

EVALUATION OF POPULATION STRUCTURE AND MIXING RATES OF ATLANTIC BLUEFIN TUNA FROM CHEMICAL SIGNATURES IN OTOLITHS

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SUMMARY

Trace elements in otoliths of Atlantic bluefin tuna (Thunnus thynnus) have been used to delineate yearlings (age-1) from eastern and western Atlantic nurseries; however, classification success for several year-classes has been moderate ranging from 60-90%, and classification functions show strong year to year variability. Here, we evaluate the utility of an alternative chemical marker in otoliths, carbon and oxygen stable isotopes, to discriminate bluefin tuna from natal regions. The discriminatory power of stable isotopes ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) in otoliths of yearlings (age-1) was high, with 91% of individuals classified correctly (based on cross-validated classification) to eastern and western nurseries. In contrast to trace element analyses, year to year variation in stable isotope signatures was minimal over five year classes (1999-2003) of yearlings. Thus, these stable isotopes and in particular $\delta^{18}\text{O}$ can be used to reliably predict nursery origin of Atlantic bluefin tuna. In an initial application, we compared otolith core material (corresponding to the first year of life) of large school, medium, and giant category bluefin tuna to reference samples of yearling signatures to determine their origin. A large fraction (~43-64%) of the Atlantic bluefin tuna collected in the western Atlantic fishery (comprised primarily of large school and medium category fish) originated from nurseries in the east. Alternatively, medium and giant category bluefin tuna from the Mediterranean were largely (~82-86%) of eastern origin. Thus, initial evidence suggests that the western fishery received high subsidy from the Mediterranean population.

RÉSUMÉ

Des éléments traces présents dans les otolithes de thon rouge de l'Atlantique (Thunnus thynnus) ont été utilisés afin de distinguer les juvéniles (âge-1) provenant des nourriceries de l'Atlantique Est et Ouest. Toutefois, le succès de classification a été modéré pour plusieurs classes d'âge, allant de 60 à 90%, et les fonctions de classification présentent une grande variabilité d'une année sur l'autre. Dans le présent document, nous évaluons l'utilité d'un autre marqueur chimique présent dans les otolithes, les isotopes stables du carbone et de l'oxygène, afin de distinguer le thon rouge originaire de diverses régions natales. La capacité de distinction des isotopes stables ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) dans les otolithes des juvéniles (âge-1) était élevée : 91% des spécimens ont été classifiés correctement (en se basant sur une classification validée par croisement) dans les nourriceries de l'Est et de l'Ouest. Contrairement aux analyses des éléments traces, la variation annuelle dans les signatures des isotopes stables était minime pour cinq classes d'âge (1999-2003) de juvéniles. Par conséquent, ces isotopes stables, et notamment le $\delta^{18}\text{O}$, peuvent être utilisés afin de prédire de manière fiable l'origine de la nourricerie du thon rouge de l'Atlantique. Dans une application initiale, nous avons comparé la substance du noyau de l'otolithe (correspondant à la première année de vie) de thons rouges juvéniles (grands bancs), de catégories moyenne et géante en vue de référencer les échantillons des signatures des juvéniles pour déterminer leur origine. Une grande fraction (~43-64%) de thons rouges atlantiques collectés dans la pêcherie de l'Atlantique Ouest (composée principalement de poissons de grands bancs et de catégorie moyenne) étaient originaires de nourriceries de l'Est. Par ailleurs, les thons rouges de catégorie moyenne ou géante de la Méditerranée étaient, dans une grande mesure (~82-86%), originaires de l'Est. Les preuves initiales suggèrent donc que la pêcherie de l'Ouest a reçu une forte proportion de la population de la Méditerranée.

