Final
Technical Report

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Assessment of natal origin and stock structure of Atlantic bluefin tuna using otolith elemental fingerprints

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Introduction

Understanding population structure and trans-oceanic movement of Atlantic bluefin tuna is critical to optimize utilization of this highly migratory species. Due in part to increased evidence of trans-Atlantic migrations, there has been increased scrutiny by scientists and resource representatives of the two-stock hypothesis, which guides stock assessments and management projections for western Atlantic bluefin tuna. There is strong belief by commercial and recreational sectors of the bluefin tuna fishery that the harvest of undersized (sublegal) juveniles in the Eastern Atlantic is curtailing the recovery of Western stock bluefin tuna, despite stringent harvest limits that have been promulgated upon North American fisheries. An Inter-sessional Conference of ICCAT on mixing will address methods to incorporate pan-oceanic migrations by Atlantic bluefin tuna in future stock assessments under the current two stock concept. There is also a call to investigate other population structures, such as metapopulations (populations linked through migration) that currently guide Pacific salmon conservation efforts in the U.S. Whichever population structure emerges as most reasonable or useful, there is a clear need for empirical methods to directly estimate the contributions of recruits originating from eastern (Mediterranean) and western nurseries (Gulf of Mexico) to the fisheries that depend upon these recruits.

The goal of this study is to examine the two-stock issue for Atlantic bluefin tuna (*Thunnus thynnus*) using otolith chemistry. Specifically, otolith chemistry (trace elements in otoliths) of juveniles from eastern and western Atlantic stocks (i.e. nurseries) is being used to assess the discriminatory power of otolith elemental fingerprints for stock identification. To date, we have examined otolith chemistry of young bluefin tuna (age-0 & age-1) from the eastern (Mediterranean Sea) and western Atlantic, and assumed that no transoceanic migration activity occurred. Spatial stability of otolith elemental fingerprints was also examined on a smaller scale by measuring the otolith elemental composition of individuals from putative sub-nurseries within the Mediterranean Sea. In addition, the temporal stability and predictive potential of these natural tags were investigated by contrasting otolith chemistry of two year-classes of age-0 *T. thynnus*. Finally, age-0 and age-1 *T. thynnus* collected from the same nursery were compared to assess age-specific differences in otolith chemistry.

Specific objectives

Objective 1. Determine the spatial stability of trace element fingerprints of age-0 and age-1 Atlantic bluefin tuna

Objective 2. Determine the temporal stability of trace element fingerprints of age-0 and age-1 Atlantic bluefin tuna

Objective 3. Develop a micromill procedure for isolating the core material for the determination natal origin of larger Atlantic bluefin tuna (age-2 to age-4+).

Work to date

ABT Collections

Sampling efforts to date have been directed at collecting juvenile bluefin tuna from discrete regions within the two principal habitats for age-0 and age-1 bluefin tuna. Drs. Jay Rooker and David Secor travel to the Mediterranean in 2002 (last years trip was cancelled due to security reasons). Drs. Rooker and Secor worked with several scientists in the Spain, Italy, and France to obtain otolith samples, including Dr. Gregorio DeMetrio (University of Bari, Italy) and Enrique Rodriquez-Marin (Spain), Dr. Alfonso Zerbi (University of Montpellier), Dr. Adolfo Lima (ICCAT, Madrid Spain). In the Mediterranean, both P.I.s sampled yearling and YOY bluefin tuna during October 2002. Dr. Gregorio DeMetrio (Bari, Italy) had retained 15 YOY bluefin from 2001 and 76 YOY from 2002 from which we extracted otoliths and tissue samples (provided to Dr. John Graves for genetic analysis). Yearlings (9) were collected from the Madrid fish market. In addition, Jay Rooker and visited with Dr. Enrique Rodriguez-Marin in Spain to obtain a larger sample on 1-year olds (~150 collected between 1998-2002). Dr. Rodriguez-Marin recently sent Dr. Rooker 170 otoliths from age-1 and age-2 bluefin collected in the eastern Atlantic and Mediterranean Sea. In addition, we are working on collection of medium size bluefin tuna through our contacts in the Mediterranean and through directed sampling and archived (NMFS) samples in the Western Atlantic Also, Drs. Rooker and Secor are working closely with NMFS scientists (Dr. Pamela Mace, Dr. Steven Turner) to collect samples in the western Atlantic. Dr. Secor chartered trips out of Point Pleasant (Hudson Canyon) and Cape Hatteras, New Jersey in the late summer and early fall. 13 yearlings were sampled by charter boat off of New Jersey. Samples were low this year, in part due to inclement weather in the fall, but also perhaps by continuing poor recruitments by western stock population as indicated by SCRS (ICCAT) stock assessments. During July-September, D. Secor frequently visited Ocean City Maryland, where he and his lab members sampled 53 medium school (FL=100 - 160 cm) bluefin tuna for otoliths.

