Characterization of Atlantic Bluefin Tuna Stock Structure Using Stable $\delta^{13}C$ & $\delta^{18}O$ Isotopes in Otoliths

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Abstract

Trace elements in otoliths of Atlantic bluefin tuna (*Thunnus thynnus*) have been used to delineate stocks; however, recent evidence suggests that classification success with this approach for discriminating eastern and western stocks is modest (60-80%). Here, we evaluate the utility of an alternative biomarker in otoliths, stable $\delta^{13}C$ and $\delta^{18}O$ isotopes. The goal of this work is to use $\delta^{13}C$ and $\delta^{18}O$ signatures to discriminate age-1 (yearling) bluefin tuna from eastern and western nurseries as well as predict the nursery origin of adults by matching signatures in otolith cores (correspond to first year of life) to our reference samples of yearlings signatures. $\delta^{18}O$ signatures in the otoliths of bluefin tuna collected in eastern and western nurseries were measured over a 5 year period, and $\delta^{18}O$ values were consistently enriched for yearlings from the Mediterranean. In contrast, $\delta^{13}C$ values in otoliths were similar between nurseries in all years investigated. Although significant interannual variation was observed for one of the stable isotopes ($\delta^{18}O$), cross-validated classification success from discriminant function analysis was still high (91-98%) when year classes were pooled by region. Stable isotope values of otolith cores from medium and giant category bluefin tuna, which represent the first year of life and correspond to whole otoliths signatures of yearlings, were assessed to determine natal origin of bluefin tuna caught in the Atlantic Ocean. Stable $\delta^{18}O$ values in the otolith cores of medium and giant category bluefin tuna were assessed from both the western Atlantic and Mediterranean Sea. Stable $\delta^{18}O$ values in otolith cores of over 50% of the medium and giant bluefin tuna collected in the U.S. recreational fishery ranged from -0.8 to -1.1 and fell within the confidence ellipse (1 SD) observed for yearling bluefin tuna from the Mediterranean. This suggests that a large fraction of bluefin tuna inhabiting the western Atlantic were produced from nurseries in the east. Conversely, otolith cores of medium and giant category bluefin tuna collected in the Mediterranean were very enriched (0 to -1.0) relative to western signatures and matched the signatures of yearlings from this region. Thus, only a small fraction (~12%) of the adult bluefin tuna collected in the Mediterranean were produced in western nurseries. Results suggest that trans-Atlantic mixing (east to west movement) of Mediterranean-born bluefin tuna is high, while movement of the western Atlantic stock to the Mediterranean waters in markedly lower.

Purpose

A. Description of Problem

Understanding population structure and mixing rates of Atlantic bluefin tuna (*Thunnus thynnus)* is critical to optimize utilization of this highly migratory species. Due in part to increased evidence of trans-Atlantic migrations from pop-up satellite archival tags (Lutcavage et al. 1999; Block et al. 2001, 2005), there has been increased scrutiny by scientists and resource representatives of the two-stock hypothesis. As a consequence, the two-stock management strategy currently used by the International Commission for the Conservation of Atlantic Tunas (ICCAT) remains unverified and there is a clear need for empirical methods to directly estimate the contributions of recruits originating from eastern (Mediterranean) and western (Gulf of Mexico) nurseries to the fisheries that depend upon these recruits. To date, the National Marine Fisheries Service and other agencies are currently supporting the development of three methods that may provide estimates of mixing rates: pop-up satellite archival tags, biochemical markers, and otolith chemistry (Magnuson 2001). While each technique may afford fishery managers with data on stock structure, future investigations using otolith chemistry may be the most viable since the approach predicts nursery origin and can be used to screen large numbers of individuals.

