities of small (30-90 cm FL) yellowfin tuna with the aid of fish aggregating devices (Wild, 1994). As a result, many yellowfin tuna are captured in this region prior to reaching reproductive maturity and before undertaking trans-Atlantic migration. While a global minimum size regulation has been set by ICCAT, the governing agency responsible for assessing and managing yellowfin tuna populations in the Atlantic Ocean, it has not been strictly enforced in this region (ICCAT, 2004). In fact, catches under the minimum size limit sometimes exceed 80% for certain fleets (ICCAT, 2004). Therefore, it is essential that managers start enforcing this size limit in the Gulf of Guinea so that more individuals are provided with the chance to reproduce in this critical spawning area. Not only would this ensure the continued supply of migrants to the Gulf of Mexico population, it could also help improve the near-overfished state of the Atlantic Ocean stock (ICCAT 2016).

In conclusion, findings from this study show that nursery areas in the Gulf of Mexico and eastern Atlantic Ocean contribute substantially to the yellowfin tuna fishery in the Gulf of Mexico. Exchange rates reported here provide strong evidence for the connectivity of eastern and western populations of yellowfin tuna in the Atlantic Ocean, and results indicate that the U.S. fishery in the Gulf of Mexico appears to be dependent, to some degree, on individuals originating from eastern Atlantic Ocean nurseries. Additionally, interannual variability in overall contribution rates was minimal over the two year classes investigated, suggesting that trans-Atlantic migration by eastern

migrants into the Gulf of Mexico may occur regularly each year. Considering that this study was based on only two year classes, additional sampling of yellowfin tuna over an extended time period is needed to help further clarify the temporal dynamics of migration rates and stock mixing in the Gulf of Mexico. Future research should also focus on extending this work to other important fisheries, such as in the Mid-Atlantic Bight, to develop a more complete understanding of the population structure of yellowfin tuna in the Atlantic Ocean.

CHAPTER V

SUMMARY AND CONCLUSIONS

Understanding population connectivity of tunas is fundamental to their effective conservation and management. The main goal of this research was to address gaps in our knowledge regarding the connectivity and mixing of yellowfin tuna populations in the Atlantic Ocean using natural chemical markers in otoliths. The general summary and conclusions of the three studies in this dissertation are discussed below.

In Chapter II, otolith chemistry of young-of-year (YOY) yellowfin tuna was examined to determine whether chemical signatures are distinct across major spawning areas in the Atlantic Ocean. YOY yellowfin tuna otoliths were collected from 4 locations in the Atlantic Ocean (Gulf of Mexico, Caribbean Sea, Cape Verde, and Gulf of Guinea) from 2013-2015 and trace element (Li, Mg, Mn, Sr, Zn, and Ba) and stable isotope ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) analyses were conducted to investigate regional variation in otolith chemical composition. Results indicated that significant regional differences in chemical signatures existed for each year class of YOY yellowfin tuna investigated. Quadratic discriminant function analysis showed that nursery assignment accuracies based on otolith trace elements and stable isotopes were 64-85% for each year class, justifying the use of these natural tracers as regional discriminators for yellowfin tuna. Particularly high classification success was observed based on combined eastern Atlantic Ocean (Gulf of Guinea + Cape Verde) and western Atlantic Ocean (Gulf of Mexico + Caribbean) nursery areas, indicating that otolith chemistry can be used for distinguishing

migrants displaying trans-ocean movement. Significant interannual variability in regional signatures was also detected, highlighting the importance of age-class matching when using the baseline of nursery signatures to estimate the origin of sub-adult and adult yellowfin tuna. In Chapter II, I clearly demonstrate that baseline chemical signatures in the otoliths of YOY yellowfin tuna are distinct and can therefore serve as an effective tool for assigning older individuals to their natal sites or place of origin, ultimately providing a way to improve our understanding of the population connectivity and mixing rates of this species in the Atlantic Ocean.

In Chapter III, I evaluated the origin and movement of sub-adult and adult yellowfin tuna collected in several regions the Atlantic Ocean using natural markers in otoliths. Specifically, I compared trace element and stable isotope signatures in the otolith cores (representing the early life period) of yellowfin tuna collected in the Gulf of Mexico, Bahamas, Martinique, and Cape Verde to the baseline of nursery signatures created in Chapter II using mixed-stock analysis. Significant mixing was observed in the Gulf of Mexico, with approximately half of the sample from this region comprised of eastern migrants and the remainder being from local production, suggesting that this fishery is heavily subsidized by migrants from the primary spawning area in the eastern Atlantic Ocean. In contrast, my sample of yellowfin tuna from adjacent waters in the Bahamas was comprised entirely of migrants from the Gulf of Mexico. Nearly all yellowfin tuna from Martinique originated in the eastern Atlantic Ocean with no local contribution detected, suggesting that the Martinique fishery also is dependent on production in the eastern Atlantic Ocean. Regional fidelity was observed for individuals

collected in Cape Verde, as results show that the majority of yellowfin tuna in this region originated from nurseries in the eastern Atlantic Ocean. This study indicates that the eastern Atlantic Ocean is an important source of yellowfin tuna to several fisheries throughout the Atlantic Ocean; therefore, effective management of fishing activity for younger tuna in this region may be key to ensuring the sustainability of the yellowfin tuna stock in the Atlantic Ocean.

