

## STOCK STRUCTURE AND MIXING OF ATLANTIC BLUEFIN TUNA: EVIDENCE FROM STABLE $\delta^{13}\text{C}$ AND $\delta^{18}\text{O}$ ISOTOPES IN OTOLITHS

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### SUMMARY

Here we evaluate the utility of an alternative geochemical marker in otoliths, stable  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopes, to discriminate yearling (age-1) *T. thynnus* from the Mediterranean Sea and the western Atlantic Ocean. Stable  $\delta^{18}\text{O}$  values in whole otoliths of *T. thynnus* collected from eastern and western nurseries were significantly different in both years examined, with  $\delta^{18}\text{O}$  values being more enriched in the Mediterranean (mean: 1999 = -1.08, 2000 = -1.30) than in the western Atlantic (mean: 1999 = -2.39, 2000 = -2.09). In contrast,  $\delta^{13}\text{C}$  values in otoliths were similar between nurseries in both years: western Atlantic (mean: 1999 = -8.2, 2000 = -8.5), Mediterranean (mean: 1999 = -8.3, 2000 = -8.4). Although significant interannual variation was observed for one of the stable isotopes ( $\delta^{18}\text{O}$ ), cross-validated classification success from discriminant function analysis was still high (98%) when year classes were pooled by region. Stable  $\delta^{18}\text{O}$  values in the otolith cores (~ first year of life) of 6 school size and medium *T. thynnus* collected in the U.S. recreational fishery ranged from -0.9 to -1.6, suggestive of Mediterranean origin for all individuals assayed. Otolith cores of 8 giant *T. thynnus* were more variable and  $\delta^{18}\text{O}$  values were indicative of yearling signatures from both nurseries.

### RÉSUMÉ

Le présent document évalue l'utilité d'un marqueur géochimique alternatif dans les otolithes, les isotopes stables  $\delta^{13}\text{C}$  et  $\delta^{18}\text{O}$ , visant à distinguer le *T. thynnus* de moins de deux ans (âge 1) de la mer Méditerranée et de l'océan Atlantique ouest. Les valeurs stables  $\delta^{18}\text{O}$  dans les otolithes entiers du *T. thynnus* prélevés dans les nourriceries orientales et occidentales étaient considérablement différentes au cours des deux années examinées, les valeurs  $\delta^{18}\text{O}$  étant plus enrichies dans la Méditerranée (moyenne : 1999 = -1.08, 2000 = -1.30) que dans l'Atlantique ouest (moyenne: 1999 = -2.39, 2000 = -2.09). Par contraste, les valeurs dans les otolithes  $\delta^{13}\text{C}$  étaient similaires entre les nourriceries au cours des deux années : Atlantique ouest (moyenne : 1999 = -8.2, 2000 = -8.5), Méditerranée (moyenne : 1999 = -8.3, 2000 = -8.4). Bien qu'une variation inter-annuelle significative ait été observée pour un des isotopes stables ( $\delta^{18}\text{O}$ ), le succès de la classification validée par croisement à partir d'une analyse de la fonction discriminante a encore été très élevé (98%) lorsque les classes d'âge ont été regroupées par région. Les valeurs stables  $\delta^{18}\text{O}$  dans les noyaux des otolithes (~ première année de vie) de 6 *T. thynnus* de taille moyenne et de taille à former des bancs recueillis par la pêche sportive des États-Unis se sont établies dans une fourchette de -0,9 à -1,6, ce qui suggère l'origine méditerranéenne de tous les spécimens examinés. Les noyaux des otolithes de 8 *T. thynnus* géants étaient plus variables et les valeurs  $\delta^{18}\text{O}$  étaient indicatives de marques d'âge inférieur à deux ans dans les deux nourriceries.

