Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry

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ABSTRACT

Chemical signatures in the otoliths of teleost fishes represent natural tags that may reflect differences in the chemical and physical characteristics of an individuals' environment. Otolith chemistry of Atlantic bluefin tuna (*Thunnus thynnus*) was quantified to assess the feasibility of using these natural tags to discriminate juveniles (age 0 and age 1) from putative nurseries. 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 85%. 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%. 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. 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*.

Key words: Atlantic bluefin tuna, Gulf of Mexico, Mediterranean, migration, otolith, stock structure

INTRODUCTION

Atlantic bluefin tuna (*Thunnus thynnus*) is a highly migratory species occurring throughout temperate and subtropical regions of the North Atlantic Ocean (Collette, 1999). Although pan-oceanic in distribution, most spawning activity appears restricted to the Gulf of Mexico and Mediterranean Sea (Magnuson *et al.*, 1994). Data from conventional tagging and microconstituent analysis suggest that transoceanic migration between the eastern and western Atlantic regions occurs but the degree of mixing is limited (Mather, 1980; Calaprice, 1986; Clay, 1991; Cort and de la Serna, 1993). However, recent evidence from pop-up satellite archival tags indicates that mixing across 45°W longitude may be markedly higher than previous estimates and that alternate spawning grounds possibly exist in the North Atlantic (Lutcavage *et al.*, 1999; Block *et al.*, 2001). Additionally, genetic studies have failed to provide compelling evidence against the hypothesis of a single stock in the North Atlantic Ocean despite ever-increasing discriminatory tools and genetic markers, further supporting the premise of significant transoceanic mixing (Magnuson *et al.*, 2001). As a consequence, the assumption of two stocks currently used for management by the International Commission for the

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Received 10 September 2001
Revised version accepted 24 May 2002

Conservation of Atlantic Tunas (ICCAT) remains unverified and additional information on migratory behaviours of *T. thynnus* is needed to resolve the issue of stock structure.

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 individuals' surroundings are integrated into the aragonite-protein matrix. As 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 (Thresher, 1999; Thorrold *et al.*, 1998, 2001). Moreover, the approach has been used recently to assess stock specificity of tunas, and findings suggest that otolith elemental analysis has promise for assessing the population connectivity of pelagic stocks (Rooker *et al.*, 2001b).

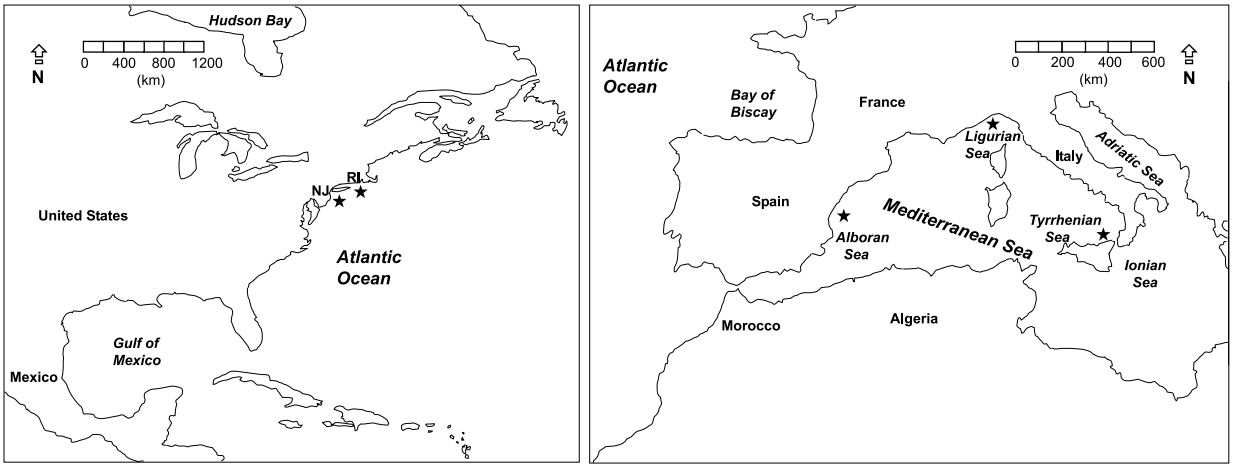
In this paper, we evaluate the stock-specificity and stability of chemical signatures in the otoliths of juvenile Atlantic bluefin tuna (*T. thynnus*) in the North Atlantic Ocean. Otolith chemistry of juveniles from eastern and western Atlantic regions (i.e. nurseries) was quantified to determine the discriminatory power of otolith chemistry for stock identification. In order to test for differences between the two primary nurseries, we examined the otolith chemistry of young *T. thynnus* (age 0 and age 1) from the eastern and western Atlantic, and assumed that no transoceanic migration activity occurred. Variability in otolith chemistry was also examined on a smaller spatial scale by measuring the otolith elemental composition of individuals from putative nurseries within the Mediterranean Sea. In addition, the temporal stability of these natural tags was investigated by contrasting otolith chemistry of 2 yr classes of age-0 *T. thynnus*. Finally, age-0 and age-1 *T. thynnus* collected from the same nursery ground were compared to assess age-specific differences in otolith chemistry.

