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Short communication

Otolith microconstituent analysis of juvenile bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea and Pacific Ocean

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Abstract

Microconstituents were measured in otoliths of juvenile northern bluefin tuna *(Thunnus thynnus)* from the Mediterranean Sea and western Pacific Ocean. Whole otoliths were analyzed by inductively coupled plasma mass spectrometry. Multivariate statistical analysis showed clear separation between the two groups. Na, Mg, Mn, and Zn were most useful in differentiating Mediterranean and Pacific samples. Findings justify further use of otolith microconstituents to investigate stock structure questions concerning Atlantic bluefin tuna.

Keywords: Otolith; Microconstituents; ICPMS; Stock structure; Bluefin tuna

1. Introduction

The northern bluefin tuna, *Thunnus thynnus*, occurring in both the Pacific and Atlantic Oceans, is a quintessential fish, epitomizing form, size, and energetics adaptive to highly migratory behavior. Extremely high demand by the Japanese market for the fatty flesh of giant bluefin tuna (>130 kg) and increased commerce of 'recreationally' captured fish have contributed to declining abundances of bluefin tuna caught in coastal waters adjacent to North America (NMFS, 1995). Abundance of adult Atlantic bluefin tuna declined ca. 80% during the period 1975–1994 (Magnuson et al., 1994; NMFS, 1995). Migratory behavior of Atlantic bluefin tuna cause them to be harvested in jurisdictional waters of many countries, as well as in interjurisdictional regions. The high worldwide demand for giant Atlantic bluefin tuna and the fish's migratory behavior dictate careful international management of this species.

Atlantic bluefin tuna occur throughout northern latitude Atlantic waters and historically ranged from Brazilian to Norwegian waters. Despite pan-oceanic distribution, spawning grounds are restricted to the Gulf of Mexico and the eastern Mediterranean Sea. In addition, tagging studies have revealed low rates (<5% on average) of transoceanic migrations by both juveniles and adults (NMFS, 1995). Despite disparate spawning grounds and evidence for low rates of transoceanic migrations, genetic studies, parasite

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markers, and microconstituent analysis of vertebrae all failed to provide compelling evidence against the null hypothesis of a single Atlantic bluefin stock (Magnuson et al., 1994).

Otolith microconstituent analysis has been applied recently to study stock structure (Edmonds et al., 1989; Campana et al., 1994, 1995; Proctor et al., 1995) and migration rates (Secor, 1992; Secor and Piccoli, 1996). The premise of this approach is that trace elements are incorporated into otoliths in direct proportion to their availability in surrounding water or food. Thus, larvae or young-of-the-year bluefin tuna exposed to either the Gulf of Mexico or Mediterranean waters might be expected to incorporate different mixtures of elements into their otoliths. Few laboratory experiments have been conducted to verify the assumption that otoliths can record environmental histories, but such studies have supported this assumption for uptake of strontium (Fowler et al., 1995; Limburg, 1995; Secor et al., 1995; Farrell and Campana, 1996). Physiological factors, temperature, and genetics may also affect uptake of specific elements into otoliths (Kalish, 1989; Thresher et al., 1994).

Here, we report the results of a feasibility study using otolith microconstituent analysis to distinguish Pacific northern bluefin tuna from the Mediterranean tuna. Our ultimate goal is to use otolith microconstituent analysis to separate eastern and western 'stocks' of Atlantic bluefin tuna. Examination of an out group – northern Pacific bluefin tuna – was used to evaluate whether the technique might be useful in future contrasts between western and eastern stocks of Atlantic bluefin tuna. It was expected that exposure of juvenile tuna (<30 kg) to waters in either the Pacific Ocean or Mediterranean Sea would result in differences in otolith composition.

2. Methods

Otoliths (sagittae) were obtained from juvenile bluefin tuna collected in 1994 from Porto Ceasaro, Italy (n=20) by Dr. J. M. Dean (FISHTECH and Belle W. Baruch Institute, Univ. South Carolina, Columbia, SC). Sizes ranged from 40 to 110 cm TL, corresponding to age classes 0+ to 3 years (Compeán-Jimenez and Bard, 1983). Additional otoliths were obtained from juveniles ranging in size from 40 to 50 cm TL (n=5; age classes 0+ or 1+) collected in the western Pacific Ocean by Dr. S. Tsuji (National Research Institute of Far Sea Fisheries, Shizuoka, Japan).