### RESUMEN

Este trabajo evalúa la utilidad de un marcador geoquímico alternativo en otolitos, isótopos estables  $\delta^{13}\text{C}$  y  $\delta^{18}\text{O}$ , para discriminar el *T. thynnus* menor de dos años (edad-1) del Mediterráneo y el Atlántico occidental. Los valores estables  $\delta^{18}\text{O}$  en otolitos completos de *T. thynnus* recopilados en zonas de cría occidentales y orientales eran significativamente diferentes en los dos años examinados, con los valores  $\delta^{18}\text{O}$  más enriquecidos en el

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*Mediterráneo (media: 1999 = -1,08, 2000 = -1,30) que en el Atlántico occidental (media: 1999 = -2,39, 2000 = -2,09). En contraste, los valores  $\delta^{13}\text{C}$  en otolitos eran similares entre las zonas de cría en ambos años: Atlántico occidental (media: 1999 = -8.2, 2000 = -8.5), Mediterráneo (media: 1999 = -8.3, 2000 = -8.4). Aunque se observó una significativa variación interanual para uno de los isótopos estables ( $\delta^{18}\text{O}$ ), el éxito de la clasificación validada con otro método a partir del análisis de la función discriminante seguía siendo muy elevado (98%) cuando las clases anuales se agrupaban por región. Los valores estables  $\delta^{18}\text{O}$  en el núcleo de los otolitos (~ primer año de vida) de 6 *T. thynnus* de tamaño cardumen y de tamaño medio recopilados en la pesquería de recreo estadounidense oscilaban entre -0,9 y -1,6, lo que sugería el origen mediterráneo de todos los individuos del ensayo. Los núcleos de los otolitos de 8 *T. thynnus* gigantes eran más variables y los valores  $\delta^{18}\text{O}$  eran indicativos de huellas de edad inferior a dos años en ambas zonas de cría.*

## KEYWORDS

*Atlantic bluefin tuna, Otolith chemistry, Stable isotopes, Stock identification, Trans-Atlantic mixing*

## 1 Introduction

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), 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). Otolith chemistry may be particularly advantageous because of its potential to identify nursery region for relatively 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 (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, resorption or 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 (Rooker et al. 2001b, Rooker et al. 2003).

While trace element signatures have been used successfully to delineate *T. thynnus* from eastern and western nurseries, classification success was moderate (60-85%) (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 ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) of otoliths appears to represent a promising tool to differentiating fish stocks. Stable isotopes have been used extensively as recorders of environmental conditions, and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  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  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopes as a tool to discriminate yearling (age-1) *T. thynnus* 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.

## 2 Methods

Sampling strategies used to procure juvenile (age-1) *T. thynnus* 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*). Conversely, collections of tuna in the western Atlantic were made in New Jersey and Rhode Island waters using hook and line from recreational activities (under special collection permits). Samples of age-1 *T. thynnus* used for stable isotope analysis were collected in the Mediterranean Sea (Ligurian Sea) and Western Atlantic (New Jersey, Rhode Island) in 1999 and 2000. All collection areas were sampled independently and on more than one occasion when possible. School and medium (69 – 183 cm FL) and giant (> 183 cm FL) category *T. thynnus* used for retrospective determination of nursery origin were collected in the U.S. recreational fishery principally in Ocean City, Maryland and Seabrooke, New Hampshire, respectively.

Sagittal otoliths were extracted from freshly caught specimens; however, 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 (Rooper et al., 2001a). Selection of single otoliths (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<sub>3</sub>) and rinsed with 18 megohm doubly deionized water (DDIH<sub>2</sub>O).

High-resolution milling was used to isolate core from sub-adult and adult *T. thynnus*. Prior to milling, sagittal otoliths were embedded in epoxy resin and sectioned using a low speed ISOMET saw to obtain a 1 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 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 *T. thynnus*), and the drill path was programmed into the MicroMill System. 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<sub>3</sub> and then rinsed in doubly deionized water. Similar to whole otoliths, cores were powdered for stable isotope analysis.