METHODS

Sample collection

Sampling strategies used to procure juvenile (age 0 and age 1) Atlantic bluefin tuna (*T. thynnus*) varied between regions. In the Mediterranean Sea, age-0 and age-1 individuals were either taken by sport fishermen using hand lines or by commercial long-line fishermen targeting albacore (*T. alalunga*). Collections were made in three regions of the Mediterranean Sea: Alboran Sea (Spain), Ligurian Sea (northern Italy), and Tyrrhenian Sea (southern Italy) (Fig. 1). Collections of tuna in the western Atlantic were made in New Jersey and Rhode Island waters using hook and line from recreational activities. Although age-0 and

Figure 1. Location of sampling sites for Atlantic bluefin tuna (*Thunnus thynnus*) in the North Atlantic Ocean. Approximate location of collection areas is shown (stars) for nurseries in the western Atlantic and three regions of the Mediterranean Sea (Alboran Sea, Ligurian Sea, Tyrrhenian Sea).



age-1 *T. thynnus* were targeted in both the Mediterranean and western Atlantic, no age-0 *T. thynnus* were collected in the western Atlantic during the study period. As a result, our assessment of spatial differences in otolith chemistry of *T. thynnus* from eastern and western nurseries was limited to comparisons of age-1 individuals. Collections of age-0 specimens within the Mediterranean Sea were made in both 1998 and 1999 in several regions to evaluate spatial and temporal patterns of otolith chemistry. All collection areas were sampled independently and on more than one occasion. Sizes of age-0 and age-1 *T. thynnus* ranged from 25 to 42 cm and 66 to 70 cm fork length (FL), respectively. In most cases, 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 (Rooker *et al.*, 2001a). Selection of single otoliths (i.e. right or left sagittae) for elemental analysis was based on random assignment.

Elemental analysis

Before elemental analysis, otoliths were carefully cleaned of surface contaminants. All reagents used were ultra-pure grade and all implements and containers were cleaned with dilute nitric acid (HNO_3) and rinsed with 18 megohm doubly deionized water ($DDIH_2O$). Otoliths were first soaked in $DDIH_2O$ to hydrate biological residue adhering to the surface of the sample and residue was then removed using fine tipped forceps. Next, otoliths were soaked in 3% hydrogen peroxide for 5 min to dissolve remaining biological residue and immersed for 5 min in 1% nitric acid to remove surface contamination. Otoliths were then flooded with $DDIH_2O$ for 5 min to remove the acid. Finally, otoliths were dried under a Class 100 laminar-flow hood and stored in plastic vials. Otolith mass was reduced by approximately 4% as a result of the decontamination procedure. In preparation for instrumental analysis, each otolith was weighed to the nearest 0.01 mg, placed in a plastic centrifuge tube and dissolved in 10 mL of 1% nitric acid. Internal standards were added to all solutions to compensate for possible instrumental drift.

Elemental concentrations were determined using a Perkin–Elmer ELAN 5000 quadrupole inductively coupled plasma mass spectrometer (ICPMS) (Perken Elmer, Inc., Shelton, CN, USA). Levels of Li, Mg, Mn and Ba were determined using external calibration standards. Levels of Ca and Sr were quantified after 100-fold dilution using external standards without matrix matching. Samples were analysed in random order to avoid possible sequence effects. Procedural blanks and two certified reference materials (CRMs) were concurrently digested and analysed following the same procedures. Limits of detection (LODs) were calculated based on three times the standard deviation of the mean procedural blank and converted to a dry weight basis. The LODs were: Li 0.01, Mg 0.19, Mn 0.06, Ba 0.01, Sr 0.90 and Ca 0.46 (values expressed as $\mu g\ g^{-1}$ dry weight; Ca in percentage). One CRM was NIST 915a (Calcium Carbonate Clinical Standard), obtained through the National Institute of Standards and Technology (Gaithersburg, MD, USA). This CRM is not certified for trace metal content, so only non-certified values are available for a few elements. Relevant values are ($\mu g\ g^{-1}$ dry weight; Ca in percentage): Mg, 1.0; Ca, 40.0; and Mn, 0.6. Per cent recovery values (mean ± SD, $n = 3$) for this CRM were: Mg 104 ± 1.0, Ca 99 ± 1.5 and Mn 103 ± 10.0. The other was an otolith CRM (Yoshinaga *et al.*, 2000) produced at the National Institute of Environmental Studies (NIES) of Japan. Certified values for this CRM are: Mg 21 ± 1, Ca 38.8 ± 0.5, Sr 2360 ± 50 and Ba 2.89 ± 0.09 (values expressed as $\mu g\ g^{-1}$ dry weight; Ca in percentage). Percent recovery values (mean ± SD, $n = 9$) for this CRM were: Mg 100 ± 3.4, Ca 99 ± 2.6%, Sr 95 ± 4.1 and Ba 97 ± 1.5. Li was also measured in the otolith CRM (0.199 ± 0.011 $\mu g\ g^{-1}$) and its concentration is not certified, so recovery cannot be computed. However, in a blind interlaboratory comparison, our method was compared with a method employing high-resolution ICPMS and isotope dilution (Secor *et al.*, 2002). Results of the Li analyses were highly similar.

