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# Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry

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ABSTRACT: Otolith chemistry of juvenile Pacific bluefin tuna *Thunnus orientalis* was measured to assess differences in composition among 3 nursery areas in the North Pacific Ocean: East China Sea, Sea of Japan and the Pacific Ocean off Shikoku. Six elements (Li, Mg, Ca, Mn, Sr and Ba) were measured in whole otoliths using solution-based inductively coupled plasma mass spectrometry. Univariate contrasts of *T. orientalis* otoliths collected in 1994 and 1995 indicated that concentrations of 5 elements (Li, Mg, Ca, Mn, Sr) differed among nurseries. Concentrations of Ca and Sr were significantly higher in the Pacific Ocean than in either marginal sea (East China Sea or Sea of Japan) nursery, while concentrations of Li, Mg and Mn were higher in fishes inhabiting marginal seas. Discriminant analysis showed clear separation of elemental fingerprints between Pacific Ocean and marginal sea nurseries, and to a lesser degree separation between the 2 marginal sea groups. Temporal stability of the elemental fingerprint was examined over a 3 yr period (1995 to 1997) in the East China Sea. Significant interannual trends were observed for 3 elements (Mg, Mn and Ba); however, elemental fingerprints of *T. orientalis* from the Pacific Ocean nursery were markedly different from all year-classes in the East China Sea.

KEY WORDS: Otolith chemistry · Pacific bluefin tuna · North Pacific Ocean



## INTRODUCTION

Pacific bluefin tuna *Thunnus orientalis* is a highly migratory species occurring throughout the North Pacific Ocean (Collette 1999). Individuals historically range from the Gulf of Alaska to Mexico and from the Sea of Okhotsk to the Philippines in the eastern and western Pacific Ocean, respectively (Collette & Nauen 1983, Bayliff 1994). Similar to their Atlantic congeners, Pacific bluefin tuna display transoceanic migratory behavior (Bayliff et al. 1991, Bayliff 1994). Tag-recapture data support the premise that juveniles (age-0 and -1) from the western Pacific Ocean commonly migrate across the ocean to areas off the coasts of the United States and Mexico. After approximately 1 to 3 yr, the majority of these fishes return to natal sites in the Pacific Ocean (Bayliff 1994). Despite their pan-oceanic distribution, spawning activity appears to be restricted to the western Pacific Ocean, and 3 nursery grounds within the region have been identified: East China Sea, Sea of Japan and Pacific Ocean (Okiyama 1974, Okiyama & Yamamoto 1979, Yonemori 1988, Kitagawa et al. 1995). While young-of-the-year bluefin tuna are commonly taken from each area, the contribution of different nursery grounds to adult stocks is unresolved.

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Analysis of the chemical composition of otoliths (ear stones) is increasingly used as a technique to differentiate stocks (see reviews by Campana 1999, Thresher 1999). Otoliths are calcium carbonate and protein accretions found in the vestibular organs of all teleost fishes, and interest in their application as recorders of time and environmental conditions has increased substantially in recent years (Secor & Rooker 2000). Otoliths are precipitated as the fish grows, and elements from the surrounding seawater are integrated into the calcium carbonate-protein matrix. Elemental concentrations in otoliths sometimes reflect the composition of ambient water, and resorption or remobilization of these elements during ontogeny is minimal (Thresher 1999). Hence, otolith elemental concentrations can serve as natural tags or 'elemental fingerprints' that reflect differences in the chemical composition of the individual fish's habitat and, therefore, provide information on natal origin and geographic association. To date, otolith elemental fingerprints have been used as indicators of population structure for several marine and estuarine-dependent fishes (Kalish 1990, Edmonds et al. 1992, Campana et al. 1999, 2000). Further, these natural tags have been used to discriminate fishes from geographically distinct nurseries and thus appear to be useful in determining the relative contributions of different nursery areas to adult stocks (Gillanders & Kingsford 1996, Milton et al. 1997, Thorrold et al. 1997).