In the laboratory, samples were decontaminated. All reagents were ultrapure grade and all implements and containers were cleaned with dilute nitric acid and rinsed with 18-MΩ doubly deionized water ($DDIH_2O$). Remnants of biological tissues and fluids were removed by immersing the otolith in $DDIH_2O$ to hydrate the residue, removing as much as possible with fine-tipped forceps, then soaking the sample in 3% H_2O_2 to remove the remainder. Otoliths were then soaked in 1% nitric acid for 5 min to remove surface chemical contamination; flooded with $DDIH_2O$ to rinse off the acid, and then dried under a HEPA Class 100 laminar flow hood.

In preparation for instrumental analysis, each otolith was weighed to the nearest 0.01 mg and placed in a plastic tube. These were digested in concentrated nitric acid and brought to volume with $DDIH_2O$. Amounts of acid used and volumes of the digests were proportional to sample weights to insure that all resulting solutions were of similar composition, thus minimizing possible matrix effects that might complicate instrumental analysis. Elemental concentrations were determined using inductively coupled plasma mass spectrometry (ICPMS). Minor element concentrations (Mn, Co, Zn, etc.) were quantified using the method of standard additions; major element concentrations (Ca, Sr, etc.) were quantified using external calibration standards after further dilution of the digests. Procedural blanks and a standard reference material (SRM) were concurrently digested and analyzed following the same procedures. The SRM was NIST 915a (calcium carbonate clinical standard), obtained through the National Institute of Standards and Technology. As this SRM is not certified for verification of trace-metal concentrations, only noncertified values are available for a few elements. Relevant values are (on a dry-weight basis): Ca, 40.0%; Mg, 1.0 ppm (ug/g); Cu, 0.95 ppm; and Mn, 0.6 ppm. Mean values (n=4) obtained for our analysis of the SRM were Ca: 38.7%, Mg: 0.99 ppm, Cu: 0.95 ppm, and Mn: 0.59 ppm.

Sample identification was coded so that specimen origin was unknown during the analysis. Differences between small bluefin tuna collected in either the Mediterranean Sea or Pacific Ocean were tested using

analysis of covariance (otolith weight used as covariate) to explore contrasts in individual elements, and by principal components analysis to identify groups of elements which explained significant variation between samples. In instances of nonrandom residual variance, the Kruskal–Wallis test was used.

3. Results

Detectable elements present in concentrations quantifiable by ICPMS included Na, Mg, Ca, Mn, Ni, Zn, Sr, and Ba. Otolith mass showed significant effects ($P<0.05$) on Mg, Mn, Sr, and Ba concentrations. Levels of both Ba and Sr increased with ontogeny (otolith mass); Mg and Mn levels were inversely related to otolith mass (Fig. 1). There was also a nonsignificant inverse trend with otolith mass apparent for Na and Zn concentrations.

Univariate contrasts between Mediterranean and Pacific samples indicated significant differences (ANCOVA – $P<0.05$) in concentrations of Na, Mg, Mn, and Zn. Because ranges of otolith mass differed substantially between sample groups, a separate statistical analysis was performed constraining the data set to otolith mass <12 mg. In the reduced data set, significant differences were observed for Mg and Mn. In the multivariate analysis, the first principal component accounted for 57% of the total variance in elemental concentrations and resulted in clear separation of the Mediterranean and Pacific groups (Fig. 2, top panel). Na, Mg, Mn, and Zn contributed positive weighting to this component (Fig. 2, bottom panel).