In Chapter IV, I assessed interannual and age-specific variability in the nursery origin of sub-adult and adult yellowfin tuna collected in the Gulf of Mexico using an expanded sample set from this region. Similar to Chapter III, nursery origin was estimated by analyzing trace elements and stable isotopes in otolith cores and comparing them with the baseline of nursery signatures created in Chapter II using mixed-stock analysis. Contribution rates of Gulf of Mexico and eastern Atlantic nursery areas were similar for both year classes of yellowfin tuna in this study, with each nursery contributing to approximately half of the sample each year. No consistent age-specific trends in stock composition were observed between the two year classes, and results show that both age classes of yellowfin tuna (age-1, age-2) were comprised of eastern migrants and locally produced individuals. Additionally, otolith element:Ca life history transects were developed to test whether the timing of trans-ocean migrations from eastern Atlantic nurseries could be estimated. Chemical signatures of migrants and residents began to merge at approximately 1-2 years of age, potentially indicating that east-to-west migration occurred during that time. Results from life history transects indicate that age-resolved chemical analysis of otoliths shows promise as a way to

estimate the timing of trans-Atlantic movement by yellowfin tuna. Overall, findings from this study suggest that the U.S. fishery in the Gulf of Mexico depends, to some extent, on eastern migrants (likely from the Gulf of Guinea) and that trans-Atlantic migration by eastern migrants into the Gulf of Mexico may occur regularly each year.

Results of this research have important implications for the management of yellowfin tuna in the Atlantic Ocean. Exchange rates reported here provide strong evidence for connectivity between populations in the eastern and western Atlantic Ocean, indicating that effective management of this species requires collaboration among multiple international agencies. Additionally, this study highlights the value of the eastern Atlantic Ocean as a critical nursery area and production zone for yellowfin tuna, as this region appears to subsidize multiple fisheries throughout the Atlantic Ocean. This finding is significant, as the eastern Atlantic Ocean is also the region in which yellowfin tuna are the most heavily exploited, accounting for >80% of the total Atlantic catch (ICCAT 2016). Therefore, continued heavy exploitation in this crucial nursery area could have ocean-wide repercussions, potentially leading to biomass declines in several regional fisheries. As a result, protection of this nursery area may be necessary to ensure the sustainability of the Atlantic yellowfin tuna stock. Overall, observations from this study significantly enhance our understanding of the population structure and connectivity of yellowfin tuna, thus providing essential information that is necessary to effectively manage and conserve the declining yellowfin tuna stock in the Atlantic Ocean.

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APPENDIX A

TABLES

Table 1. Summary data for young-of-the-year (YOY) yellowfin tuna collected from four regions of the Atlantic Ocean. Mean (\pm SD) fork length (FL) is provided for each region and year class.

Region	Year class	N	Mean FL (cm)
<i>Cape Verde</i>	2012	34	47.4 (5.3)
	2013	15	46.0 (2.7)
	2014	21	38.3 (3.1)
<i>Gulf of Guinea</i>	2012	35	37.2 (3.4)
	2013	22	46.6 (2.2)
<i>Gulf of Mexico</i>	2012	20	35.9 (4.0)
	2013	16	38.3 (3.3)
	2014	6	34.2 (5.1)
<i>Caribbean Sea</i>	2012	23	29.0 (2.3)
	2013	26	28.9 (2.4)
	2014	20	39.1 (5.2)

Table 2. Summary table for all yellowfin tuna collected in the Atlantic Ocean. Sample size, mean (\pm SD) fork length (FL), age range, mean (\pm SD) age, and collection dates are shown for each region.

Region	N	Mean size (cm FL)	Age range (years)	Mean age (years)	Collection dates
<i>Cape Verde</i>	47	111.5 (8.9)	1.7-2.7	2.1 (0.2)	7/15/2014-3/19/2015
<i>Martinique</i>	43	147.9 (10.9)	2.4-4.2	3.4 (0.4)	5/2/2015-7/30/2015
<i>Gulf of Mexico</i>	50	117.6 (14.6)	1.7-3.2	2.5 (0.4)	2/18/2014-7/21/2015
<i>Bahamas</i>	7	101.6 (7.3)	1.9-2.4	2.1 (0.2)	6/3/2016-6/4/2016

Table 3. Nursery origin estimates of yellowfin tuna captured in four regions in the Atlantic Ocean: Cape Verde, Martinique, Gulf of Mexico, and Bahamas. Percentage (\pm SD) of individuals originating from each nursery area (Eastern Atlantic, Gulf of Mexico, or Caribbean Sea) were obtained by direct maximum-likelihood estimation (MLE) and maximum classification likelihood (MCL) within HISEA, a mixed-stock analysis program (Millar, 1990).