The purpose of the current study was to evaluate the feasibility of using otolith chemistry to delineate juvenile *Thunnus orientalis* from different nursery grounds. Our primary objectives were to describe quantitatively the elemental composition of whole otoliths of juveniles (age-0) collected from the 3 primary nurseries in the North Pacific Ocean and to test for stock-specific differences in elemental fingerprints among nurseries. In addition, recent studies indicate that interannual variation in water chemistry affects otolith elemental fingerprints (Milton et al. 1997, Patterson et al. 1999, Campana et al. 2000). In response, we examined elemental fingerprints of *T. orientalis* over several years to assess the temporal 'stability' and predictive potential of these natural tags.

## MATERIAL AND METHODS

**Field collections.** Juvenile (age-0) *Thunnus orientalis* were collected from the 3 known nurseries in the western Pacific Ocean. Two of the seas are marginal (East China Sea, Sea of Japan) while the third location was off Shikoku, Japan, in the Pacific Ocean (Fig. 1). Juveniles used to assess spatial differences in elemental fingerprints were collected in 1995 except from the Sea of Japan; samples for this nursery were available only in 1994. Samples from the East China Sea were collected over 2 additional years (1996, 1997) to assess interannual patterns of otolith chemistry. Sizes ranged from 23 to 54 cm forklength (FL) (mean ± SD 38.3 ± 9.5), and weights were typically between 0.5 and 1.0 kg. Sagittal otoliths were extracted from freshly caught specimens. Although otolith elemental concentration between pairs is symmetrical for tunas (*Thunnus* spp.) (Rooker et al. 2001), selection of single otoliths for elemental analysis was based on random assignment.

**Elemental analysis.** All reagents used were ultrapure grade and all implements and containers were cleaned with dilute nitric acid and rinsed with 18 MΩ doubly deionized water ($DDIH_2O$). Before analysis, otoliths were carefully decontaminated. First, they were soaked in $DDIH_2O$ to hydrate biological residue adhering to the surface of the sample; this residue was removed using fine-tipped forceps. Next, the otoliths were soaked in 3% hydrogen peroxide for 5 min to dissolve any remaining biological residue. They were then immersed for 5 min in 1% nitric acid to remove surface contamination and then flooded with $DDIH_2O$ for 5 min to remove the acid. Finally, they were dried under a Class 100 laminar flow clean-air hood and stored in plastic vials. Otolith weight was reduced by approximately 4% as a result of the decontamination procedure. In preparation for instrumental analysis, each otolith was weighed to the

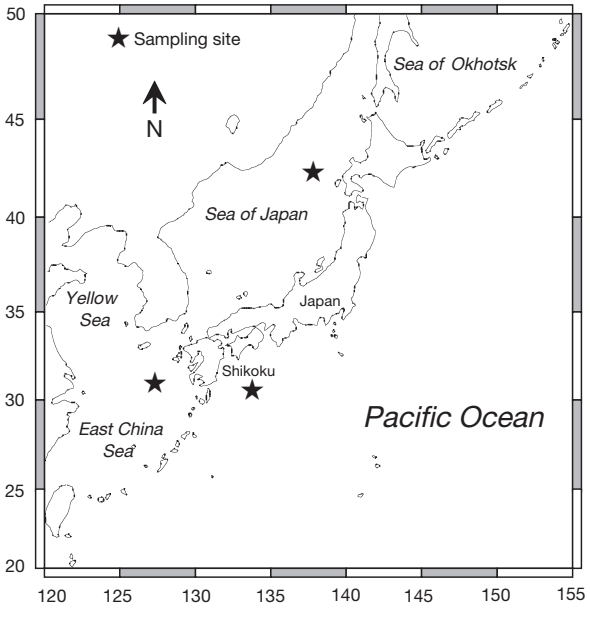


Fig. 1. Location of sampling sites in the North Pacific Ocean. Approximate location of collection areas is shown for each of the 3 putative nurseries: East China Sea, Sea of Japan and Pacific Ocean off Shikoku, Japan

nearest 0.01 mg and placed in a plastic centrifuge tube. They were digested in concentrated nitric acid. Quantities of acid used and volumes of the digests were proportional to sample weights to ensure that all resulting solutions were of similar composition in order to minimize possible matrix effects. The digests were diluted with $DDIH_2O$ to a final acid concentration of 1% nitric acid. Internal standards were added to all solutions to compensate for possible instrument drift.