Multivariate analysis of variance (MANOVA) was used to test for spatial and temporal differences in otolith chemistry. Nursery ground and year were used as fixed factors in separate MANOVA models. Pillai trace (V) was chosen as the test statistic as it is the most robust to violations of homogeneity of covariance (Wilkinson *et al.*, 1996). Univariate tests for each element were analysed using analysis of covariance (ANCOVA), and a preliminary model (interaction regression) was used to determine if slopes of regression lines (homogeneity of slopes assumption) differed. The main significance test of ANCOVA (homogeneity of *y*-intercepts) was performed for all elements because the assumption of parallel slopes was met. Tukey's HSD test was used to find *a posteriori* differences ($\alpha = 0.05$) among sample means. Linear discriminant function analysis (LDFA) was used to classify juveniles from different nurseries and/or year classes. Small differences in otolith weights and fish lengths occurred among sites and years, and thus we

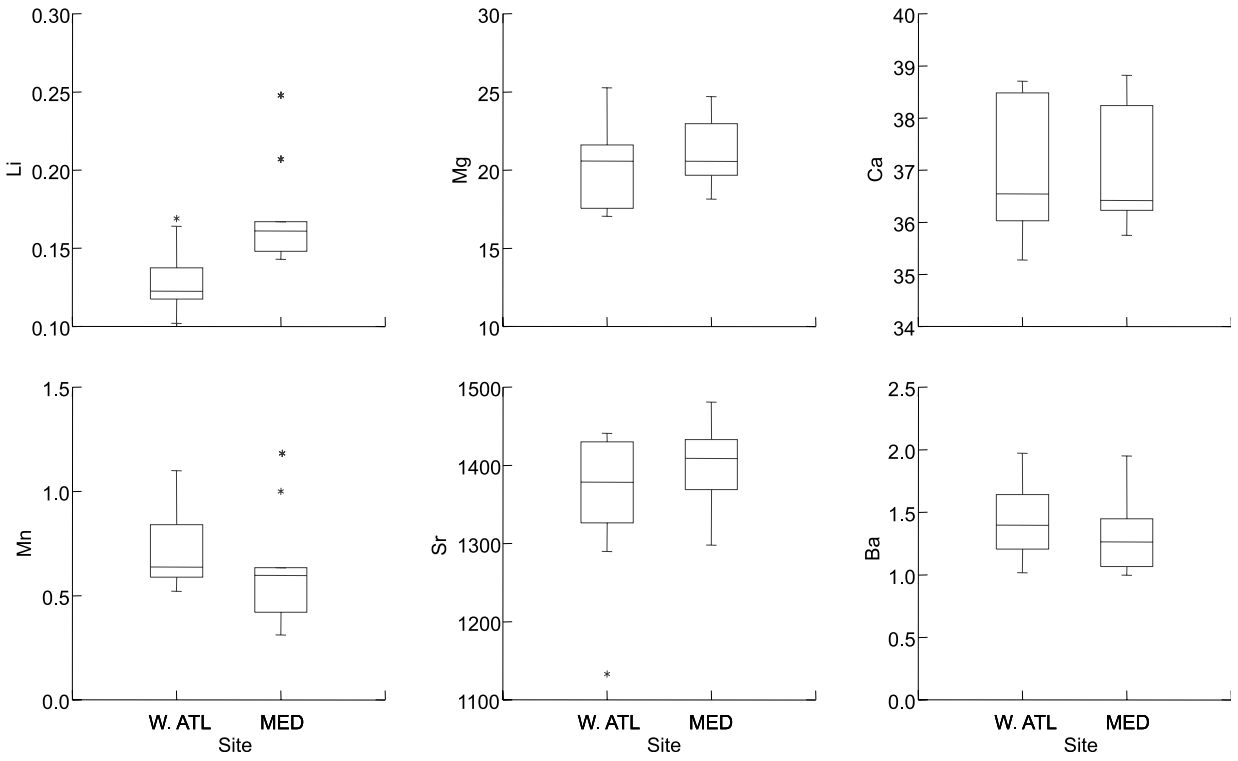
examined relationships between elemental concentration and otolith weight prior to performing LDFA. We removed the effect of size (otolith weight used as a proxy for fish size) to ensure that differences in fish size among samples did not confound any site-specific differences in otolith chemistry. Concentrations were weight-detrended by subtraction of the common within-group linear slope from the observed concentration (Rooker *et al.*, 2001b). The relative importance of individual elements in discriminating across spatial and temporal scales was assessed using the *F-to-remove* statistic (estimated during discriminant analysis procedure; Wilkinson *et al.*, 1996). Elements with large *F-to-remove* values in a discriminant model are most helpful for discriminating among nurseries or year classes. Correlation of elements used in the discriminant function model was evaluated using the Tolerance statistic. Such estimates range from 0 to 1 and a small value indicates that a variable is highly correlated with one or more of the other variables (Wilkinson *et al.*, 1996). Prior to statistical testing, residuals were examined for normality and homogeneity among factor levels. Within group distribution and variance were examined and an outlier was removed in one case (high Mn value) to meet parametric assumptions.