Region		Percent composition (\pmSD)		
		<i>Eastern Atlantic</i>	<i>Gulf of Mexico</i>	<i>Caribbean Sea</i>
<i>Cape Verde</i>	MLE	84.9 (14.2)	1.2 (3.0)	13.9 (13.0)
	MCL	84.2 (12.5)	2.3 (4.1)	13.6 (11.2)
<i>Martinique</i>	MLE	96.5 (6.8)	3.5 (6.8)	0.0 (0.0)
	MCL	94.0 (8.6)	6.0 (8.6)	0.0 (0.0)
<i>Gulf of Mexico</i>	MLE	53.9 (10.1)	46.1 (10.1)	0.0 (0.0)
	MCL	48.5 (11.5)	51.5 (11.5)	0.0 (0.3)
<i>Bahamas</i>	MLE	0.0 (0.0)	100.0 (0.0)	0.0 (0.0)
	MCL	0.0 (0.0)	100.0 (0.0)	0.0 (0.0)

Table 4. Summary table for yellowfin tuna collected in the Gulf of Mexico in 2014 and 2015. Mean (± 1 SD) age and fork length ranges (FL) are provided for each year class. Ages were estimated based on published age-length curves for yellowfin tuna in the Atlantic Ocean (Driggers et al. 1999, Shuford et al. 2007), and estimated ages were used to back-calculate the spawning date of each individual to determine year class assignments.

Year class	N	Size range (cm FL)	Mean age (years)	Collection dates
2012	130	78-141	2.4 (0.4)	2/7/2014 - 7/21/2015
2013	47	69-104	1.7 (0.2)	3/3/2015 - 7/21/2015

Table 5. Size-specific nursery contribution estimates (mean \pm SD) of age-1 and age-2 yellowfin tuna collected in the Gulf of Mexico in 2014 and 2015. Results for the 2012 year class were obtained from mixed-stock analysis using the 2012 baseline dataset, while the 2013 year class was analyzed using the 2013 baseline (Chapter II). Percent composition estimates using two methods are provided for each year class and age class: direct maximum-likelihood estimation (MLE) and maximum classification likelihood (MCL).

Year class	Age	N	Nursery of origin	Percent composition (\pm SD)	
				MLE	MCL
2012	1-2 years	21	<i>Eastern Atlantic</i>	69.1 (10.9)	67.3 (14.1)
			<i>Gulf of Mexico</i>	30.9 (10.9)	32.7 (14.1)
			<i>Caribbean Sea</i>	0.0 (0.0)	0.0 (0.0)
	2-3 years	107	<i>Eastern Atlantic</i>	42.0 (7.5)	35.7 (8.2)
			<i>Gulf of Mexico</i>	58.0 (7.5)	64.4 (8.2)
			<i>Caribbean Sea</i>	0.0 (0.0)	0.0 (0.0)
2013	1-2 years	41	<i>Cape Verde</i>	1.3 (1.8)	3.0 (3.2)
			<i>Gulf of Guinea</i>	48.5 (8.4)	47.7 (8.4)
			<i>Gulf of Mexico</i>	45.2 (8.2)	48.0 (8.2)
			<i>Caribbean Sea</i>	5.1 (4.0)	1.4 (2.7)
	2-3 years	6	<i>Cape Verde</i>	0.0 (0.0)	0.0 (0.0)
			<i>Gulf of Guinea</i>	67.5 (21.2)	69.0 (19.8)
			<i>Gulf of Mexico</i>	18.1 (19.3)	16.6 (17.6)
			<i>Caribbean Sea</i>	14.5 (14.5)	14.4 (14.8)

APPENDIX B

FIGURES

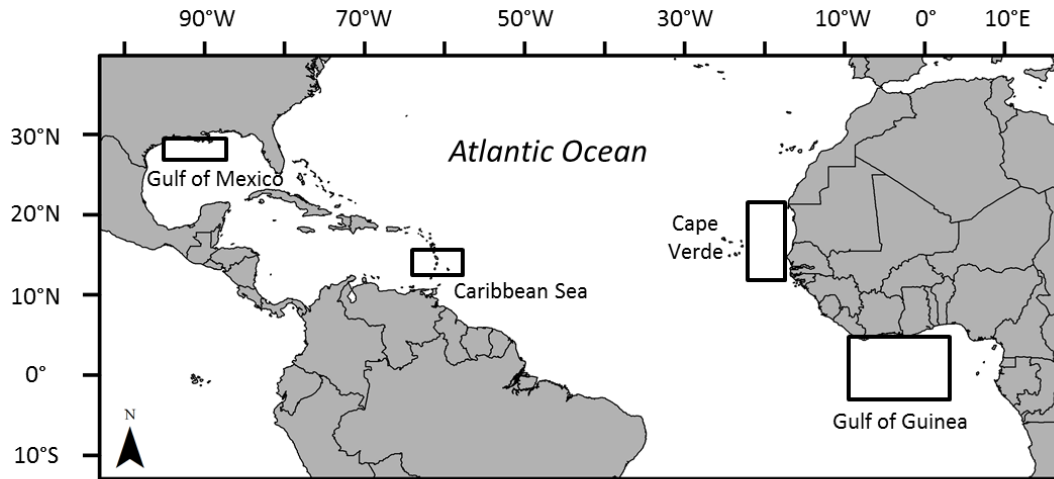


Figure 1. Map showing locations of the four nursery areas sampled for young-of-year (YOY) yellowfin tuna in the Atlantic Ocean: Gulf of Mexico, eastern Caribbean Sea, Cape Verde, and Gulf of Guinea. Approximate collection areas are denoted by black boxes.