Carbon and oxygen stable isotopes were determined from powdered samples of whole otoliths or otolith cores using stable isotope mass spectrometers at the University of Houston (Department of Department of Geosciences, Stable Isotope Laboratory) and the University of Maryland (Department of Geology, Stable Isotope Laboratory). Analytical precision of the mass spectrometer was 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 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 CO<sub>2</sub> 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

## 3 Results and Discussion

Multivariate analysis of variance indicated that stable isotopes of age-1 *T. thynnus* collected in the Mediterranean and western Atlantic nurseries differed significantly (Pillai's trace = 93.54,  $p < 0.01$ ). Univariate contrasts indicated that  $\delta^{18}\text{O}$  values differed significantly between regions (ANCOVA,  $p \leq 0.01$ ), with  $\delta^{18}\text{O}$  values being more enriched in the Mediterranean (mean: 1999 = -1.08, 2000 = -1.30) than in the western Atlantic (mean: 1999 = -2.39, 2000 = -2.09 (Figure 1). In contrast,  $\delta^{13}\text{C}$  values were similar between the two nurseries in both years: western Atlantic (mean: 1999 = -8.2, 2000 = -8.5), Mediterranean (mean: 1999 = -8.3, 2000 = -8.4). Our results for *T. thynnus* are in accord with empirical evidence since the cooler waters of the Mediterranean would be expected to produce enriched levels of  $\delta^{18}\text{O}$  (e.g., Thorrold et al. 1997, Campana 1999). A significant interannual

effect was detected for  $\delta^{18}\text{O}$ ; however,  $\delta^{13}\text{C}$  values were similar between years. Cross-validated classification success was 100% when year classes were evaluated separately and 98% when year classes were pooled.

Otolith cores of school/medium and giant category *T. thynnus* collected in the western Atlantic (U.S. recreational fishery, New Hampshire and Maryland) were isolated and assayed to predict nursery origin and identify trans-Atlantic movement patterns. Stable  $\delta^{18}\text{O}$  values in the otolith cores of school/medium *T. thynnus* ranged from -1.6 to -0.9 (Figure 2), suggestive of Mediterranean origin since otoliths from yearlings ranged from approximately -1.6 to -0.4. Otolith cores of giant *T. thynnus* were more variable ranging from -2.1 to -1.1. Stable  $\delta^{18}\text{O}$  isotopes values fell into the observed ranges of yearlings from both nurseries, indicative of nursery origin in both the Mediterranean Sea and western Atlantic Ocean.

Results presented here indicate stable isotopes appear to hold considerable promise as a tool to discriminate stocks of *T. thynnus*, and appear to be more reliable predictor of nursery origin than trace elements in otoliths (Secor et al. 2002, Rooker et al. 2003). Moreover, 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. However, precaution must be exercised when interpreting these data. Clearly, sample sizes must be increased prior to drawing inferences about mixing and relative contribution rates of eastern and western nurseries to specific fisheries or regions. Care must also be invested to ensure that samples are drawn representatively from principal fisheries. Most critical however in the short term is evaluation of potential bias in the core isolation procedure. Sectioning and milling may introduce bias if cores do not adequately represent whole otoliths of yearlings (i.e., the entire first ~12-18 months of life). In response, we are currently evaluating milling effects (e.g., by comparing sectioned and milled otolith to whole otolith from the same yearling individuals) and expanding the library of inter-annual record of isotopic concentrations associated with bluefin tuna nurseries. The latter point will require additional data on spatial and temporal variability in isotopic signatures of yearlings from both regions. Once these issues are addressed properly, we believe the approach may be used to effectively discriminate stocks among regional fisheries and lead to a full-scale investigation of the stock structure and mixing rates of *T. thynnus*.