Otolith chemistry is increasingly used as a technique to differentiate stocks, and interest in its application as a recorder of time and environmental conditions has increased substantially in the past decade (Campana 1999, Thresher 1999; Secor and Rooker 2000; Campana and Thorrold 2001). Otoliths (ear stones) precipitate as the fish grows and elements from the water surrounding an individual are integrated into the aragonite-protein matrix. Since otoliths are metabolically inert, remobilization of newly deposited elements during ontogeny is negligible. Consequently, the chemical composition of otoliths may serve as natural tags or chemical signatures that reflect differences in the chemical composition of the individuals' habitat. Recent work suggests that otolith chemistry can be used to identify natal origin and assess the relative contribution of different nursery areas to mixed adult stocks (Thorrold et al. 1998, 2001). Moreover, the approach has been used recently to assess stock specificity of tunas, and findings suggest otolith elemental analysis has promise for assessing the population connectivity of pelagic stocks. Still, scientific evidence has been insufficient to support stock structure assumptions in the management of Atlantic bluefin tuna. Through past SK support, we have, (1) established field and laboratory protocols for chemical analysis of bluefin tuna otoliths (Rooker et al. 2001a); (2) used an otolith reference material to measure within and between laboratory precision of otolith chemical assays (Rooker et al. 2001a; Secor et al. 2002); (3) Conducted an inter-laboratory test for elemental signature differences between juvenile Atlantic bluefin tuna collected in western and eastern Atlantic nursery regions (Secor et al. 2002). (4) Investigated intra-nursery stability in elemental fingerprints across different spatial and time scales (Rooker et al. 2001b; Rooker et al. 2003). Early results indicated that otolith elemental fingerprints are significantly different between bluefin tuna nurseries, but insufficiently distinct to allow precise study of mixing rates (Secor et al. 2002).

While trace element signatures have been used successfully to delineate bluefin tuna from eastern and western nurseries, classification success was modest (60-80%) (Secor et al. 2002, Rooker et al. 2003), suggesting that accuracies must be improved before full-scale investigations of stock structure are attempted. The resolving power of the approach could be increased using two approaches. First, preconcentration procedures can be used in the future to eliminate matrix interferences on the inductively coupled plasma mass spectrometer (ICPMS), allowing analysts to accurately determine transition metal concentrations at nM to pM levels. This approach increases the pool of reliable elements that can be effectively quantified and is currently under investigation. Alternatively, stable isotope analysis ($\delta^{13}C$ and $\delta^{18}O$) of otoliths appears to represent a promising tool to differentiating fish stocks. Stable isotopes have been used extensively as recorders of environmental conditions, and $\delta^{13}C$ and $\delta^{18}O$ values in otoliths have been used successfully to discriminate individuals from different estuarine nurseries or stocks (Thorrold et al. 2001). Here, we assess the utility of stable $\delta^{13}C$ and $\delta^{18}O$ isotopes as a tool to discriminate yearling (age-1) bluefin tuna from eastern and western nurseries, and apply the approach to predict the nursery origin of sub-adults and adults collected in the western Atlantic Ocean.

B. Objectives of Project

1) Quantify stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in whole otoliths of age-1 Atlantic bluefin tuna from eastern and western nurseries

2) Assess the temporal stability of stable $\delta^{13}C$ and $\delta^{18}O$ isotope signatures over a three-year period in both eastern and western nurseries

3) Analyze stable isotopes for the juvenile portion of otoliths from sub-adult and adult bluefin tuna (medium and giant category) captured from eastern and western nurseries

4) Predict mixing rates of sub-adult or adult (medium and giant category) Atlantic bluefin tuna using stable isotope signatures

Approach

Description of work

Samples of age-1 bluefin tuna used for stable isotope analysis were collected in the Mediterranean Sea and Western Atlantic in 2002-2004 (combined with 1999 and 2000 samples). Sampling strategies used to procure age-1 (yearling) Atlantic bluefin (*Thunnus thynnus*) tuna varied between regions. In the Mediterranean Sea, age-1 individuals were either taken by sport fishermen using hand lines or by commercial long-line fishermen targeting albacore (*Thunnus alalunga*). Additional samples were provided by European colleagues (Dr. Gregorio DeMetrio, Dr. Enrique Rodriquez-Marin). Conversely, collections of tuna in the western Atlantic were made in New Jersey and Rhode Island waters using hook and line from recreational activities. . Collections of large school (66-135 lbs), medium (135-310 lbs) and giant (310+ lbs) category bluefin tuna were collected