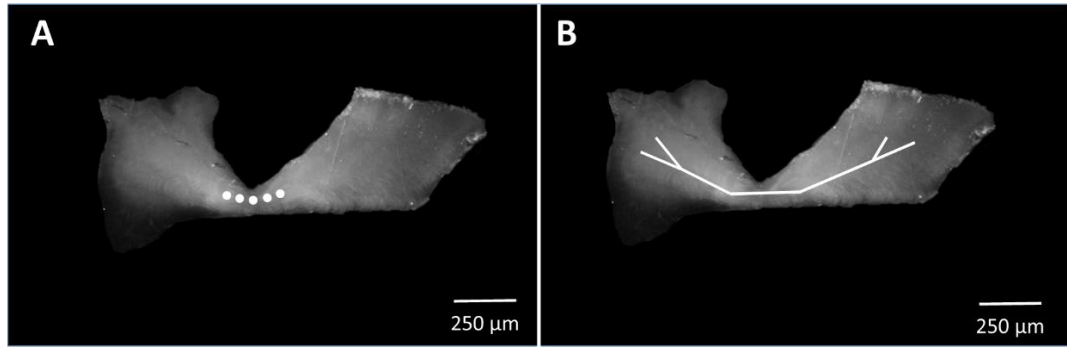


Figure 2. Transverse section of a young-of-year (YOY) yellowfin tuna otolith displaying A) the approximate location of laser ablation spots for trace element analysis and B) the MicroMill drill path used for stable isotope analysis. All material within 175 μm on each side of the drill path was isolated due to the width of the drill bit.

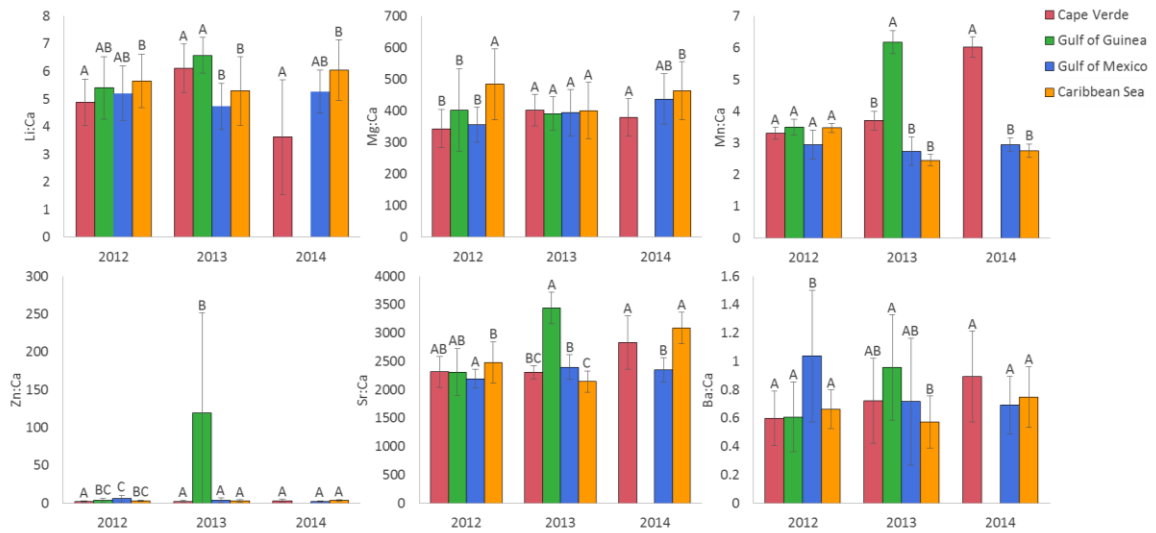


Figure 3. Mean (\pm SD) element:Ca ratios ($\mu\text{mol mol}^{-1}$) in otolith cores of young-of-the-year (YOY) yellowfin tuna collected from four nursery areas in the Atlantic Ocean. Lettering above each bar indicates Tukey's HSD pairwise comparisons results; for each region, values with different letters are significantly different ($p < 0.05$).