Elemental concentrations were determined using a Perkin-Elmer ELAN 5000 quadrupole inductively coupled plasma mass spectrometer (ICPMS). Concentrations of Li, Mg, Mn and Ba were quantified using the method of standard additions; concentrations of Ca and Sr were determined using external calibration standards. Samples were analyzed in random order to avoid possible sequence effects. Procedural blanks and a standard reference material (SRM) were concurrently digested and analyzed following the same procedures. Limits of detection were calculated based on 3 standard deviations of the mean (n = 7) and converted to a dry weight (dry wt) basis; mean limits of detection were Li (0.01), Mg (0.19), Mn (0.06), Ba (0.01), Sr (0.90) and Ca (0.46) (values expressed as µg $g^{-1}$ dry wt; Ca in %). The SRM was NIST 915a (calcium carbonate clinical ctandard), obtained through the National Institute of Standards and Technology (Gaithersburg MD, USA), and was used to estimate the recovery, precision and accuracy of the method. This SRM is not certified for trace metal content, so only non-certified values are available for a few elements. Percentages of recovery (± SD, n = 3) of SRM were Mg (104 ± 1.0%), Ca (99 ± 1.5%) and Mn (103 ± 10.0). Samples of an otolith certified reference material (CRM) (Yoshinaga et al. 2000) produced at the National Institute of Environmental Studies of Japan have been analyzed on several occasions, although not concurrently with *Thunnus orientalis* otoliths, using this method. Certified values for the CRM ranged accordingly: Mg (21 ± 1), Ca (38.8 ± 0.5), Sr (2360 ± 50) and Ba (2.89 ± 0.09). Percentages of recovery (% ± SD, n = 18) of CRM were Mg (100 ± 11.9%), Ca (97 ± 4.6%), Sr (95 ± 5.3%) and Ba (98 ± 18.3%).

Multivariate analysis of variance (MANOVA) was used to test for spatial and temporal differences in otolith elemental fingerprints. Nursery ground and year were used as fixed factors in separate MANOVA models. Pillai trace ($V$) was chosen as the test statistic since it is the most robust to violations of homogeneity of covariance (Wilkinson et al. 1996). Univariate tests for each element were analyzed using MANOVA (covariate: otolith weight), and a preliminary model (interaction regression) was used to determine whether slopes of regression lines (homogeneity of slopes assumption) differed. The main significance test of ANCOVA (homogeneity of $y$-intercepts) was performed for all elements since 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 year classes. Small differences in otoliths weights and fish lengths occurred among sites and years, and thus we examined relations between elemental concentration and otolith weight before performing LDFA. An effect was observed for only 1 element (Ba), with concentrations positively associated with otolith weight (ANCOVA, $p \leq 0.05$). Similar to Campana et al. (2000), we removed the effect of size (otolith weight used as a proxy for fish size) to insure that differences in fish size among samples did not confound any site-specific differences in otolith chemistry. Concentrations of Ba were weight-detrended by subtraction of the common within-group linear slope from the observed concentration (concentration – $b_i \cdot$ otolith weight). 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 1 or more of the other variables (Wilkinson et al. 1996). A tolerance estimate of 0 indicates that 100% of the variance in that element can be explained by other independent variables (i.e., elements). Before statistical testing, residuals were examined for normality and homogeneity among factor levels. Within-group distribution and variance were examined and ln-transformations were used in a few cases to meet parametric assumptions.