in both the western Atlantic and Mediterranean Sea. Samples of large school, medium, and giant category bluefin tuna from the western Atlantic were provided by NOAA Fisheries (Steve Turner) collected under the Bluefin Tuna Program. Medium and giant bluefin tuna from a New England fishery were collected by Dr. Secor in cooperation with the Seabrook NH Yankee Fisherman's Fishing Cooperative. In the Mediterranean Sea, medium and giant category bluefin tuna were collected by Dr. Rooker from tuna farming operation in Marsaxlokk, Malta (Fish and Fish LTD) and Murcia, Spain (T.F.M-Tuna Farms of the Mediterraneo S.I.) in 2003 and 2004, respectively. In addition to collecting otoliths, tissue samples for genetic analysis were provided to Dr. John Graves, Virginia Institute for Marine Science).

Sagittal otoliths were extracted from freshly caught specimens, and a small number of samples were frozen prior to otolith extraction. Previous work on *Thunnus* spp. suggests that the effect of short-term freezing on otolith composition is negligible (Rooker et al., 2001a). Selection of a single otolith (i.e., right or left sagitta) for stable isotope analysis was based on random assignment. Before stable isotope analysis, whole otoliths and otolith cores were carefully cleaned. All reagents used were of ultra pure grade and all implements and containers were cleaned with dilute nitric acid (HNO_3) and rinsed with 18 megohm doubly deionized water ($DDIH_2O$). Whole otoliths of age-1 (yearling) bluefin tuna were powdered and used to further develop our library of signatures for eastern and western nurseries. Alternatively, high-resolution milling was used to isolate core from medium and giant category bluefin tuna. Prior to milling, sagittal otoliths were embedded in epoxy resin and sectioned using a low speed ISOMET saw to obtain a 1.5 mm transverse section through the core. This section was attached to a "blank" plastic section with thermoplastic glue and then attached to the plate of the Merchantek MicroMill System. An intervening piece of plastic between the slide and the sectioned otolith allowed the drill to completely pass through the otolith without striking the sample plate. Following attachment to the sample plate, the portion of the otolith corresponding to the first year of life was identified (via measurements from sectioned otoliths of yearling bluefin tuna), and the drill path was programmed into the MicroMill System (Fig. 1). Approximately 20 passes were made at 40-50 microns depth per path to isolate the core material, and surface profiling was used to correct for beveling in the section. Drill and path speed were stipulated based upon past previous work. The cored material was then displaced from the section and transferred to an acid washed vial. Following micro-milling (sub-adult and adult) cored otoliths were rinsed (20 s) in ultrapure HNO_3 and then rinsed in doubly deionized water. Similar to whole otoliths, cores were powdered for stable isotope analysis.

Carbon and oxygen stable isotopes were measured using a Finnegan Mat Delta Plus Mass Spectrometer maintained at the University of Maryland College Park Stable Isotope Laboratory. All analyses were conducted under supervision of Dr. Jay Kaufman (Department of Geology, Univ. MD College Park). Analytical precision of the mass spectrometer is 0.2 per mil. Stable δ^{13} C and δ18 O isotopes are reported relative to the PDB scale after comparison to an in-house laboratory standard that has been calibrated to PDB. Stable $\delta^{13}C$ and $\delta^{18}O$ isotopes are reported relative to the PDB scale after comparison to laboratory standards that were calibrated to PDB. Carbon dioxide gas was

evolved from each sample and standard powder by reaction with 100% phosphoric acid in an evacuated reaction tube. The tubes were suspended in a temperature-controlled circulating water bath at 50°C for a minimum of 30 minutes allowing time for complete reaction. Before analysis, evolved CO2 gas was transferred from the acid-reaction tube to a clean, dry, gas collection tube through a series of cold traps (liquid nitrogen and alcohol/dry ice) on a glass vacuum line. This transfer step protects the mass spectrometer from water vapor, acid vapor, carbonate powder, and incondensable gases.

To evaluate the reliability of our analytical and coring techniques, three tests were performed. First, an inter-laboratory comparison of the two labs processing our samples (University of Houston Lab versus University of Maryland Lab) was performed. This was deemed necessary since the UH lab processed 1999-2000, while samples (whole otoliths and otolith cores) from 2001 to the present were analyzed at the University of Maryland facility. Second, contamination related to handling and milling procedures are expected to be trivial for stable isotope analysis. Still, tests of embedding media were performed. Finally, we compared whole otoliths of yearlings to otolith cores of transverse sections of the same individuals to assess our ability to consistently micro-mill the core region associated with the first year of life. Whole otoliths and paired milled otolith "wings" were pulverized (powdered) using acid washed mortar and pestle.