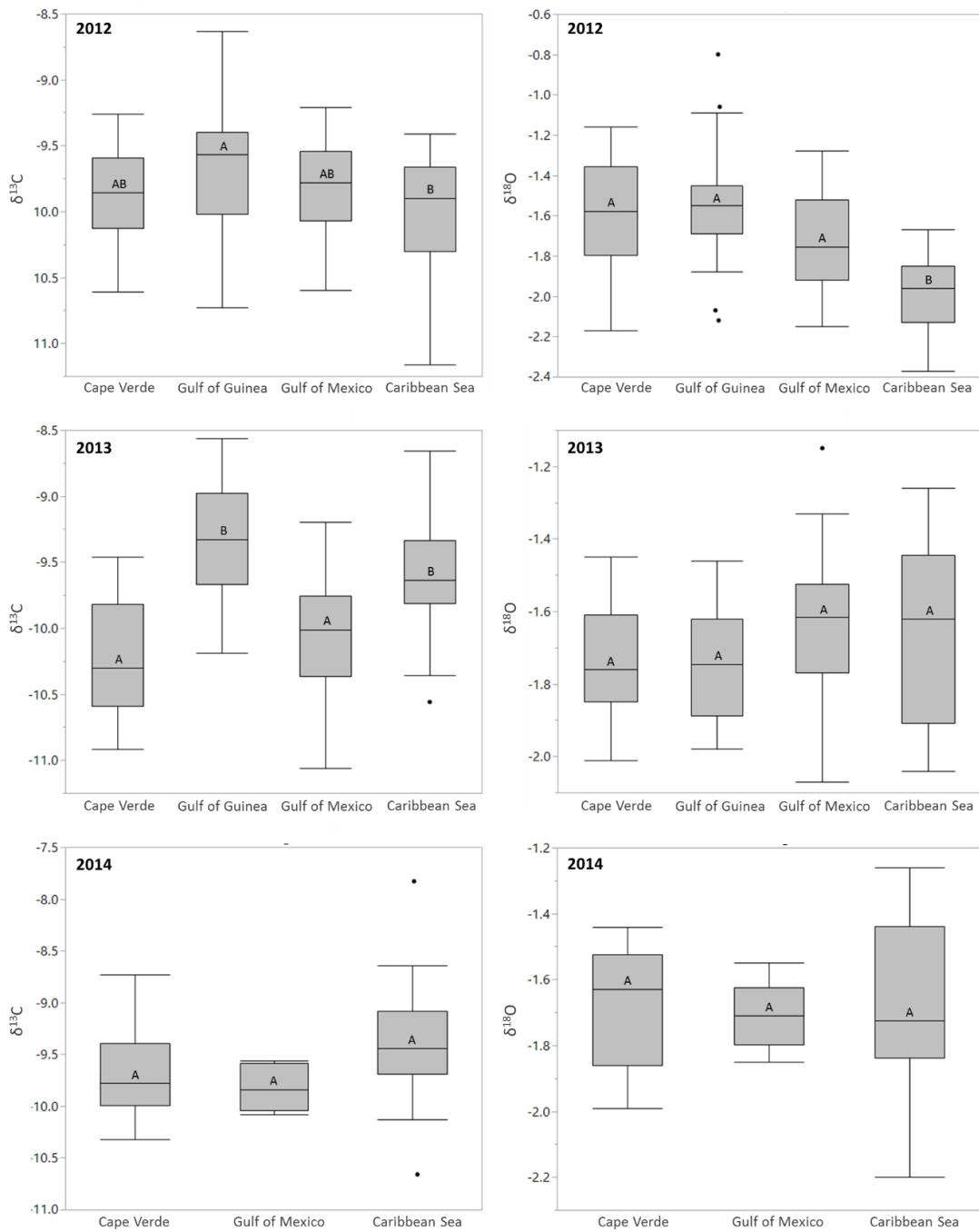


Figure 4. Box plots of otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for young-of-the-year (YOY) yellowfin tuna from four nursery areas in the Atlantic Ocean. Interquartile range (25th and 75th percentile) is shown by the extent of the boxes, and error bars extend to the outermost data points (excluding outliers). Median values are shown in boxes as black lines and lettering depicts significant regional differences (Tukey's HSD, $p < 0.05$).

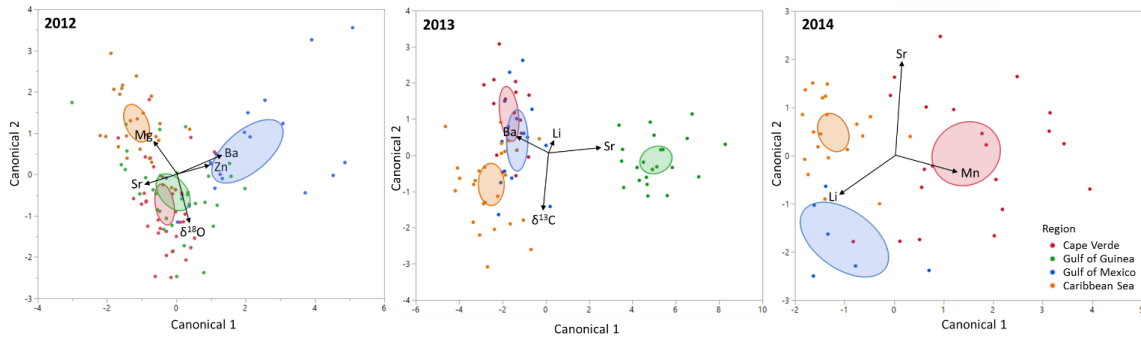


Figure 5. Canonical scores based on trace elements (Li:Ca, Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca, Ba:Ca) and stable isotopes ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) in young-of-the-year (YOY) yellowfin tuna otoliths collected from four nursery areas in the Atlantic Ocean: Cape Verde, Gulf of Guinea (2012+2013 only), Gulf of Mexico, and Caribbean Sea. Ellipses represent 95% confidence limits around each multivariate mean and biplot vectors show the relative influence of each element on regional discrimination.