## RESULTS

Six elements (Li, Mg, Ca, Mn, Sr and Ba) in the whole otoliths of *Thunnus orientalis* were present at levels above detection limits. Mean concentrations and natural variability of elements examined across year classes and nurseries ranged widely (Table 1). MANOVA indicated that elemental signatures varied significantly among nursery grounds ($p \leq 0.05$). Further, results from univariate contrasts showed that concentrations of 5 elements (Li, Mg, Ca, Mn and Sr) differed significantly (ANCOVA, $p \leq 0.05$) among nursery areas (Fig. 2). Concentrations of Ca and Sr were significantly

Table 1. Mean concentrations of elements present in the sagittal otoliths of juvenile *Thunnus orientalis* from 3 nurseries in the North Pacific Ocean examined from 1994 to 1997. Data are mean ± SD. Concentrations are given in μg $g^{-1}$ dry wt (ppm) ±1 SD with the exception of Ca (%)

| Element | Pacific Ocean (1995) | Sea of Japan (1994) | E. China Sea (1995) | E. China Sea (1996) | E. China Sea (1997) |
|---|---|---|---|---|---|
| Li | 0.141 ± 0.072 | 0.188 ± 0.082 | 0.191 ± 0.052 | 0.235 ± 0.077 | 0.258 ± 0.069 |
| Mg | 17.26 ± 2.29 | 44.83 ± 6.09 | 38.16 ± 10.12 | 50.27 ± 14.90 | 36.50 ± 6.44 |
| Ca | 38.13 ± 1.22 | 37.45 ± 0.44 | 36.70 ± 1.32 | 37.20 ± 0.92 | 37.08 ± 1.10 |
| Mn | 0.808 ± 0.296 | 1.904 ± 0.578 | 1.274 ± 0.411 | 1.431 ± 0.331 | 1.083 ± 0.259 |
| Sr | 1298 ± 82 | 1181 ± 25 | 1243 ± 69 | 1248 ± 48 | 1288 ± 43 |
| Ba | 1.036 ± 0.238 | 1.224 ± 0.207 | 1.045 ± 0.192 | 0.997 ± 0.137 | 1.016 ± 0.379 |

higher in the Pacific Ocean than in either marginal sea nursery. In contrast, concentrations of Li, Mg and Mn were significantly higher in fishes inhabiting marginal seas. Mean concentrations of 2 elements (Mg and Mn) were approximately 2-fold higher in marginal sea groups (Table 1). Significant interannual differences in elemental fingerprints were observed among 3 successive cohorts in the East China Sea (MANOVA, $p \leq 0.05$; Fig. 3). Levels of 3 elements, Mg, Mn and Ba, differed among age-0 year cohorts (ANCOVA $p \leq 0.05$ for Mn and Ba; interaction regression $p \leq 0.05$ for Mg). Tukey's HSD test indicated that concentrations of Mg, and Mn in 1995 were significantly higher than in 1996 or 1997. Mean concentration of each element was approximately 20 to 30% higher in 1995 than in 1996 or 1997 (Table 1). Conversely, levels of Ba were significantly higher in 1997 than in earlier years.

Discriminant analysis, based on concentrations of all 6 elements, produced clear separation of Pacific Ocean and marginal sea groups, and to a lesser degree separation between the 2 marginal sea groups (Fig. 4). Canonical variable 1 (*x*-axis) separated the Pacific Ocean group from others and accounted for 91% of the total dispersion; canonical scores of group means were −3.3 (Pacific Ocean) and approximately 1.5 for marginal sea groups. The second canonical variable (*y*-axis) contrasted the 2 marginal sea groups; scores of group means for the East China Sea and Sea of Japan were 0.8 and −0.9, respectively. Cross-validated or jack-knifed classification (group assignment excluded case being classified) indicated classification success of 100% for the Pacific Ocean group and reduced levels for the East China Sea (75%) and Sea of Japan (80%) groups. Classification success based on pooled samples (marginal seas versus Pacific Ocean) was 100%. *F-to-remove* values of Mg, Li and Mn were markedly higher than other elements suggesting that these elements were most useful for discriminating groups. Tolerance of all 6 elements was greater than 0.6 (range 0.6 to 0.9) and thus no problems with colinearity were perceived.