RESULTS

Variability in otolith chemistry between eastern and western nurseries

Multivariate analysis of variance indicated that otolith chemistry of age-1 Atlantic bluefin tuna (*T. thynnus*) collected in the Mediterranean (Ligurian Sea) and western Atlantic nurseries differed significantly (Pillai's trace = 3.55, $P < 0.01$). Univariate contrasts indicated that concentrations of only one element (Li) differed significantly (ANCOVA, $P < 0.05$) between nursery areas (Fig. 2). The concentration of Li was higher for *T. thynnus* collected in the Mediterranean than in the western Atlantic. Discriminant analysis, based on concentrations of all six elements, indicated that 71% (based on cross-validated or jackknifed classification) of these individuals were correctly assigned

Figure 2. Box plots of elemental concentrations of otoliths from age-1 Atlantic bluefin tuna (*Thunnus thynnus*) collected in 1999 from western Atlantic ($n = 12$) and Mediterranean (Ligurian Sea) ($n = 9$) nurseries. Interquartile ranges (25th and 75th percentile) are shown by extent of boxes and horizontal lines represent medians (50th percentile). Whiskers range from 10th to 90th percentiles and values outside this range are plotted with asterisks. Concentrations given in parts per million with the exception of Ca (%).



to nursery sites (Mediterranean Sea 67%, western Atlantic 75%). One record of otolith Mn from the Ligurian Sea was identified as an outlier (studentized residual > 3.0) and classification success was improved to 85% by removing this case from the discriminant model. The *F-to-remove* value of Li was markedly higher than other elements (10.3). Values of two other elements (Mg, Ba) were moderately high (1.8 and 5.1, respectively), suggesting that these elements may also be useful for discriminating Mediterranean and western Atlantic juveniles. Correlation of elements in the discriminant model was moderate (Tolerance: 0.3–0.5).

Variability in otolith chemistry within a regional nursery

Assessments of within-nursery variability in otolith chemistry were conducted for age-0 *T. thynnus*. Results of MANOVA showed that elemental signatures differed significantly among the three putative nurseries within the Mediterranean Sea (Pillai's trace = 4.32, $P < 0.001$). In addition, univariate contrasts indicated that concentrations of four elements (Li, Mg, Mn, and Sr) differed significantly among the three nurseries (ANCOVA, $P < 0.05$) (Fig. 3). Mg and Mn levels in the otoliths of *T. thynnus* were significantly higher from the Alboran Sea than the Ligurian Sea or Tyrrhenian Sea (Tukey's HSD, $P < 0.05$). Li levels were significantly higher in *T. thynnus* from the Ligurian Sea while Sr concentrations were significantly lower for individuals from the Ligurian Sea (Tukey's HSD, $P < 0.05$). Results from LDFA indicated that classification success was higher for individuals collected in the Ligurian Sea (80%), and lower for individuals originating from the Alboran Sea (67%) and Tyrrhenian Sea (62%) (Fig. 4). Univariate tests indicated that Li, Mg, Mn and Sr differed among nurseries, and *F-to-remove* values from the discriminant model were highest for these four elements. Tolerance statistics were high for all six elements (0.70–0.83) indicating that elements used in the model were not highly correlated with other elements.

Interannual variability in otolith chemistry

Otolith chemistry of age-0 *T. thynnus* differed significantly between year classes in the Alboran Sea (Pillai's

Figure 3. Box plots of elemental concentrations of otoliths from age-0 Atlantic bluefin tuna (*Thunnus thynnus*) collected in 1999 from three putative nurseries in the Mediterranean Sea: Alboran Sea (ALB, $n = 11$), Ligurian Sea (LIG, $n = 10$), Tyrrhenian Sea (TYR, $n = 22$). Concentrations given in parts per million with the exception of Ca (%). Definition of boxes, lines, and whiskers as in Figure 2.