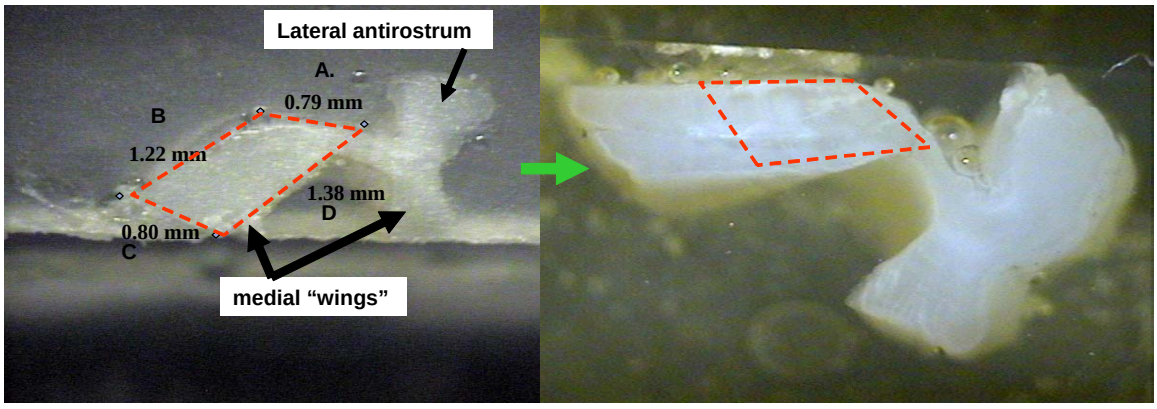


Figure 1. Micromilling procedure showing the location of the medial wing of an age-1 Atlantic bluefin tuna (*Thunnus thynnus*); measurements from transverse sections of age-1 specimens were used to develop the drilling path for medium and giant category bluefin tuna (example shown in right frame).

B. Project management

Drs. Jay R. Rooker (Texas A&M University, Department of Marine Biology & Department of Wildlife and Fisheries Sciences) and Dr. David Secor (University of Maryland, Chesapeake Biological Laboratory) served as Principal Investigators on the proposed study. Drs. Rooker and Secor managed all fisheries-related activities on the project (e.g. tuna collections and otolith extractions, cleaning, and preparation for the stable isotope mass spectrometer, micromilling core sections, data analysis). Dr. Jay Kaufman (University of Maryland-College Park, Department of Geology) performed stable isotope runs on powdered samples provided by Drs. Rooker or Secor.

Findings

A. Actual accomplishments and findings

Evaluation of Protocol

We initially estimated precision by examining separate mass spectrometry runs on the same otolith. Mean machine error for medium-category Atlantic bluefin tuna was 2±1% and 8±6%, respectively for $\delta^{13}C$ and $\delta^{18}O$. In addition, paired sagittal otoliths (n =14) were run at two different stable isotope facilities (University of Houston versus University of Maryland) to see if differences occurred between labs. Paired tests between labs showed there was no inter-laboratory effect for $\delta^{13}C$ (mean difference = 0.03 mil; $p = 0.73$); however, a significant effect was observed for $\delta^{18}O$ (mean difference = 0.25 mil; $p < 0.001$). Thus, a correction factor was applied to yearling signatures from the University of Houston to standardize our signature data (correction factor: 0.25 per mil addition to $\delta^{18}O$ from UH).

Since cored regions of otoliths from medium and giant category bluefin tuna were used to represent the age-1 $\delta^{13}C$ and $\delta^{18}O$ signature, paired core versus whole otolith comparisons (based on using the left and right sagittal otolith from the same individual) were conducted for yearling bluefin tuna No consistent depletion or enrichment in core material in comparison to the entire otolith occurred, and paired t-tests showed no significant difference between core and whole otoliths in either $\delta^{13}C$ (mean difference = 0.12 mil; $p=0.33$); or $\delta^{18}O$ (mean difference = 0.07 mil; $p=0.26$). Mean error between core and whole otoliths was 2% and 6% for $\delta^{13}C$ and $\delta^{18}O$, which was similar to the estimated precision of replicated runs.