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RESUMEN

Los elementos traza en otolitos del atún rojo del Atlántico (*Thunnus thynnus*) se han utilizado para revelar ejemplares de un año (edad 1) de las zonas de cría del Atlántico oriental y occidental; sin embargo, el éxito en la clasificación de varias clases de edad ha sido moderado, oscilando entre el 60 y 90%, y las funciones de clasificación muestran una fuerte variabilidad en los diferentes años. En este documento, se evalúa la utilidad de un marcador químico alternativo en los otolitos, los isótopos estables de oxígeno y carbono, para discriminar el atún rojo a partir de sus regiones natales. La potencia discriminatoria de los isótopos estables ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) en otolitos de ejemplares de un año (edad 1) fue elevada, se clasificó correctamente el 91% de los ejemplares (basándose en una clasificación de validación cotejada) en las zonas de cría oriental y occidental. A diferencia de los análisis de elementos traza, la variación interanual en las firmas isotópicas estables fue mínima en cinco clases anuales (1999-2003) de ejemplares de un año. Por tanto, los isótopos estables y, en particular el $\delta^{18}\text{O}$, pueden utilizarse para predecir con bastante seguridad el origen del atún rojo en cuanto a su zona de cría. En una aplicación inicial, comparamos el material del núcleo del otolito (que se corresponde con el primer año de vida) de atunes rojos de categoría gigante, mediana y juveniles (grandes bancos) con muestras de referencia de firmas de un año de edad para determinar su origen. Una importante proporción (~43-64%) de atún rojo atlántico recogido en la pesquería del Atlántico occidental (formada sobre todo por peces de categoría mediana a juveniles (grandes bancos) procedía de las zonas de cría del Este. A su vez, los atunes rojos de categoría gigante y mediana del Mediterráneo eran en su mayor parte (~82-86%) de origen oriental. Por tanto, la evidencia inicial sugiere que la pesquería occidental recibe un flujo considerable de la población del Mediterráneo.

KEY WORDS

Otolith chemistry, Mixed stock fishery, Stable isotopes, Stock identification, Population structure

1 Introduction

Management of Atlantic bluefin tuna (*Thunnus thynnus*) is currently based on the premise of two principal zones of spawning and juvenile production, which occur in the Mediterranean Sea and Gulf of Mexico. Although trans-Atlantic migration of members from both production zones is well documented through conventional and electronic tags (Magnuson et al. 1994, Lutcavage et al. 1999, Block et al. 2001, 2005), some degree of residency to natal areas is assumed, justifying ICCAT's separate assessments and regulations for "eastern" and "western" stocks. This assumption has been challenged in recent times due to the seasonal progression of Atlantic bluefin tuna across the 45°W meridian management boundary, as observed through recent landings data and electronic tagging results. Further, some scientists have suggested that the supposition of only two natal zones with a high degree of natal homing to each may be flawed. As a consequence, 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.

Several approaches are currently being used to quantify the extent of mixing between eastern and western stocks, including electronic tags, genetics, and otolith chemistry. Although corroborative data from all three approaches will be needed to properly validate population models and determine the level of mixing, otolith chemistry may be the most useful approach for determining contribution rates of recruits from putative nurseries since it predicts nursery origin. Chemical signatures in otoliths are linked to ambient conditions, and material deposited in the otolith during the first year of life serves as a "birth certificate" or natural tag of an individual's nursery habitat. Previous studies have demonstrated that trace elements in otoliths can be used to determine the origin of highly migratory species, including bluefin tuna; however, the discriminatory power was modest and interannual variation in classification functions would require application of year-class specific elemental signatures (Secor et al. 2003, Rooker et al. 2003). In response, the utility of alternative chemical markers in otoliths have been examined, and recent work suggests that stable isotopic signatures in otolith of Atlantic bluefin tuna can be used to effectively distinguish Atlantic bluefin tuna from eastern and western nurseries (Rooker and Secor 2004). Here, we evaluate the discriminatory power of stable isotopic signatures in otoliths for 5 year-classes (1999-2003) of yearlings from both eastern and western nurseries, and report trans-Atlantic movement patterns of medium and giant category Atlantic bluefin tuna.