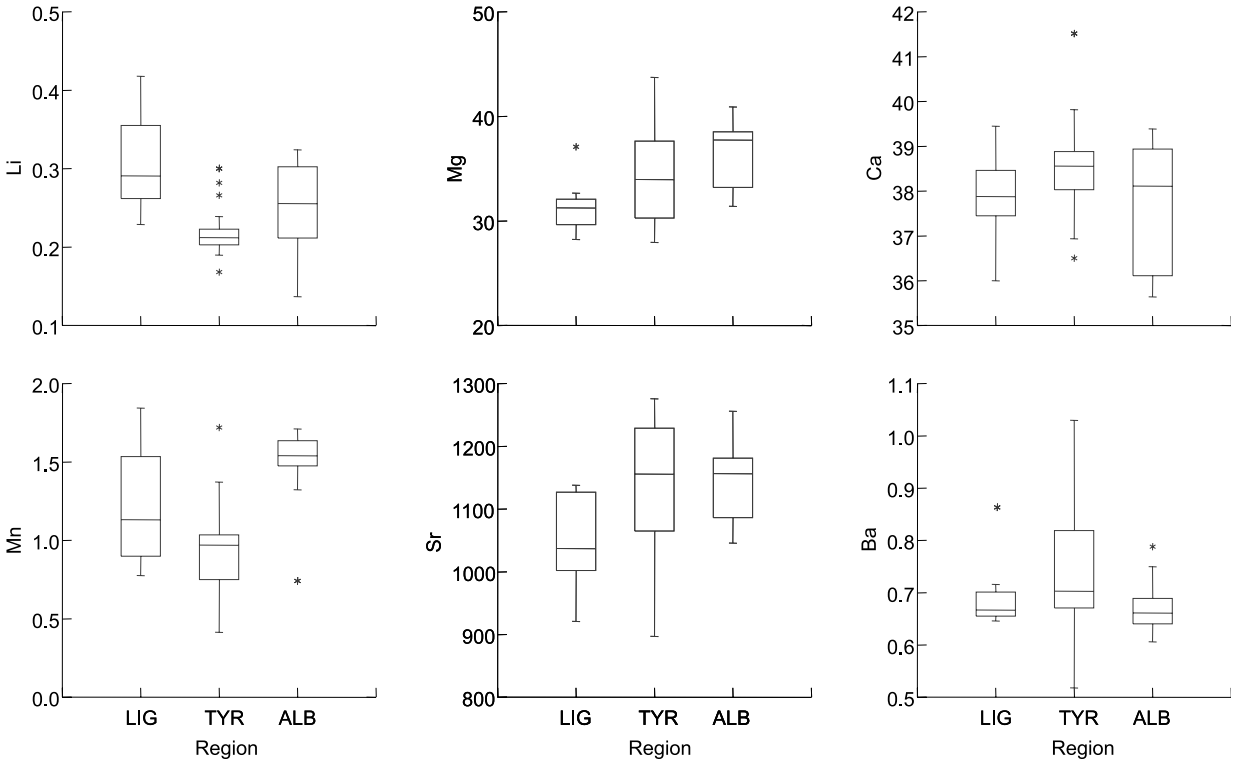
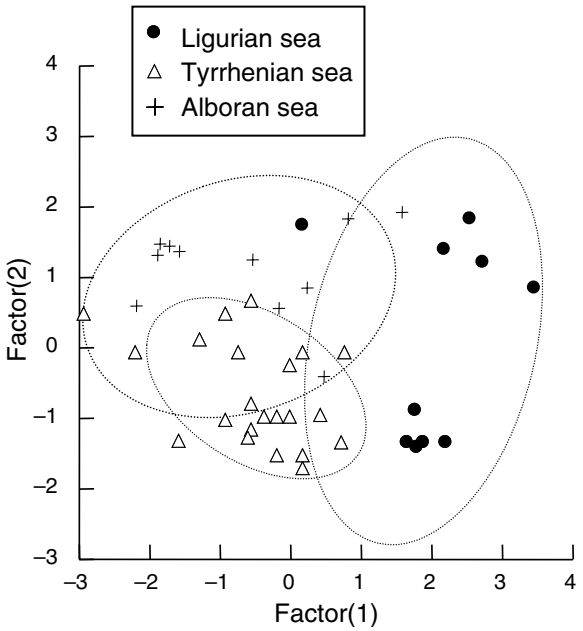


Figure 4. Canonical plot scores and 95% confidence ellipses from discriminant analysis of otolith chemical signatures of age-0 Atlantic bluefin tuna (*Thunnus thynnus*) collected in 1999 from three nurseries in the Mediterranean Sea: Alboran Sea, Ligurian Sea, Tyrrhenian Sea. Discriminant analysis based on six elements: Li, Mg, Ca, Mn, Sr, and Ba.



trace = 10.38, $P < 0.001$). Univariate contrasts indicated that concentrations of three elements (Mg, Mn, Ba) differed significantly (ANCOVA, $P < 0.05$) between 1998 and 1999 year classes (Fig. 5). Concentrations of Mg and Mn were higher for age-0 *T. thynnus* in the 1999 group while Ba levels were higher for the 1998 group. Interannual differences in otolith chemistry were not observed between 1998 and 1999 year classes in the Tyrrhenian Sea (Pillai's trace = 0.282, $P > 0.05$); univariate contrasts for all six elements were statistically similar (ANCOVA, $P > 0.05$). Despite the occurrence of interannual differences in the otolith chemistry of individuals from the Alboran Sea, discriminant analysis showed that cross-validated accuracies of nursery origin (Alboran Sea vs. Tyrrhenian Sea) were slightly higher (80%) when year classes were pooled; classification success in 1998 and 1999 between the two nurseries was 78 and 71%, respectively.