MANOVA of consecutive year classes from a marginal sea nursery showed that differences in elemen-

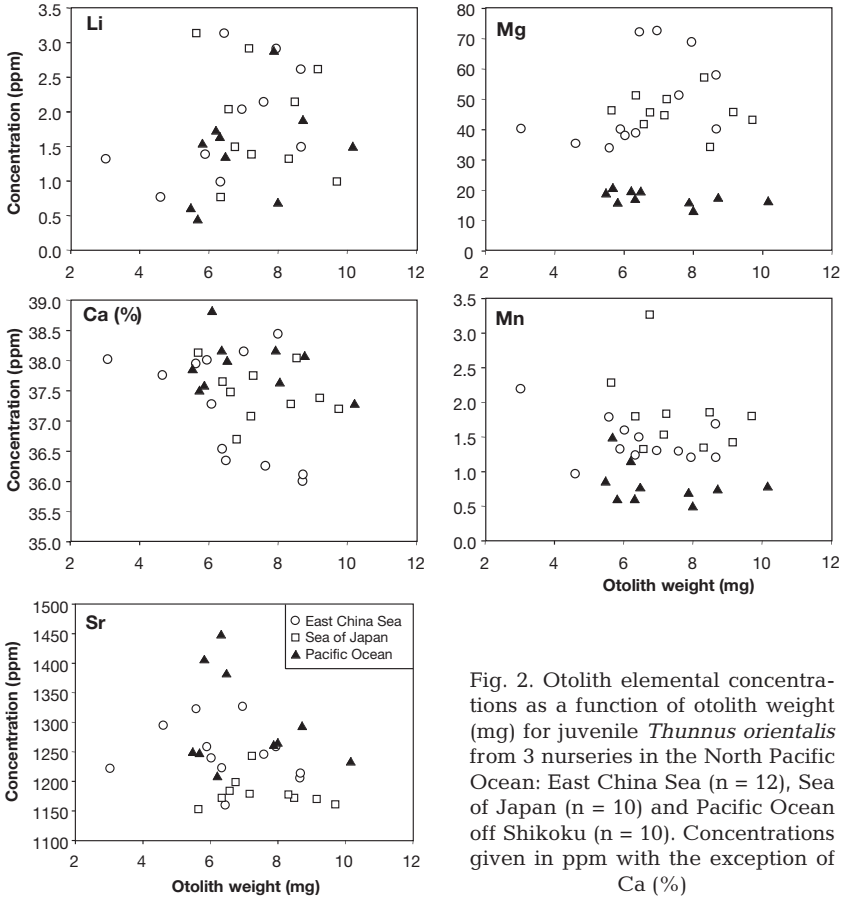


Fig. 2. Otolith elemental concentrations as a function of otolith weight (mg) for juvenile *Thunnus orientalis* from 3 nurseries in the North Pacific Ocean: East China Sea (n = 12), Sea of Japan (n = 10) and Pacific Ocean off Shikoku (n = 10). Concentrations given in ppm with the exception of Ca (%)