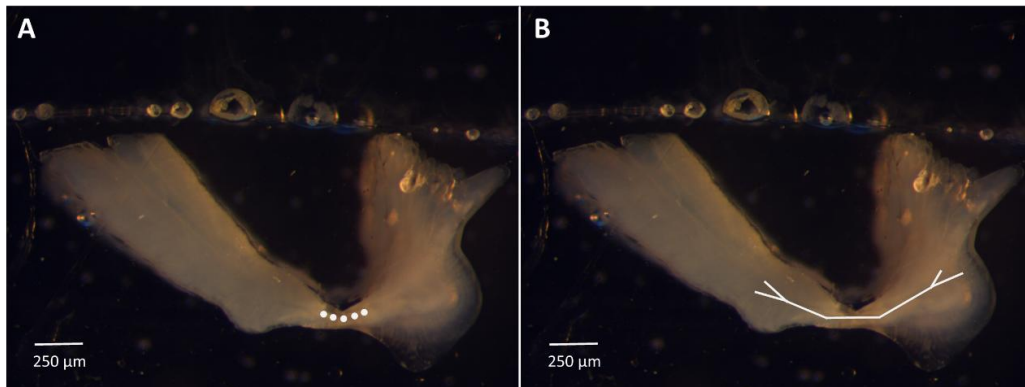


Figure 6. Transverse section of a sub-adult yellowfin tuna sagittal otolith showing A) the location of trace element ablation points and B) the drill path used to isolate the core region of the otolith for stable isotope analysis.

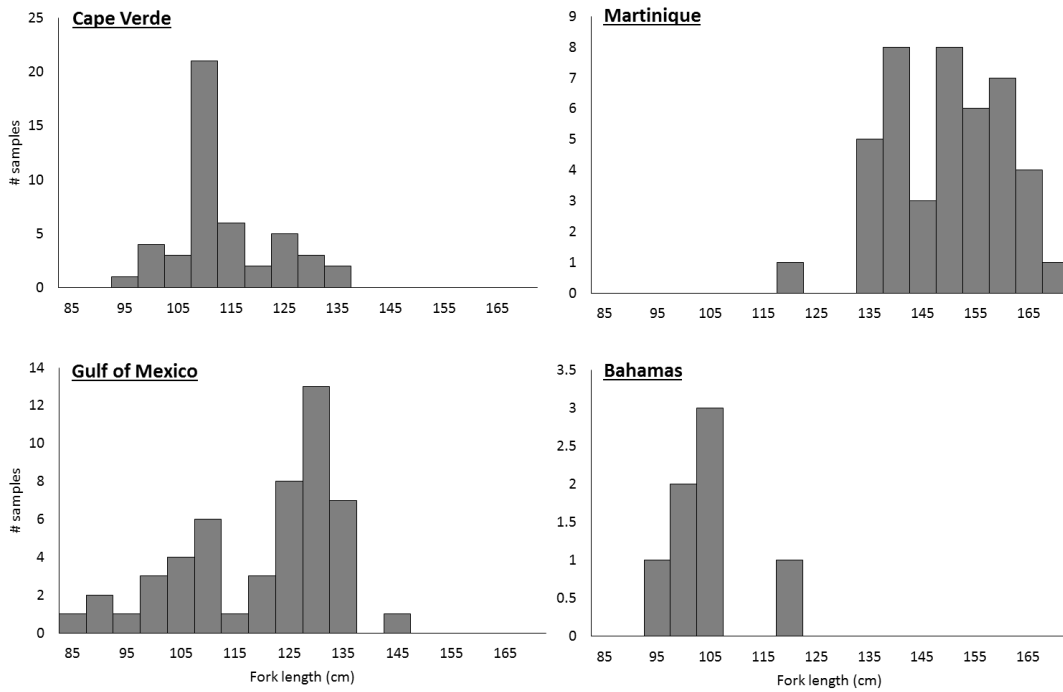


Figure 7. Fork length frequency by region for all sub-adult and adult yellowfin tuna collected in the Atlantic Ocean from 2014 to 2016.