Age-specific differences in otolith chemistry

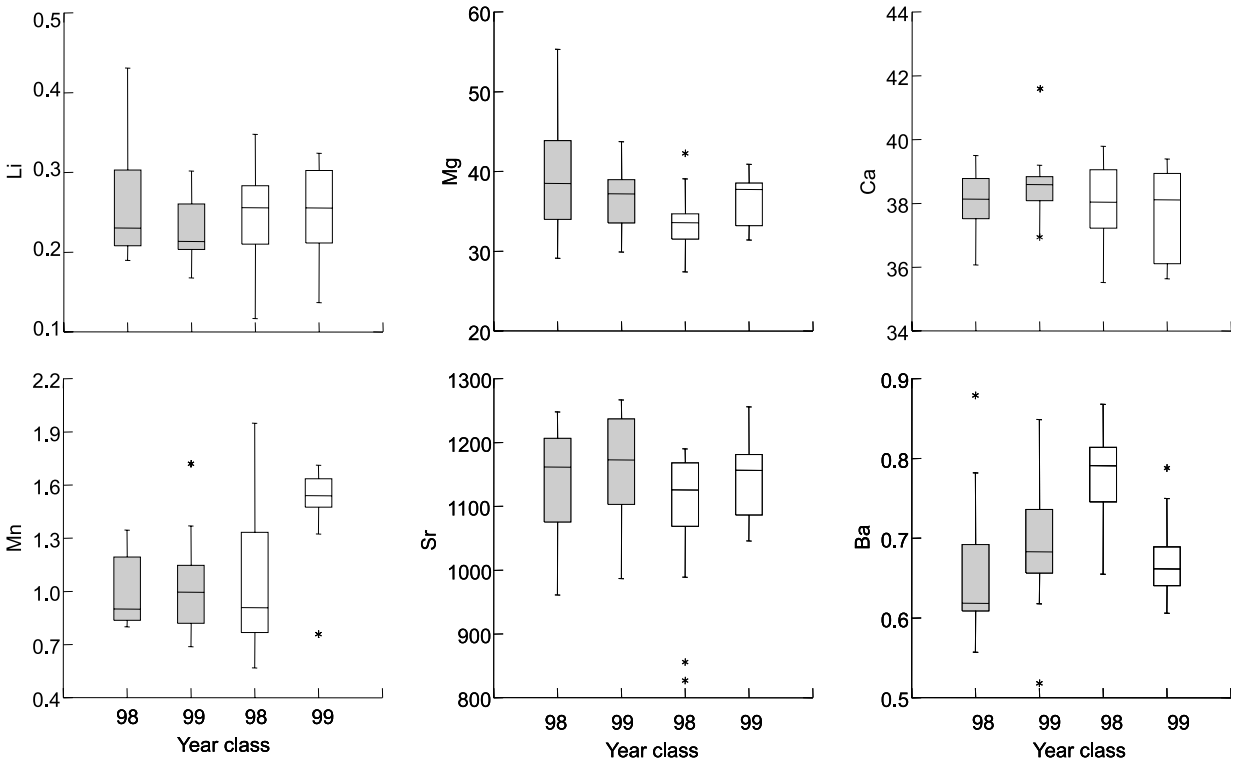
Age-0 and age-1 *T. thynnus* collected from the same region of the Ligurian Sea were compared and distinct differences in otolith chemistry were observed (Figs 2 and 3). Results of MANOVA showed that elemental signatures of age-0 and age-1 *T. thynnus* were significantly different (Pillai's trace = 23.62, $P < 0.001$). Univariate tests indicated that otolith concentrations of Li, Mg, Sr, and Ba were significantly different between age classes in the Ligurian Sea (ANCOVA, $P < 0.05$). Concentrations of Li and Mg were approximately twofold greater in age-0 *T. thynnus* while Sr and Ba were markedly higher in age-1 individuals.