Analysis of Western versus Eastern Atlantic bluefin tuna

Stable isotopic composition of otoliths of age-1 Atlantic bluefin tuna from 2001-2003 were analyzed at the University of Maryland-College Park (Facility Manager: Dr. Jay Kaufman, Department of Geology) and added to our reference samples of yearlings, which included yearlings from 1999 and 2000. Multivariate analysis of variance indicated that stable isotopes of age-1 bluefin tuna collected in the Mediterranean and western Atlantic nurseries differed significantly (MANOVA $p < 0.001$). Univariate contrasts of several year classes of stable isotopic composition of yearling bluefin tuna

otoliths indicated that $\delta^{18}O$ values of individuals collected from the Mediterranean were enriched relative to the western Atlantic. Cross-validated classification success from discriminant function analysis (DFA) for all year classes combined was 98% (Fig. 2). It should be noted that samples from 2001 from the western Atlantic were not included in our original assessment shown in Figure 2 because the data appeared too enriched relative to signatures of yearlings from all other years assessed. In fact, $\delta^{18}O$ values were enriched and fell into the general range of yearlings collected in the Mediterranean, possibly indicating trans-Atlantic movement occurred (east to west movement during the first 12+ months of life). Yearling bluefin tuna in the Pacific commonly display trans-oceanic movement behaviors and thus it is possible that these individuals were of Mediterranean origin. Nevertheless, we opted for a model that included all year classes and samples (Fig. 3), and cross-validated classification success from DFA was only slightly reduced from previous model (MANOVA $p < 0.001$; classification success: total = 91%, east = 98%, west = 79%).

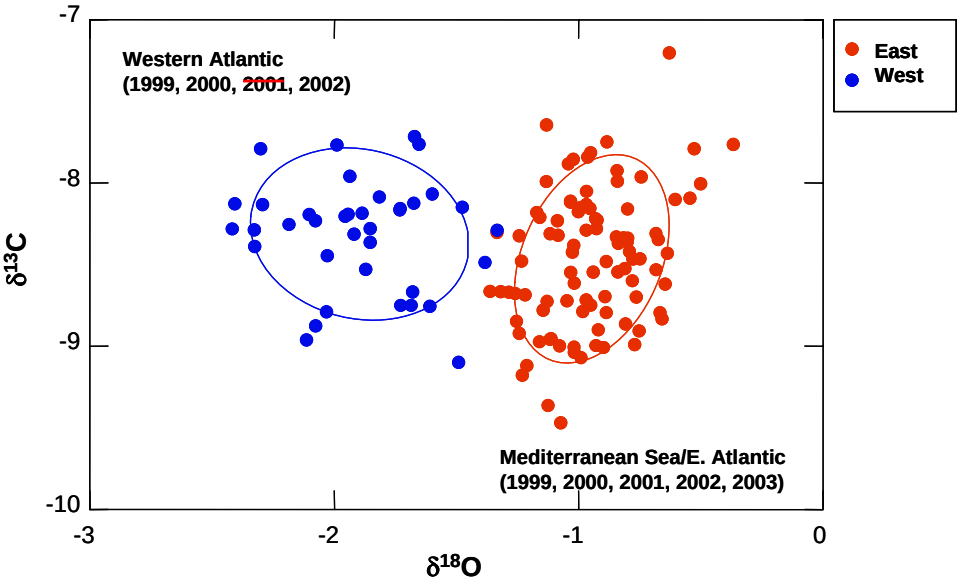


Figure 2. Stable $\delta^{13}C$ and $\delta^{18}O$ signatures in the whole otoliths of age-1 (yearling) bluefin tuna collected in eastern and western nurseries from 1999-2003. Confidence ellipses (1 SD) shown for east and west groups. Note: 2001 year class of yearlings not included here.