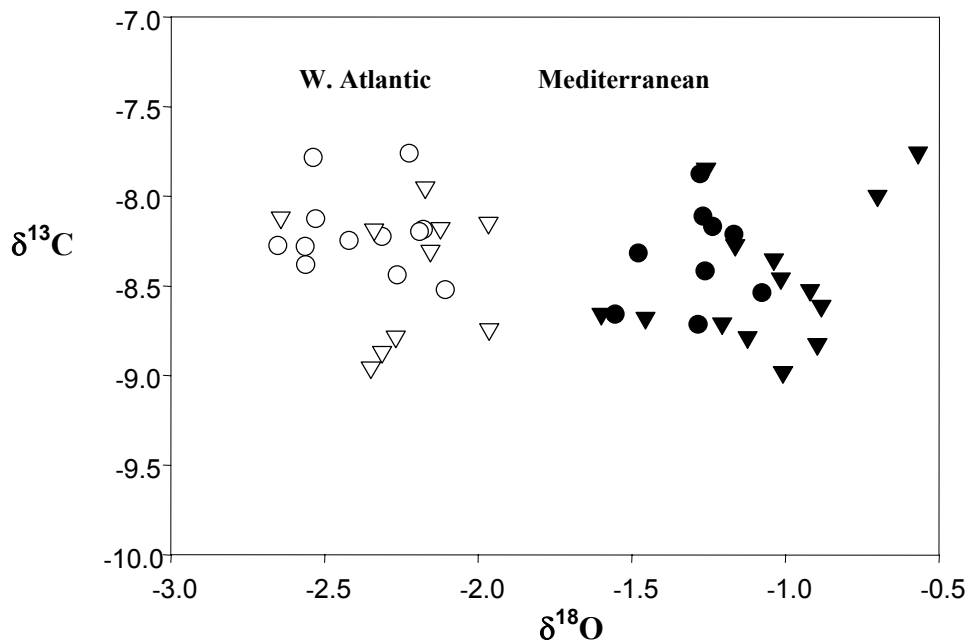
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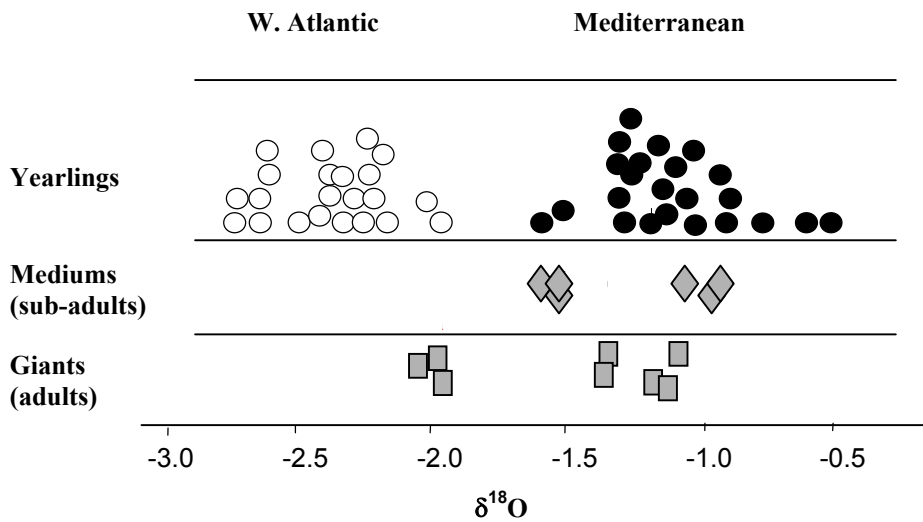
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**Figure 1.** Stable  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotope values from whole otoliths of yearling (age-1) Atlantic bluefin tuna (*Thunnus thynnus*) collected from the Mediterranean Sea (solid symbols, n = 23) and Western Atlantic (open symbols, n = 22) in 1999 (circles) and 2000 (triangles).



**Figure 2.** Jitter diagram of stable isotopes corresponding to the first year of life of Atlantic bluefin tuna (*Thunnus thynnus*). Yearlings (age-1) were collected over two years (1999, 2000) from W. Atlantic (open circles) or Mediterranean (solid circles) and whole otoliths analyzed. School and medium size-class sub-adult tuna (99 – 148 cm FL, n = 6) were collected from Ocean City, MD and giant adult tuna (199 – 270 cm FL, n = 8) were collected from Seabrooke, NH in 2001-2002.