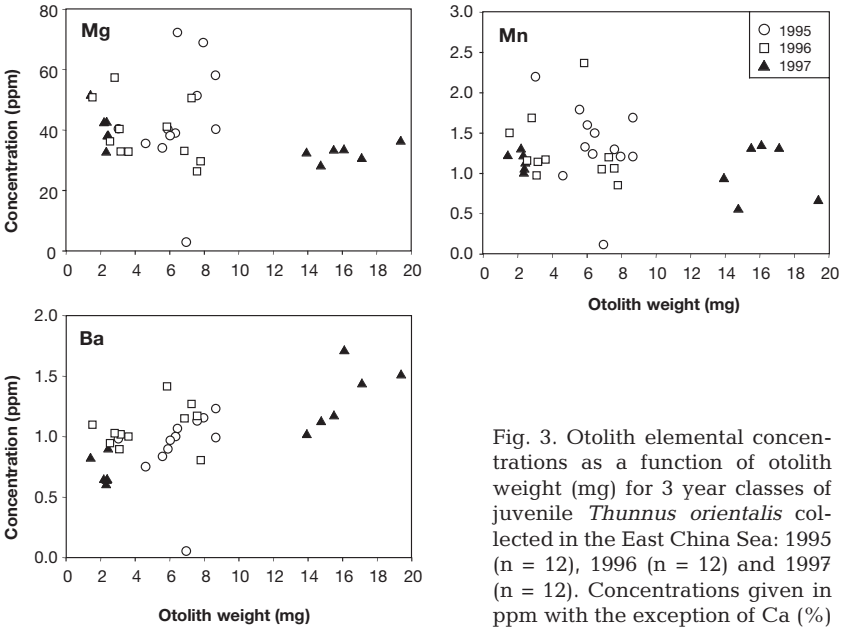


Fig. 3. Otolith elemental concentrations as a function of otolith weight (mg) for 3 year classes of juvenile *Thunnus orientalis* collected in the East China Sea: 1995 (n = 12), 1996 (n = 12) and 1997 (n = 12). Concentrations given in ppm with the exception of Ca (%)

tal fingerprints between Pacific Ocean and East China Sea groups are greater than temporal changes within a region. Six-element discriminant analysis showed clear separation of the Pacific Ocean group from all year classes in the East China Sea (Fig. 5). The first canonical variable (*x*-axis) differentiated the Pacific Ocean group from all others, explaining over 84% of the total dispersion; canonical scores of group means were 2.8 (Pacific Ocean) and –0.1 to 1.6 (East China Sea). Separation among year classes within the East China Sea was poor on all 3 canonical variables. Jackknifed classification success was 100% for the Pacific Ocean and less than 50% for each year class in the East China Sea. Similar to above, the largest contributing elements to the model were Mg and Li, and tolerance was moderate to high (range 0.7 to 0.9) for all 6 elements.

## DISCUSSION

Elemental analysis of juvenile *Thunnus orientalis* otoliths indicated that concentrations of certain elements varied among putative nursery grounds in the North Pacific Ocean. Further, trace element fingerprints from each nursery ground are distinct and vary enough to distinguish individual fishes from different regions. Results of this nature suggest that exposure to environmental conditions in the pelagic realm (chemical and physical characteristics of ambient water) is different among nursery grounds and produces distinct otolith elemental signatures. However, researchers must recognize that otolith chemical composition is not a simple representation of ambient water chemistry, and that the relation between the environmental and physiological processes are poorly understood (Thresher 1999). Despite inherent problems associated with understanding the causal factors of incorporation, ele-

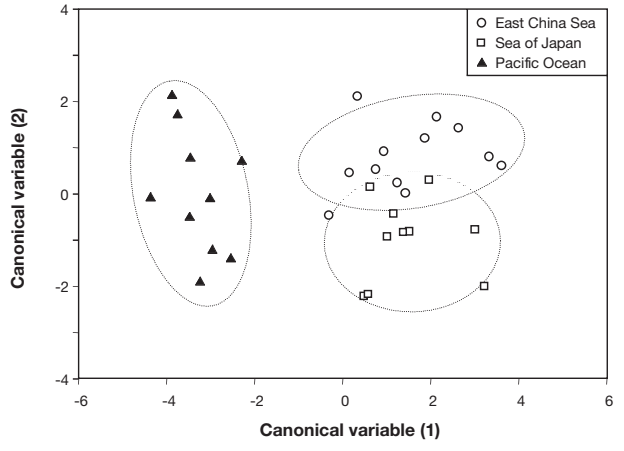


Fig. 4. Canonical plot scores and 95% confidence ellipses from discriminant analysis of otolith elemental fingerprints of juvenile *Thunnus orientalis* from 3 nurseries in the North Pacific Ocean. Discriminant analysis based on 6 elements: Li, Mg, Ca, Mn, Sr and Ba