Otolith elemental fingerprinting

To date, have successfully completed all three objectives outlined above and are currently isolating core material for predicting the origin of age 4+ Atlantic bluefin tuna. Thus, we are close to providing a direct test of the two-stock hypothesis (i.e. is there significant mixing between eastern and western nurseries?). Otolith chemistry of Atlantic bluefin tuna (*Thunnus thynnus*) from three cohorts (year classes) has been quantified to assess the feasibility of using these natural tags to discriminate juveniles (age-0 and age-1) from putative nurseries. Specifically, a suite of six elements (Li, Mg, Ca, Mn, Sr and Ba) was measured in whole otoliths using solution-based inductively coupled plasma mass spectrometry. Otolith chemistry of age-1 *T. thynnus* collected from the two primary nurseries in the Mediterranean Sea and western Atlantic Ocean differed significantly, with a cross-validated classification accuracy of 70-85% (Fig. 1).

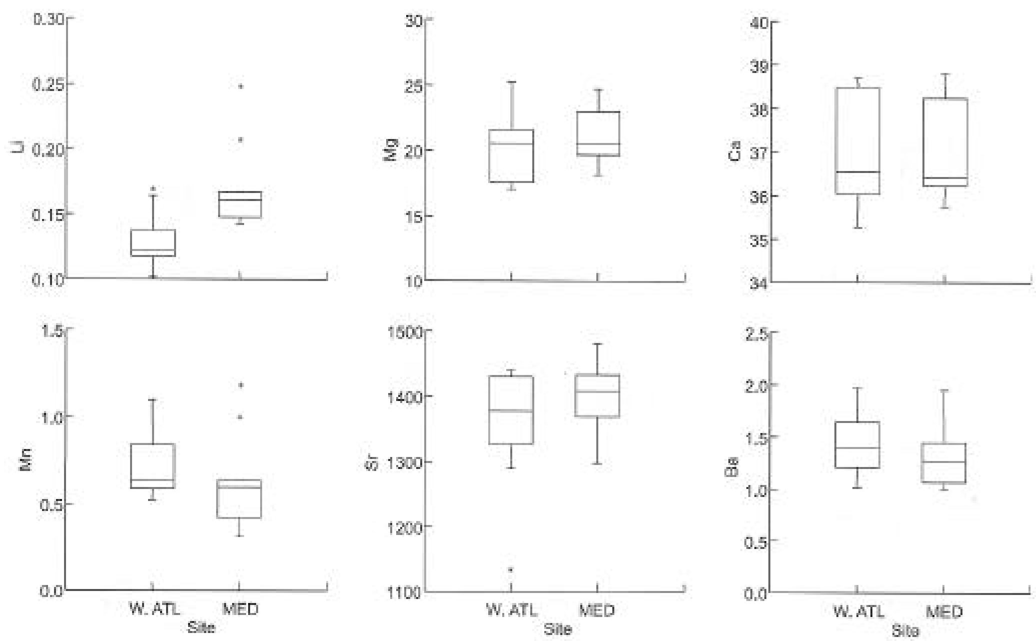

Figure 1. Box plots of elemental concentrations of otoliths from age-1 Atlantic bluefin tuna collected in 1999 from western Atlantic and Mediterranean nurseries. Interquartile ranges (25th and 75th percentile) are shown by extend of boxes and horizontal line represent medians (50th percentile). Concentration in ppm with the exception of Ca (%).