2 Methods

Samples of yearling (age-1) bluefin tuna used for stable isotope analysis were collected from the eastern Atlantic (Bay of Biscay)/Mediterranean Sea and the western Atlantic (East coast of U.S. from Maryland to Rhode Island) in 2001-2003. Sampling strategies used to procure age-1 (yearling) Atlantic bluefin tuna varied between regions. In the east, yearlings were either taken by sport fishermen using hand lines or by commercial long-line fishermen targeting albacore (*Thunnus alalunga*), while samples in the western Atlantic were from the recreational hook-and-line fishery. Collections of large school (66-135 lbs), medium (135-310 lbs) and giant (310+ lbs) category bluefin tuna from the western Atlantic were provided by NOAA Fisheries under the Bluefin Year Program (BYP), with additional samples from the New England fishery provided by the Seabrook NH Yankee Fisherman's Fishing Cooperative. In the Mediterranean Sea, medium and giant category bluefin tuna harvested by purse seine vessels were collected from tuna farming operation in Marsaxlokk, Malta (Fish and Fish LTD) and Murcia, Spain (T.F.M-Tuna Farms of the Mediterraneo S.L.) in 2003 and 2004, respectively.

Whole otoliths were carefully cleaned with dilute nitric acid (HNO_3), rinsed with doubly deionized water (DDIH_2O). High-resolution milling was used to isolate core from large school, medium and giant category bluefin tuna. Prior to milling, sagittal otoliths were embedded in Stuers epoxy resin and sectioned using a low speed ISOMET saw to obtain 1.5 mm transverse sections through the core. 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 New Wave MicroMill System. Approximately 25-30 passes were made at 40-50 microns depth per path to isolate core material from each otolith, and surface profiling was used to correct for beveling in the section. Drill depth and path speed were stipulated based upon past previous work. Cored material was then displaced from the section and transferred to an acid washed vial. Following micro-milling, otolith cores were rinsed (20 s) in ultrapure HNO_3 and then rinsed with DDIH_2O . Whole otoliths and cores of transverse sections were pulverized (powdered) using acid washed mortar and pestle.

Carbon and oxygen stable isotopes were measured using a Finnegan Mat Delta Plus Mass Spectrometer maintained at the University of Maryland (UM) 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 $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotopes are reported relative to the PDB scale after comparison to an in-house laboratory standard that has been calibrated to PDB. Two protocol tests were performed to assess the reliability and consistency of the stable isotope approach. First, an inter-laboratory comparison of stable isotope laboratories was performed because otoliths from yearlings collected in 1999-2000 were processed at the University of Houston (UH) (see Rooker and Secor 2004), while all other samples (whole otoliths and otolith cores) from 2001 to the present were analyzed at the University of Maryland facility. Since cored regions of otoliths from large school, medium, and giant category bluefin tuna were used to represent the yearling signature, we also compared $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures in whole otoliths of yearlings to transverse sections of the other paired otolith from the same individual.

3 Results and discussion

Assessment of stable isotope protocols

Mean machine error at the UM stable isotope facility for yearling Atlantic bluefin tuna otoliths was $2\pm 1\%$ and $8\pm 6\%$, respectively for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. No inter-laboratory effect was observed for paired otoliths run at UM and UH for $\delta^{13}\text{C}$ (mean difference = 0.03 mil; $p = 0.73$); however, a significant effect was observed for $\delta^{18}\text{O}$ (mean difference = 0.25 mil; $p < 0.001$). Thus, a correction factor was applied to yearling signatures processed at UH to standardize our signature data (correction factor: 0.25 per mil addition to $\delta^{18}\text{O}$ from UH). Paired t-tests showed no significant difference between cores from transverse sections and whole otoliths in either $\delta^{13}\text{C}$ (mean difference = 0.12 mil; $p=0.33$) or $\delta^{18}\text{O}$ (mean difference = 0.07 mil; $p=0.26$), indicating that our micro-milling protocol did not deplete or enrich the core signatures. In fact, mean error between core and whole otoliths for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was similar to the estimated precision of replicated runs.