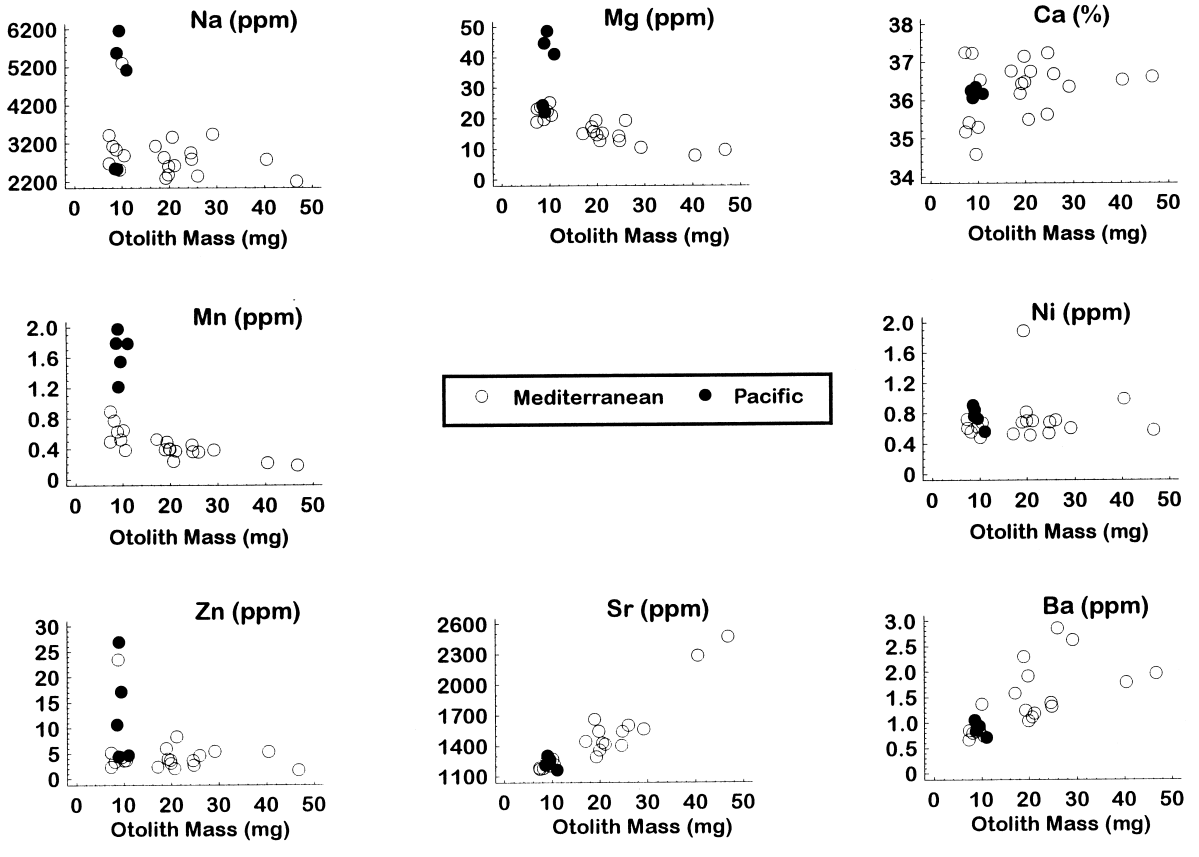

Fig. 1. Otolith elemental concentrations vs. otolith mass for Mediterranean Sea and Pacific Ocean juvenile bluefin tuna. Concentrations given in parts per million with the exception of calcium which is given in parts per hundred.