Temporal variability in yearling signatures was examined over several years in both the east and west (5 year classes and 4 year classes, respectively). A significant interannual effect was detected for $\delta^{18}O$; however, $\delta^{13}C$ values were similar among all years examined. Regional variability (within Mediterranean) was also examined (Fig. 4), and chemical signatures of yearling bluefin tuna from different seas (Tyrrhenian Sea, Ligurian Sea, Adriatic Sea, and East Atlantic-Bay of Biscay) were relatively similar and the difference in signatures between nurseries (east and west) was greater than temporal or regional variability.

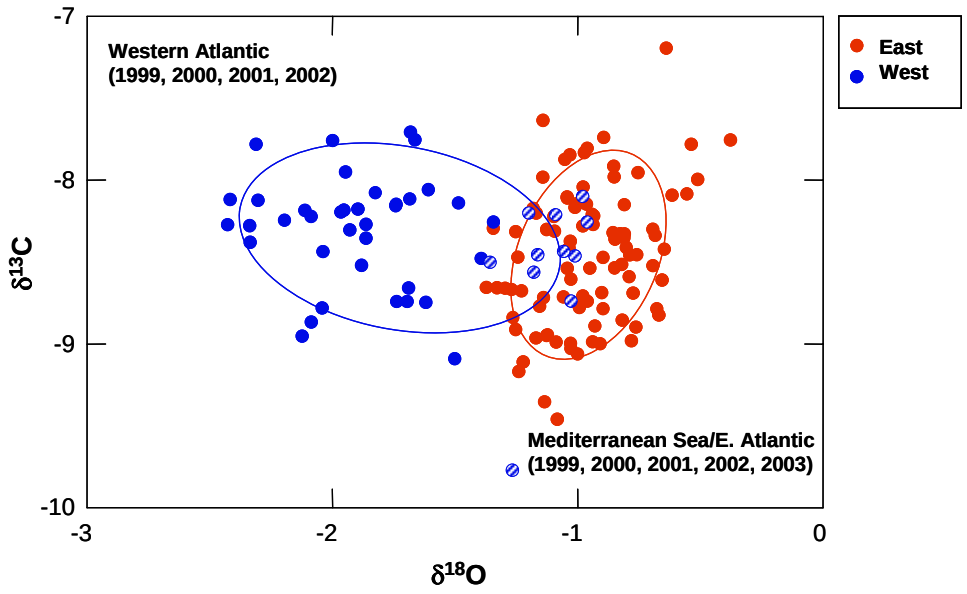


Figure 3. Stable $\delta^{13}C$ and $\delta^{18}O$ signatures in the whole otoliths of age-1 (yearling) bluefin tuna collected in eastern and western nurseries from 1999-2003. Confidence ellipses (1 SD) shown for east and west groups. 2001 year class from western Atlantic included in this model (blue circles with diagonal lines).