Analysis of Western versus Eastern Atlantic bluefin tuna

Multivariate analysis of variance indicated that stable isotope signatures in the otoliths of yearling Atlantic bluefin tuna from eastern and western nurseries differed significantly (MANOVA $p < 0.001$). Univariate contrasts indicated that $\delta^{18}\text{O}$ signatures in the otoliths of yearling from each nursery were distinct, with enriched

values observed for bluefin tuna from cooler waters in the eastern Atlantic and Mediterranean Sea (**Figure 1**). Total cross-validated classification success of yearlings to eastern and western nurseries was 91% (east = 98%, west = 79%) over 4-5 year classes of samples. Although classification success was high using all year classes (1999-2003), $\delta^{18}\text{O}$ values for one year class from the western Atlantic (2001) were enriched relative to all other signatures from this region. $\delta^{18}\text{O}$ values of yearlings from 2001 were within the observed range of the yearlings from the Mediterranean, indicating trans-Atlantic movement (east to west) may have occurred during the first 12+ months of life. Even though total classification success was higher (>98%) when the 2001 samples from the western Atlantic were excluded or re-classified in the DFA model, we opted for a more conservative approach and assumed that no trans-Atlantic mixing occurred during the first year of life.

Stable isotopic signatures in the otolith cores of large school, medium, and giant category bluefin tuna (N=146) were compared to reference samples to determine natal origin. The majority of $\delta^{18}\text{O}$ values in otolith cores of large school/ medium (N = 76) and giant (N = 12) category bluefin tuna from the west ranged from -0.8 to -1.8, and over 50% of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures fell within the confidence ellipses of eastern or western yearlings (points within region where ellipses from the east and west were excluded). Of these signatures, 47% of the bluefin tuna collected in the west were from natal sites in the west, while a large fraction (53%) originated from eastern nurseries (**Table 1**). Results from DFA classifications (based upon different posterior probability error scenarios) were similar, suggesting that a large fraction (43-64%) of bluefin tuna inhabiting the western Atlantic were produced from nurseries in the east. Otolith cores of medium (N = 15) and giant (N = 43) category bluefin tuna collected in the Mediterranean were enriched relative to western cores, with most the values between -0.6 to -1.2. A large fraction (86%) of the core signatures fell within the confidence ellipse of the eastern yearling. Similarly, all three DFA classifications indicated that adult bluefin tuna collected in the Mediterranean were comprised primarily (82-84%) of individuals from natal sites in the east.

Our findings indicate that stable isotopic signatures in otoliths hold considerable promise as a tool to determine natal origin of Atlantic bluefin tuna. Signatures in the cores of sub-adult and adult bluefin tuna provide strong evidence of trans-Atlantic mixing, particularly for the western fishery sample. While results suggest high rates of subsidy of the western fishery by bluefin tuna of Mediterranean origin, precaution must be exercised when interpreting these initial data. First, the reference data set (signatures of yearlings) may not include all possible signatures for eastern and western nurseries. Further, we saw evidence in 2001, that the western yearling sample may have already undergone a trans-Atlantic migration from the Mediterranean. Still, in four of five years stable isotope signatures were quite distinct and invariant. With 2-3 additional year-class samples, we expect that the 2001 sample may be shown to be anomalous. Secondly, our sample sizes from mixed stock fisheries were small and not a complete or unbiased representation of regional Atlantic bluefin tuna fisheries. For instance, age and sizes of bluefin tuna collected in the east and west differed in our study; only 14% of the western samples were giant category bluefin, while over 74% of the bluefin tuna from tuna cages in the Mediterranean were giants.

Clearly, information on natal homing is needed to fully understand the implications of trans-Atlantic movement on the dynamics of Atlantic bluefin tuna populations and their ability to sustain eastern and western stock fisheries. Here, we have shed some light on possible mixing amplitudes and rates of subsidy. In the future, we recognize two important applications of these natural tags: 1) tests of the origin of adults present on spawning grounds in both the Mediterranean and Gulf of Mexico; and 2) tests of mixing rates for representative samples of important bluefin tuna fisheries.