DISCUSSION

Otolith chemistry of age-1 Atlantic bluefin tuna (*T. thynnus*) varied significantly between eastern and western nurseries. However, concentrations of only one element (Li) differed significantly between the two primary nurseries and cross-validated classification success was moderately high. Elemental analysis of age-1 *T. thynnus* collected in other areas of eastern and western Atlantic nurseries in 1998 has also been recently examined at two different ICPMS facilities (National Marine Fisheries Service ICPMS Laboratory, Sandy Hook, NJ, USA, and National Research Council of Canada's Institute of Environmental Research and Technology, Ottawa, Canada) and results were consistent with findings from the present study. Inter-laboratory discrimination of individuals from the two nurseries ranged from 68 to 81%, and univariate contrasts showed significant differences for Li and Mg at both laboratories (Secor *et al.*, 2002). In the present study, a significant site difference was observed for Li but not for Mg. Nonetheless, Mg was influential in the classification function, and levels of this element were higher in the Mediterranean as reported in the interlaboratory study. Chemical signatures in otoliths also have been used recently to discriminate Pacific bluefin tuna (*T. orientalis*) from coastal seas and offshore nurseries in the Pacific Ocean (Rooker *et al.*, 2001b). In contrast to results for *T. thynnus*, five of six elements examined (Li, Mg, Ca, Mn, Sr) differed among nurseries and classification success was greater than observed in the present study.

Differences in otolith chemistry were not entirely unexpected because hydrography and trace element fluxes differ between eastern and western Atlantic nurseries (Send *et al.*, 1999; Solis and Powel, 1999). The Gulf of Mexico and coastal regions of the western Atlantic are heavily influenced by riverine and terrestrial inputs of metals. Almost half of the riverine discharge of the continental USA flows into the Gulf of Mexico, with the Mississippi River serving as the primary source of anthropogenic and lithophilic elements (Wen *et al.*, 1999). In addition, geochemical reactions that occur following the burial of shelf

Figure 5. Box plots of elemental concentrations of otoliths from 2 yr classes (1998, 1999) of age-0 Atlantic bluefin tuna (*Thunnus thynnus*) from two nursery areas in the Mediterranean Sea: Alboran Sea (1998: $n = 12$, 1999: $n = 12$), Tyrrhenian Sea (1998: $n = 15$, 1999: $n = 12$). Plots of otolith elemental concentrations for *T. thunnus* from Alboran Sea (open) and Tyrrhenian Sea (shaded) are given in parts per million with the exception of Ca (%). Definition of boxes, lines, and whiskers as in Figure 2.



sediments (diagenesis) appear to further enrich waters of the Gulf and western Atlantic with metals. In contrast, Atlantic waters feed the Mediterranean Sea through the Strait of Gibraltar and supply metal-depauperate water to the region (Minas and Minas, 1993; Lascaratos *et al.*, 1999; Send *et al.*, 1999). As a result, concentrations of certain elements in the Mediterranean Sea are lower than in waters of the Gulf of Mexico and coastal areas in the north-west Atlantic Ocean. Still, otolith chemistry of age-1 *T. thynnus* was relatively similar for several elements, suggesting that differences in ambient water chemistry were relatively minor.

Otolith chemistry of age-0 *T. thynnus* among the three nurseries in the Mediterranean Sea (Alboran Sea, Ligurian Sea, Tyrrhenian Sea) was also distinct, demonstrating that the approach has promise for assessing connectivity or exchange rates among *T. thynnus* from different natal sources within the eastern nursery. Although significant differences were present for Li, Mg, Mn, and Sr, classification success based upon chemical signatures was only moderate (62–80%). Concentrations of Li, Mg and Mn were higher in the otoliths of *T. thynnus* from the northern and western Mediterranean (Alboran Sea, Ligurian Sea) than in the central region (Tyrrhenian Sea), and observed concentrations may be related to oceanographic conditions. Riverine discharge accounts for over 80% of particulate matter in the western Mediterranean (*c.* 20% because of atmospheric sources), higher than in the central or eastern Mediterranean (Guerzoni *et al.*, 1999). Consequently, rivers in the north-west Mediterranean (e.g. Rhône, Ebro) are likely sources of lithophilic elements (Price *et al.*, 1999). In addition, metal-enriched inputs from the south-west coast of Spain (Tinto and Odiel Rivers) are transported through the Strait of Gibraltar, and mix with waters of the western Mediterranean. The western region is also well fertilized by upwelling at the northern boundaries of the anticyclonic gyre (Minas and Minas, 1993; Dafner *et al.*, 2001), and concentrations of elements displaying nutrient-type distributions may be higher in these cold, nutrient-rich waters. Thus, elevated concentrations of certain elements (i.e.

Mg, Mn) in otoliths of juvenile *T. thynnus* collected from this region may be due in part to variation in water chemistry.