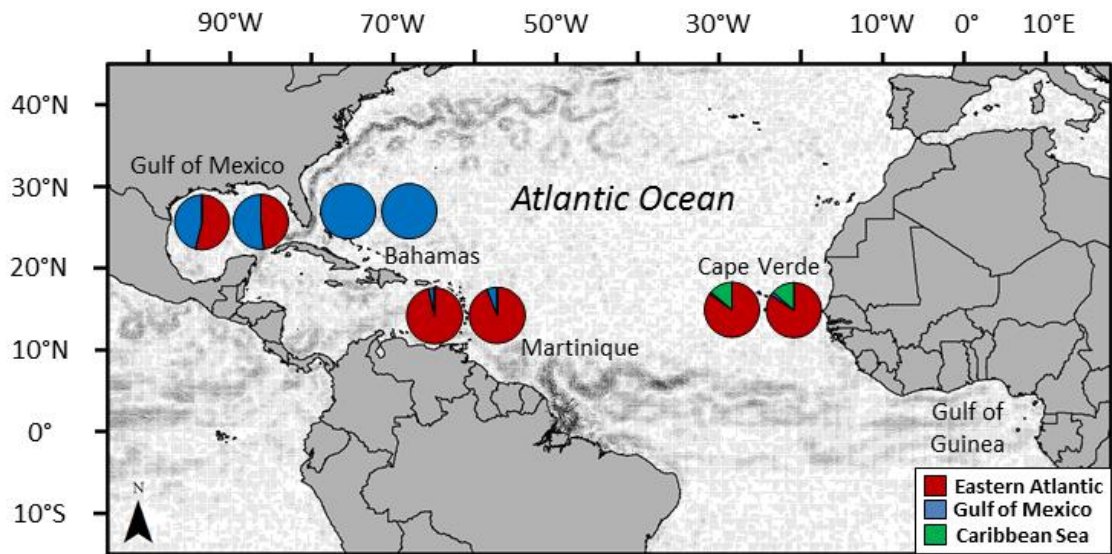


Figure 8. Map showing estimates of natal origin for sub-adult and adult yellowfin tuna captured in four regions in the Atlantic Ocean: the Gulf of Mexico, Martinique, Bahamas, and Cape Verde. Pie charts (MLE: left, MCL: right) indicate percentage of individuals originating from each nursery area (Eastern Atlantic, Gulf of Mexico, or Caribbean Sea). Vector lines show general ocean current patterns (downloaded from the Aviso database using the Marine Geospatial Ecology Toolbox in ArcGIS 10.2; Roberts et al., 2010).

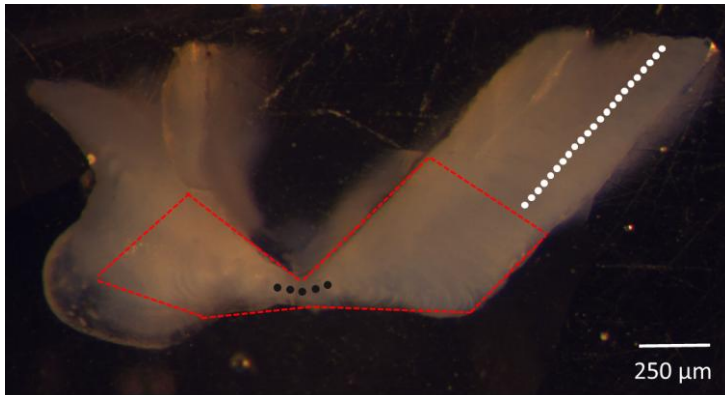


Figure 9. Transverse section of a sagittal otolith from a yellowfin tuna showing the position of laser ablation spots (white circles) in a life history transect. The red dashed lines outline the core region milled for stable isotope analysis and dark circles represent core ablation spots analyzed to determine nursery origin.

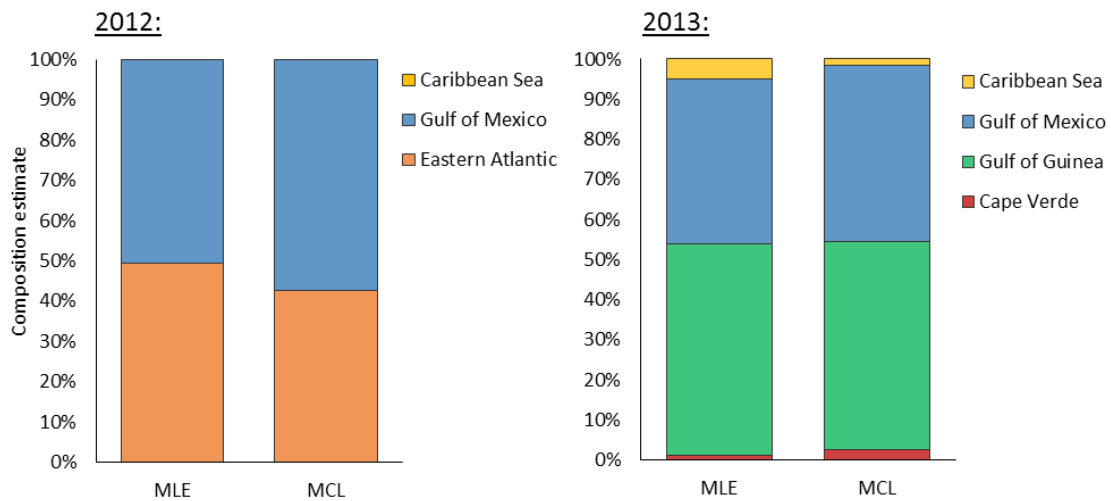


Figure 10. Predictions of nursery origin (percent by region) of sub-adult and adult yellowfin tuna collected in the Gulf of Mexico. Nursery origin estimates are shown for each year class; estimates for the 2012 year class were derived using the 2012 young-of-year baseline, while the 2013 year class was analyzed using the 2013 baseline. Results of direct maximum-likelihood estimation (MLE) and maximum classification likelihood (MCL) are shown on the left and right, respectively.