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Table 1. Classification of medium and giant category bluefin tuna of unknown origin from the east (N =58) and west (N =88) based on stable isotopic signatures in cores of otoliths. Four classification procedures were used: 1) based on whether points fell within confidence ellipses (1 SD) of reference samples of yearling bluefin tuna (note: points falling outside the either confidence ellipse were classified as unknown); 2) Discriminant function analysis-DFA (quadratic), 3) DFA with classification restricted to individual signatures with posterior probabilities being 90% for one of the nurseries; 4) DFA with classification restricted to individual signatures with posterior probabilities being 80% for one of the nurseries.

| | <u>East</u> | <u>West</u> | <u>Unknown</u> |
|---------------------------|-------------|-------------|----------------|
| <i>Confidence Ellipse</i> | | | |
| From East | 86% | 14% | 29 |
| From West | 53% | 47% | 43 |
| <i>DFA (ALL)</i> | | | |
| From East | 83% | 17% | 0 |
| From West | 64% | 36% | 0 |
| <i>DFA (PP 90%)</i> | | | |
| From East | 82% | 18% | 19 |
| From West | 43% | 57% | 51 |
| <i>DFA (PP 80%)</i> | | | |
| From East | 84% | 16% | 7 |
| From West | 59% | 41% | 24 |

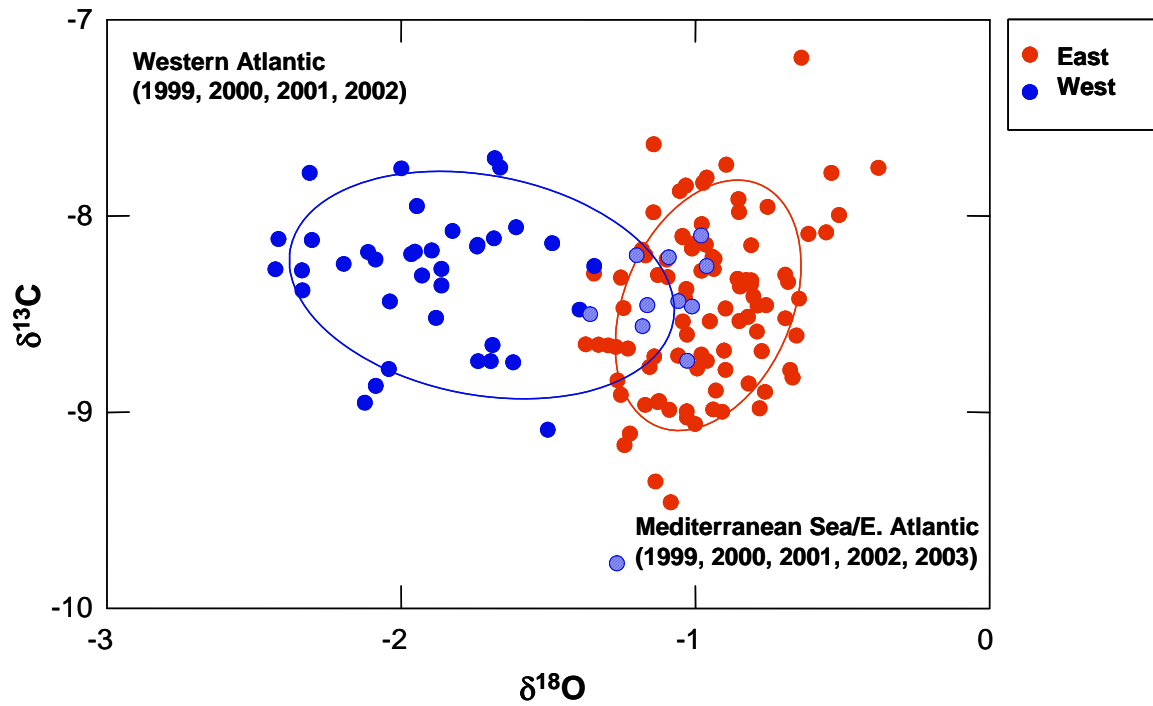


Figure 1. Stable $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures in the whole otoliths of yearling (age-1) Atlantic bluefin tuna collected in eastern and western nurseries from 1999-2003. Confidence ellipses (1 SD) shown for east and west groups. 2001 year class from western Atlantic included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