Our results suggest that spatial differences in chemical signatures in the otoliths of *T. thynnus* can be used to establish natal origin. Nevertheless, classification accuracies were moderate and need to be improved before full-scale investigations of stock structure are attempted. Enhancing the resolving power of the approach is possible and several options are available. First, preconcentration procedures can be used in the future to eliminate matrix interferences on the ICPMS, allowing analysts to accurately determine transition metal concentrations at nM to pM levels. Application of solid phase preconcentration procedures as a means for interference-free measurement of trace elements in otoliths has been reported (Arslan and Paulson, 2002). We avoided inherent problems associated with spectral interference by focusing efforts on a limited number of elements deemed reliable (Thresher, 1999; Rooker *et al.*, 2001a), and omitted suspect elements from our analyses. Development of separation and preconcentration procedures will remedy such concerns and increase the number of elements that can be used to discriminate stocks reliably. Secondly, as demonstrated by Thorrold *et al.* (2001), stable isotopes (δ^{13}C and δ^{18}O) may be used in conjunction with trace element chemistry to improve classification success. Stable isotopic ratios have been used extensively as recorders of environmental conditions, and δ^{13}C and δ^{18}O values in otoliths have been linked to physiological processes (diet, metabolic rate) and temperature, respectively (Gauldie *et al.*, 1994; Edmonds and Fletcher, 1997; Thorrold *et al.*, 1997, 1998). Consequently, the integration of stable isotopes into future assessments of *T. thynnus* may be warranted, especially if the addition of new elements derived from preconcentration methods fails to adequately delineate stocks. Thirdly, as a result of migratory behaviour, initial assessments of natal origin could be restricted to young-of-the-year (age-0) *T. thynnus*, particularly individuals collected within the first 6 months of life. Tunas and other highly migratory species often move 100s of kilometres during the first year(s) of life in search of food and optimal thermal conditions (Clay, 1991; Bayliff, 1994; Polovina, 1996; Kitagawa *et al.*, 2000). Therefore, chemical signatures in the otoliths of age-1 *T. thynnus* may reflect several water masses and it is even possible that our reference sample included migratory yearlings from mixed stocks. In response, the resolving power of the technique may be improved in future assessments by sampling individuals during the first 6 or 12 months of life or by analysing only the portion of the otolith (i.e., core) corresponding to this early life interval.

Another potential source of bias that may compromise the predictive value of the approach is temporal variability in otolith composition. Retrospective determination of natal source depends on the premise that chemical signatures are sufficiently stable across time to allow for accurate classification. To date, a limited number of studies have examined the issue of temporal stability of otolith chemistry and results indicate that stock-specific signatures vary among years (e.g. Patterson *et al.*, 1999; Campana *et al.*, 2000). Long-term stability of otolith chemical signatures is not evident in any study to date, suggesting that chemical signatures may serve only as short-term natural tags (1–3 yr). Temporal stability of otolith chemical signatures of Pacific bluefin tuna (*T. orientalis*) was recently examined over a 3-yr period in the North Pacific Ocean (Rooker *et al.*, 2001b). Although interannual trends were evident, differences in otolith chemistry between Pacific Ocean and coastal sea nurseries were greater than temporal variability within a nursery. In the present study, otolith chemistry of *T. thynnus* differed between 2 yr classes in the Alboran Sea; however, univariate contrasts for all six elements were statistically similar in the Tyrrhenian Sea. While interannual differences in otolith chemistry were observed in the Alboran Sea, classification accuracies were not negatively affected. In fact, our ability to discriminate individuals from the Alboran Sea and Tyrrhenian Sea was improved by pooling year classes (cross-validate accuracy of 80%), indicating spatial differences were sufficient to counter temporal variability.

In summary, this study demonstrates that chemical signatures in the otoliths of juvenile *T. thynnus* from eastern and western Atlantic nurseries are relatively distinct and show some degree of temporal persistence. In addition to water chemistry, other environmental factors (e.g. temperature, diet, genetics) may generate variability in otolith chemistry (Campana, 1999; Thresher, 1999), and detailed evaluations of these mechanisms are needed to fully assess the utility of otoliths as natural tracers. Furthermore, classification accuracies should be improved prior to attempting investigations of natal origin. Once achieved, elemental signatures in otolith cores (age-0 portion of otolith) from larger *T. thynnus* can be quantified, providing a means of identifying source(s) and relative contributions of different nursery grounds in the Mediterranean Sea and western Atlantic Ocean. Due in part to the increased evidence of *trans*-Atlantic

migrations of *T. thynnus* (Lutcavage *et al.*, 1999; Block *et al.*, 2001), data are critically needed to estimate contributions of recruits originating from eastern and western Atlantic nurseries, and findings from future otolith-based assessments will most likely play a central role in determining the population connectivity of *T. thynnus*.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Marine Fisheries Service to JR and DS (Saltonstall-Kennedy Grant Award no. NA07FD0176 and NA86FD0110). We thank Drs P. Mace, S. Turner, and E. Prince for assisting with collection efforts in the Mediterranean and western Atlantic. M. Deflorio, B. Falterman, S. Larsen, G. Palandri, M. Relini, N. Santamaria, and B. Sarak provided support in the laboratory and field.

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