Spatial and temporal variation in otolith chemistry was evaluated for age-0 *T. thynnus* collected from three nurseries within the Mediterranean Sea: Alboran Sea (Spain), Ligurian Sea (northern Italy), and Tyrrhenian Sea (southern Italy). Distinct differences in otolith chemistry were detected among Mediterranean nurseries and classification accuracies ranged from 62 to 80% (Fig. 2). Interannual trends in otolith chemistry were observed between year classes of age-0 *T. thynnus* in the Alboran Sea; however, no differences were detected between year classes in the Tyrrhenian Sea (Fig. 3). Age-0 and age-1 *T. thynnus* collected from the same region

(Ligurian Sea) were also compared and distinct differences in otolith chemistry were observed, indicating ontogenetic shifts in habitat or elemental discrimination. Findings suggest that otolith chemistry of juvenile *T. thynnus* from different nurseries are distinct and chemical signatures show some degree of temporal persistence, indicating the technique has considerable potential for use in future assessments of population connectivity and stock structure of *T. thynnus* (Please see attached galley proofs of upcoming article in the journal *Fisheries Oceanography* by Rooker et. al. 2003).

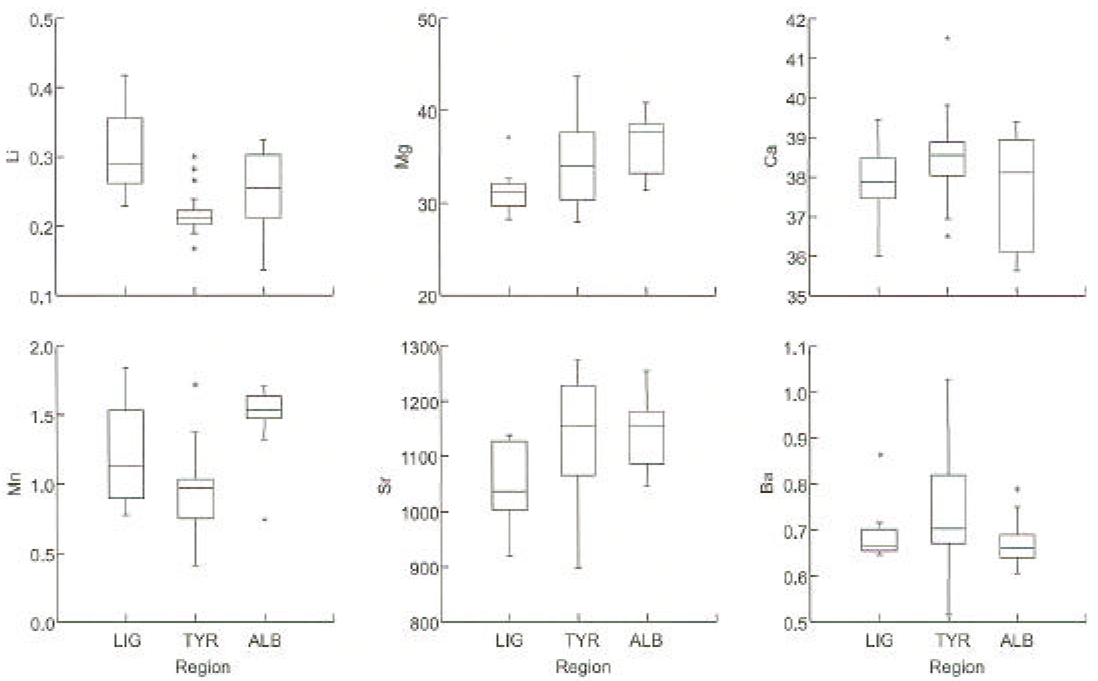

Figure 2. Box plots of elemental concentrations of otoliths from age-0 Atlantic bluefin tuna collected in 1999 from three putative nurseries in the Mediterranean Sea: Alboran (ALB), Ligurian (LIG), Tyrrhenian (TYR).

Contract products

Several peer-reviewed papers resulted from SK funded research on Atlantic bluefin tuna. Three manuscripts (#1-3) are products of the current SK grant (*NA07FD0176)*.

1. Rooker JR, Secor DH, Zdanowicz VS, DeMetrio G, Relini LO (*2003*) Identification of northern bluefin tuna stocks from putative nurseries in the Mediterranean Sea and western Atlantic Ocean using otolith chemistry. *Fisheries Oceanography* 12: 75-84

2. Secor DH, Campana SE, Zdanowicz VS, Lam JWH, McLaren JW, Rooker JR (*2002*) Inter-laboratory comparison of Atlantic and Mediterranean bluefin tuna otolith microconstituents. *ICES Journal of Marine Science59:* 1294-1304