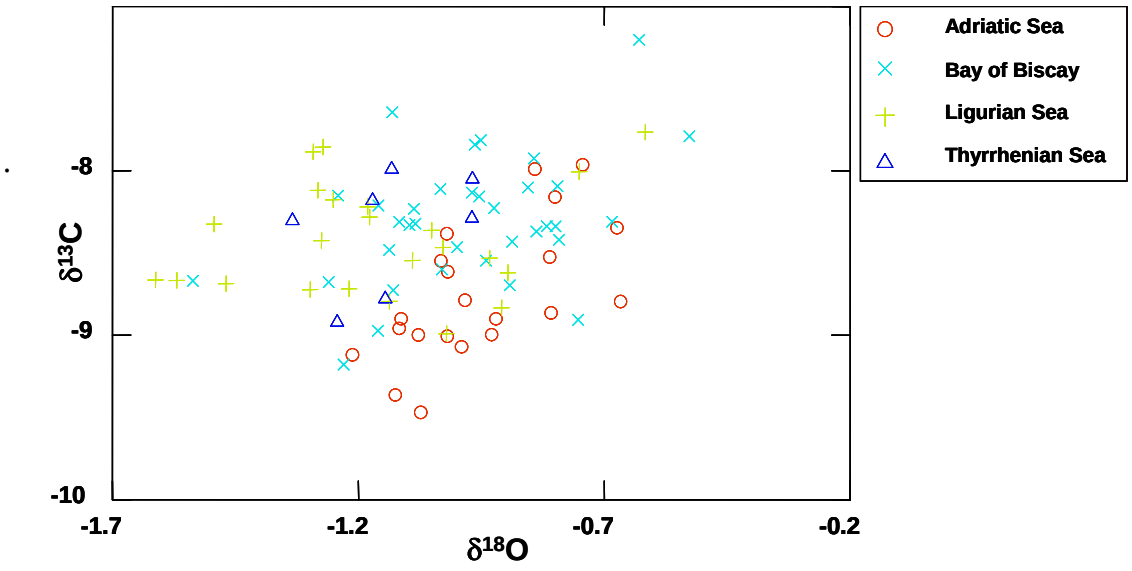
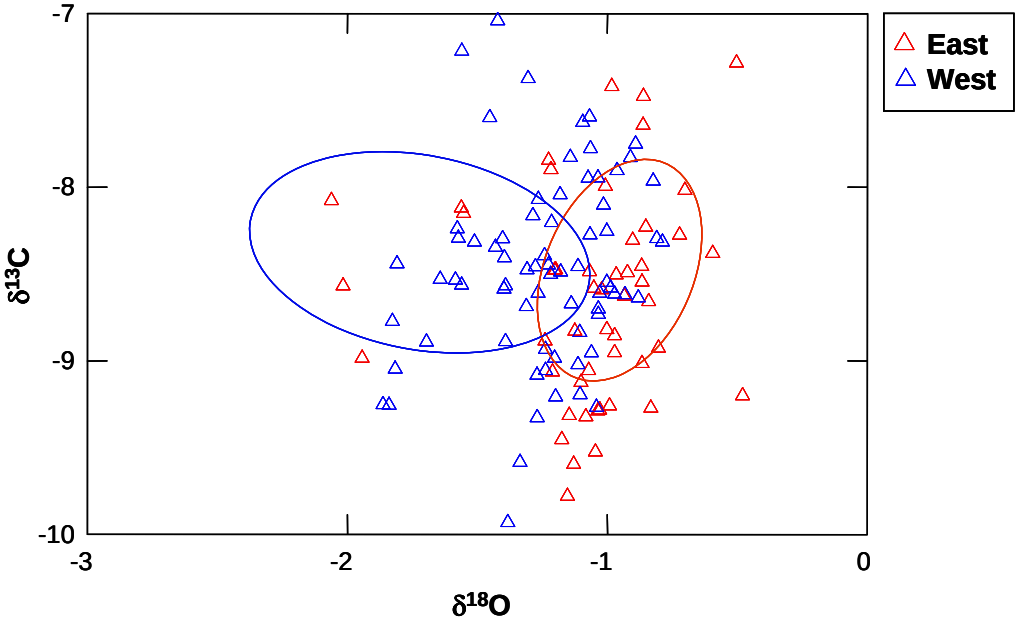


Figure 4. Stable $\delta^{13}C$ and $\delta^{18}O$ signatures in the whole otoliths of age-1 (yearling) bluefin tuna collected in the Eastern Atlantic from 1999-2003.

Otolith cores of over 200 medium and giant category Atlantic bluefin from the western Atlantic and Mediterranean have been isolated. To date, stable isotopes have been measured in the otolith cores in over 120 of these individuals. Stable isotope values of otolith cores from medium and giant category bluefin tuna, which represent the first year of life and correspond to whole otoliths signatures of yearlings, were assessed to determine natal origin of bluefin tuna caught in the Atlantic Ocean. Stable $\delta^{18}O$ values in the otolith cores of medium and giant category bluefin tuna were assessed from both the western Atlantic and Mediterranean Sea. Stable $\delta^{18}O$ values in otolith cores of over 50% of the medium and giant bluefin tuna collected in the U.S. recreational fishery ranged from -0.8 to -1.1 and fell within the confidence ellipse (1 SD) observed for yearling bluefin tuna from the Mediterranean (Fig. 5). This suggests that a large fraction of bluefin tuna inhabiting the western Atlantic were produced from nurseries in the east. Conversely, otolith cores of medium and giant category bluefin tuna collected in the Mediterranean were enriched (0 to -1.0) relative to western signatures and matched the signatures of yearlings from this region. Thus, only a small fraction (~12%) of the adult bluefin tuna collected in the Mediterranean were produced in western nurseries. Results suggest that trans-Atlantic mixing (east to west movement) of Mediterranean-born bluefin tuna is high, while movement of the western Atlantic stock to the Mediterranean waters in markedly lower.