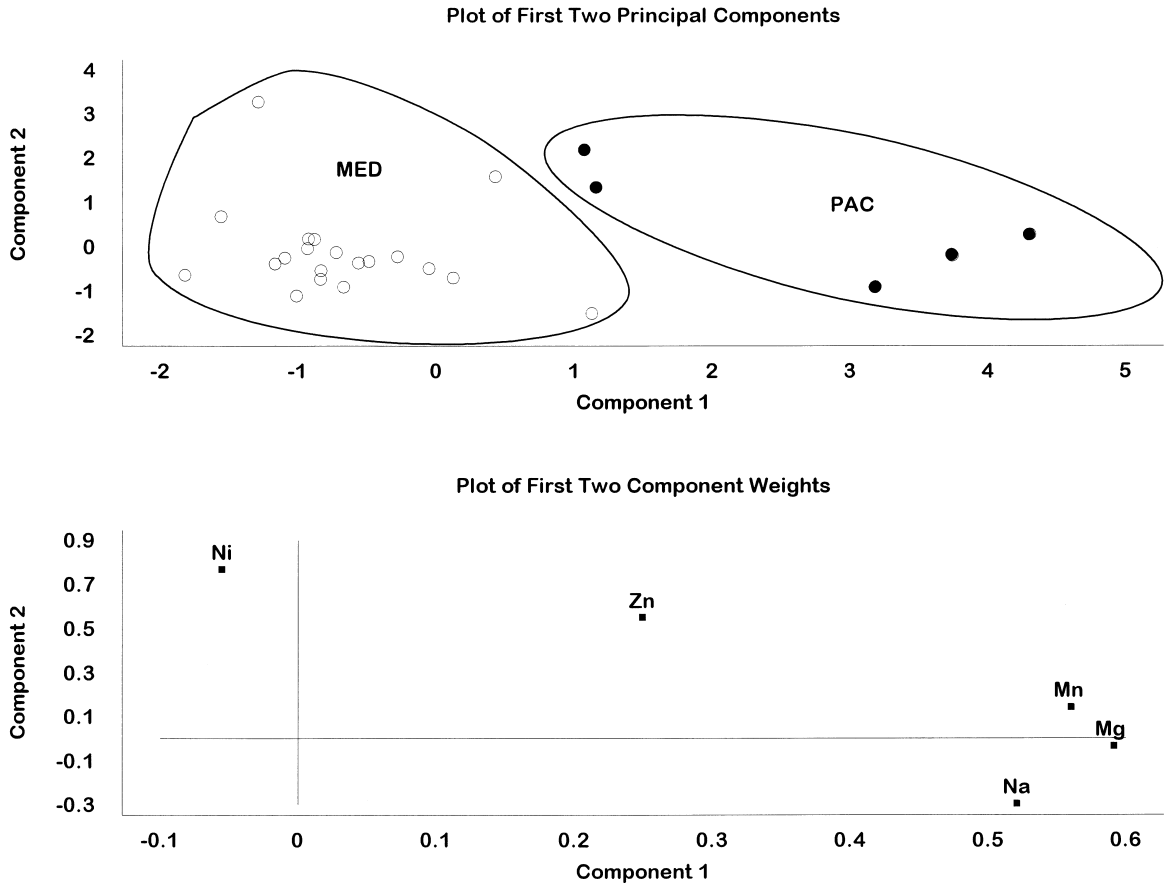

Fig. 2. Principal components analysis (PCA) of otolith microconstiutents (Na, Mg, Mn, Ni, and Zn) of Mediterranean Sea and Pacific Ocean juvenile bluefin tuna. Top panel shows bivariate plots of PCA scores for the first two components. Bottom panel shows weightings of elements to the first two components.

4. Discussion

Spawning by eastern stock Atlantic bluefin occurs throughout the summer in the western Mediterranean and Adriatic Seas (Magnuson et al., 1994). Seven of our Mediterranean specimens were yearlings and can be assumed to have resided in the Mediterranean Basin throughout their lives. Older juveniles are known to migrate into the Atlantic northward to the Bay of Biscay and southward to the Canary Islands; however, the majority of small tuna are probably residents of the Mediterranean Basin, where commercial fisheries target them (Magnuson et al., 1994). Results of tagging studies indicated 84% retention of small tuna (<44 kg) in the Mediterranean (Cort and de la Serna, 1994 as reported in Magnuson et al., 1994). Because we cannot assume that all Mediterranean tuna experienced Mediterranean Sea conditions over their entire life history, elemental signatures may have been influenced by conditions occurring elsewhere in the Atlantic Ocean. Northern Pacific bluefin tuna spawn in the western Pacific Ocean and disperse rapidly as juveniles. All specimens used in the current study were yearlings collected in the western Pacific Ocean.

Recent work has shown that relatively few elements have been needed to characterize elemental fingerprints. Campana et al. (1995) found that Mg, Li, Zn, Sr, Ba, and Pb explained sufficient variation in distinguishing among groups of Atlantic cod. In studies by Edmonds et al., 1989, 1991, 1992), useful elements

were Na, Mg, Si, P, S, K, Fe, and Sr. In juveniles (*Achoerodus viridis* Labridae) which recruited into estuarine or coastal nurseries, Gillanders and Kingsford (1996) found that Mn, Ba, and Co contributed most to discriminating elemental signatures. In our ICPMS analysis of Mediterranean and Pacific bluefin tuna otoliths, Na, Mg, Mn, and Zn contributed most to variation between groups. Wavelength dispersive X-ray spectrometry and proton induced X-ray emission spectrometry analyses of southern bluefin tuna (Proctor et al., 1995) found detectable levels of Na, K, S, Cl, Ca, and Sr. Univariate and multivariate analyses of otolith microconstituents failed to show significant stock structure in southern bluefin tuna.

Discrimination between Pacific and Mediterranean bluefin tuna in this pilot study supports development of the otolith microconstituent approach to investigate stock structure within the Atlantic Ocean. Otoliths from young-of-the-year, or yearling specimens, from the Western Atlantic and Mediterranean should be examined to reduce the likelihood of transoceanic migrations from nursery areas. Unfortunately, these age-classes have not been observed in the western Atlantic in recent years (S. Turner, NMFS, Southeast Fisheries Science Center, Miami FL, personal communication). However, two- and three-year old juveniles are routinely captured in recreational fisheries and could be sampled and compared with Mediterranean-caught juveniles of similar age under the assumption that trans oceanic migrations are limited in these age-classes. Successful application of otolith microconstituent analysis to stock structure questions assumes that all life stages can be identified according to nursery of origin. Therefore, the core region (part of the otolith corresponding to the first year of life) may need to be isolated from the otoliths of juvenile and adult bluefin tunas. Further research is also needed to standardize probe-based techniques and develop clean coring techniques (Secor and Chesney, 1997).

Otolith microconstituent analysis is novel but appears to be capable of distinguishing groups based upon their elemental fingerprints. However, further standardization among laboratories should be undertaken if it is to have wide utility in resolving issues of stock structure in the future. We recommend the involvement of multiple laboratories in developing reference materials and conducting intercomparison exercises (e.g Campana et al., 1997) to validate this emerging technique and develop acceptable protocols.

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