3. Rooker JR, Secor DH, Zdanowicz VS, Relini LO, Santamaria N, Deflorio M, Palandri G, Relini M (2002) Otolith elemental fingerprints of Atlantic bluefin tuna from eastern and western nurseries. *Col. Vol. Sci. Pap. ICCAT 54:498-506*

4. Rooker JR, Secor DH, Zdanowicz VS, Itoh T (2001a) Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. *Marine Ecology Progress Series* 218: 275-282

5. Rooker JR, Zdanowicz VS, Secor DH. (2001b) Otolith chemistry of tunas: assessment of base composition and post-mortem handling effects. *Marine Biology* 139: 35-43

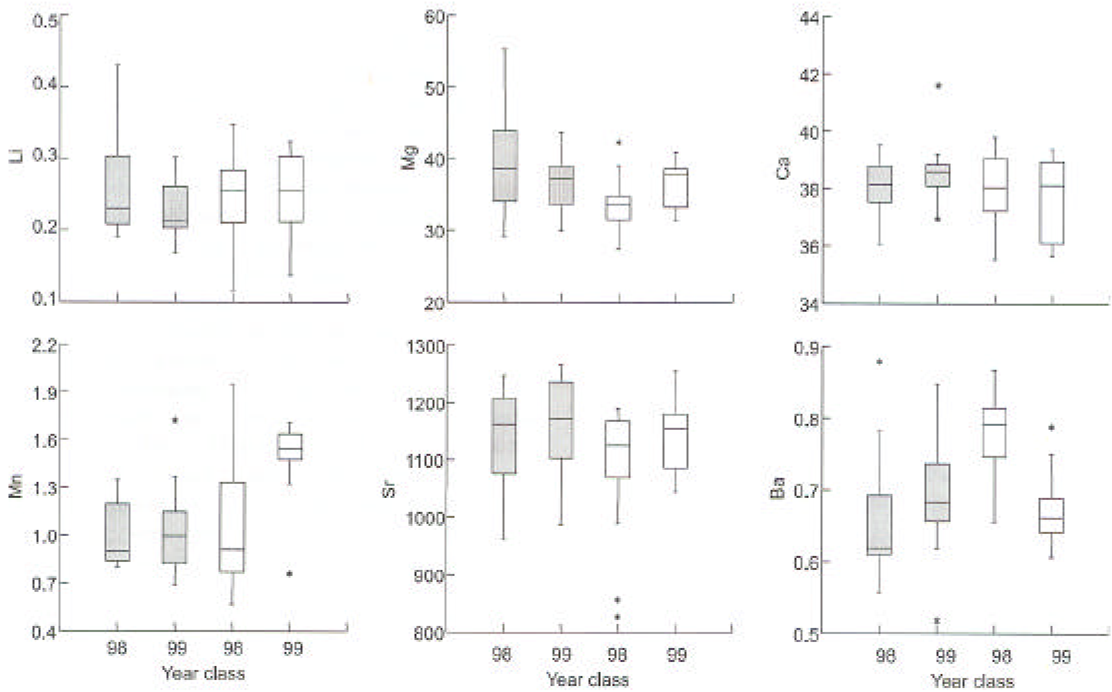

Figure 3. Box plots of elemental concentrations of otoliths from 2 year classes of age-0 Atlantic bluefin tuna from two nurseries in the Mediterranean Sea: Alboran (open) and Tyrrhenian (shaded).

Work in progress

Stable isotopes

In addition to trace element fingerprinting of Atlantic bluefin tuna, we recently assessed the utility of stable isotopes and preliminary results suggest that stable $\delta^{13}C$ and $\delta^{18}O$ isotopes are powerful and reliable markers of nursery origin. Stable $\delta^{13}C$ and $\delta^{18}O$ isotope values from otoliths of age-1 Atlantic bluefin tuna collected from eastern and westerns nurseries in 1999 and 2000 were distinct, and total classification success (cross validated) for individuals from the east and west was 100% (based on discriminant analysis of $\delta^{13}C$ and $\delta^{18}O$ values). Interestingly, Radtke (1987) reported $\delta^{13}C$ and $\delta^{18}O$ values from otolith cores of giant Atlantic bluefin tuna (2.2 –2.7 m fork length) caught in the Western Atlantic and, when compared to our signatures, otolith cores of all three giants examined matched the Mediterranean signature, suggesting that they crossed the Atlantic. Based on the these findings, we plan to use both trace element chemistry and stable isotopes in future predictions of natal origin.