	Nursery		
	East	West	Unknown
ABT Collected in East	21 (88%)	4 (12%)	25
ABT Collection in West	23 (52%)	21 (48%)	27

Figure 5. Stable $\delta^{13}C$ and $\delta^{18}O$ signatures in otoliths cores of medium and giant category Atlantic bluefin tuna collected from the east and west. Confidence ellipses (1 SD) from age-1 model (east = red; west = blue).

Results presented here indicate stable isotopes appear to hold considerable promise as a tool to discriminate stocks of bluefin tuna and appear to be more useful predictors of nursery origin than trace elements in otoliths (Secor et al. 2002, Rooker et al. 2003). Stable isotopes are much less likely to be contaminated by the drilling procedure than are trace elements, and thus contamination effects that often complicate trace element interpretation may not apply to stable isotope analysis. Still, we believe precaution must be exercised when interpreting these data and a conservative approach is warranted, particularly since the reference data set (signatures of yearlings) may not include all possible signatures for eastern and western nurseries. Further, there is a need to fine tune the core isolation procedure to minimize the observed variability in the core signatures to improve assignment procedures.

B. Description of need for additional work

Otolith cores of approximately 70 additional medium and giant category bluefin tuna from the western Atlantic are at the stable isotope facility in Maryland, and will likely be processed in the next 4-6 weeks. Once the final batch of samples is processed, more sophisticated assignment methods or mixed stock analysis procedures (maximum likelihood and Bayesian) will be employed to determine the nursery origin of medium and giant category bluefin tuna collected in the east and west. Also, to avoid assignment errors, posterior probabilities will be examined from discriminant function analysis to determine the confidence with which otolith cores from adults are classified to their respective nursery.

VII. Evaluation

A. Goals met; modifications required

No significant problems were encountered and goals of the project were met.

B. Dissemination of Project Results

Publications and Project Outcomes

In addition to the papers and presentations listed below, we are currently working on two separate papers (ICCAT document, Marine Ecology Progress Series) that will be submitted in the early fall.

Papers

Rooker JR, Secor DH (2004) Stock structure and mixing of Atlantic bluefin tuna: evidence from stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in otoliths. ICCAT Collective Volume of Scientific Papers 56(3) 1115-1120.

Rooker, J.R., D.H. Secor, V.S. Zdanowicz, G.De Metrio, and L.O. Relini (2003) Identification of Atlantic Bluefin tuna stocks from putative nurseries using otolith chemistry. Fish. Oceanogr. 12 (2): 75-84.

Platform Presentations

Rooker JR, Secor DH (2005) Evidence of trans-Atlantic movement of bluefin tuna from chemical signatures in otoliths. 56th International Tuna Conference, Lake Arrowhead California.

Rooker JR, Secor DH (2005) Trans-Atlantic mixing of Atlantic bluefin tuna: evidence from stable isotopes in otoliths. 135th Annual Meeting of the American Fisheries Society, Anchorage Alaska.

Rooker JR (2004) Ecology and stock structure of fishes in the Gulf of Mexico: application of novel approaches to answer age old questions? Department of Zoology, University of Bari, Italy

Rooker JR (2004) Stock structure and mixing rates of Atlantic bluefin tuna. Italian Large Pelagic Fish Working Group Meeting, University of Genoa, Italy

Rooker JR (2004) Ecology and stock structure of Atlantic bluefin tuna. Department of Animal Health and Well-being, University of Bari, Italy

Rooker JR (2004) Stock structure of Atlantic bluefin tuna determined from stable isotope analysis. Spanish Institute of Oceanography— Centro Oceanografico de Murcia, Spain

Rooker JR (2004) Ecology of pelagic fishes in the western Atlantic: application of novel approaches to answer age old questions?" Population structure of are the answers in the ear stones? Spanish Institute of Oceanography— Centro Oceanografico de Santander, Spain

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