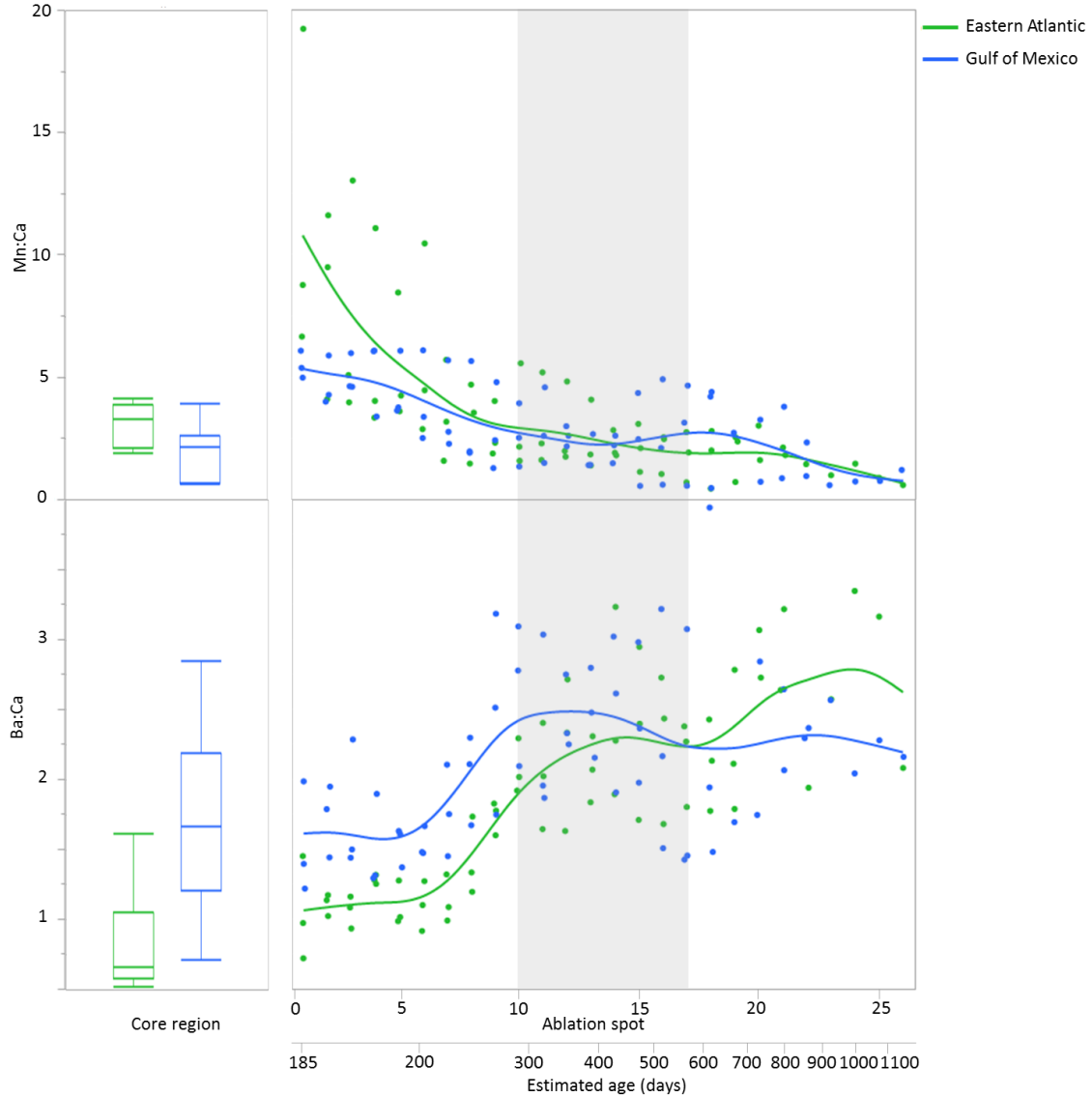


Figure 11. Mn:Ca and Ba:Ca ratios ($\mu\text{mol mol}^{-1}$) in otolith cores and life history transects of six yellowfin tuna collected in the Gulf of Mexico. Based on mixed-stock analysis, three of these individuals originated in the Gulf of Mexico (blue) and three were migrants from the eastern Atlantic (green). The box plot on the left shows the median core value for individuals from each region. Interquartile range (25th and 75th percentile) is shown by the extent of the boxes, and error bars extend to the outermost data points. Life history transects are shown on the right; the x-axis indicates the position of the ablation spot in each transect (with spot 1 being closest to the core of the otolith) and the corresponding estimated age (in days) based on otolith microstructure analysis. Points represent element:Ca values for each ablation spot, and each group of points was fitted with a cubic spine ($\lambda=0.05$) to show general trends. The gray shaded area highlights the portion of the transect where element:Ca values of both local and migrant yellowfin tuna appear to converge.