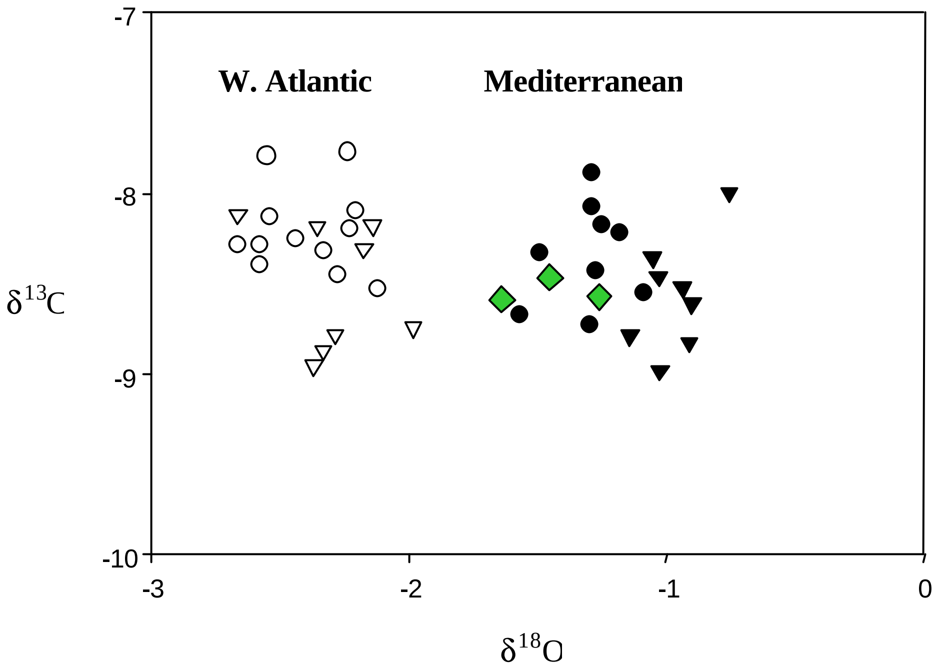

Figure 4. Stable $\delta^{13}C$ and $\delta^{18}O$ isotope values from otoliths of age-1 Atlantic bluefin tuna (*Thunnus thynnus*) collected from Eastern (solid symbols) and Western (open symbols) Atlantic in 1999 (circles) and 2000 (triangles) (J. Rooker unpublished data). Green diamonds represent values reported by Radtke (1987) for cores of giant Atlantic bluefin tuna (2.2 –2.7 m fork length) caught in the Western Atlantic.

Development of procedure to isolate otolith core

To establish the nursery origin of sub-adult or adult Atlantic bluefin tuna (age-3 to age-6), otolith chemistry in the core of the otolith must be determined. To isolate the otolith core, a high resolution MicroMill System will be utilized (www.merchantek.com/micromill.htm). The system includes a high-speed precision drill mounted on an automated stage that controls X-Y-Z motion. A microscope is built around the drill and stage and the otolith is visualized through an imaging system. The imaging system allows extremely precise < 1 µm control of drill paths across daily and seasonal structures in otolith section and will be used to isolate milligrams of material collected from otolith cores of Atlantic bluefin tuna (Fig. 1). Earlier prototypes of the system have seen productive use in the isolation of material used for otolith stable isotope studies.

Interestingly, 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 are inconsequential for stable isotope analysis. We will use the MicroMill System to isolate the portion of otolith growth corresponding to nursery habitat use (i.e. first year of life).

Otolith samples from the Bluefin Program (ICCAT) were requested (February 2003) for prediction of nursery origin and trans-Atlantic mixing. This initial assessment will be based on over 200 Atlantic bluefin tuna (age-3 to age-6) collected in the western Atlantic, and approximately 40 samples we obtained in 1997, 1998, 1999, and 2000. Consequently, our prediction will be based on approximately 160 individual bluefin tuna. Otoliths are currently being cleaned and sectioned for core isolation on the MicroMill. Otoliths from these sub-adult or adult Atlantic bluefin tuna (age-3 to age-6) will be used to obtain the first otolith-based estimate of stock structure and mixing. We anticipate that core isolation will be finished by the summer of 2003 and stable $\delta^{13}C$ and $\delta^{18}O$ isotopes will be quantified in the fall of 2003.