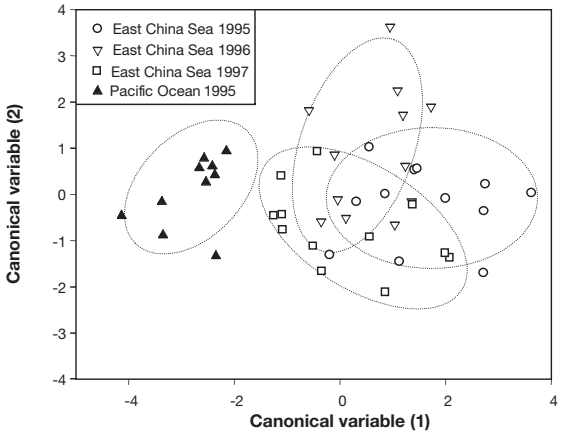


Fig. 5. Canonical plot scores and 95% confidence ellipses from discriminant analysis of otolith elemental fingerprints of juvenile *Thunnus orientalis*. Results are shown for 3 year classes from the East China Sea (1995, 1996, 1997) and 1 year class from the North Pacific Ocean (off Shikoku). Discriminant analysis based on 6 elements: Li, Mg, Ca, Mn, Sr and Ba

mental fingerprints of juvenile *T. orientalis* appear to be useful tracers of nursery origin.

Previous studies have shown that otolith composition varies among groups of fishes collected from different geographic regions (e.g., Campana & Gagne 1995, Campana et al. 1995, Edmonds et al. 1995, Secor et al. 2001), including work on *Thunnus* spp. (Proctor et al. 1995, Secor & Zdanowicz 1998). In addition, analyses of the otolith core or whole otoliths of young-of-the-year fishes have been used to establish natal origin (Kalish 1990, Gillanders & Kingsford 1996, Milton et al. 1997, Thorrold et al. 1998). While the underlying mechanisms responsible for geographic differences in otolith composition and water chemistry (metal:Ca ratios) are not completely understood, our data indicate that otolith composition is strongly linked to the location of the nursery. Conspicuous trends in otolith elemental composition were observed for several elements, and results suggest that the ambient water chemistry of the Pacific nursery (off Shikoku) differs markedly from the 2 marginal sea nurseries. Specifically, we found that concentrations of several 'indicator' elements (Li, Mg and Mn) were higher in the otoliths of juvenile *T. orientalis* collected from the Sea of Japan and East China Sea, while levels of others (Ca and Sr) were higher in the Pacific Ocean nursery.

Differences in oceanographic features between the Pacific Ocean and marginal sea nurseries are likely to result in substantial differences in ambient water chemistry. East China Sea and Sea of Japan are heavily influenced by riverine and estuarine inputs of terrestrial metals. Two of the largest rivers of the world, Yellow River (Huanghe) and Yangtze River (Changjiang), feed into the East China Sea (Yang et al. 1983). Similarly, rivers near the nursery in the Sea of Japan (e.g., Razdol'naya River, Ishikari River) are likely sources of anthropogenic and lithophilic elements. Studies assessing the geochemical compositions of suspended and trapped particulate matter in these marginal seas indicate that concentrations of certain elements (Mg, Mn and Ba) decrease with increasing distance from land or river mouth (Arakaki et al. 1994, Hung et al. 1999). Therefore, it is probable that elevated levels of certain elements in the otoliths of juvenile *Thunnus orientalis* are due to variations in water chemistry. A second potential source for high levels of elements in marginal seas is coastal upwelling, and these events occur regularly within the Sea of Japan and East China Sea (e.g., Hung & Chan 1998). Upwelling zones are characterized by cold, nutrient-rich water, and certain elements (e.g., Ba) display nutrient-type distributions. Accordingly, concentrations of such elements are typically higher in areas close to the upwelling zone and decline as the distance from the zone increases. Conversely, the nursery off Shikoku is far removed from the upwelling-influenced shelf breaks and affected by oceanographic features such as the Kuroshio Current. The Kuroshio supplies warm, metal-depauperate water to Shikoku nursery grounds, and ambient metal concentrations are typically lower (Hung & Chan 1998). Therefore, it is not surprising that concentrations of certain metals are lower in the otoliths of juvenile *T. orientalis* from this region.

Trends in Sr levels present in *Thunnus orientalis* otoliths may also be associated with differences in ambient chemistry among nursery grounds. Sr concentration in seawater is positively correlated with salinity (Ingram & Sloan 1992, Limburg 1995) and, since Sr has a similar ionic radius and valence to Ca, it is often incorporated into otoliths in direct proportion to ambient conditions (Farrell & Campana 1996, Secor & Rooker 2000). As mentioned above, surface waters in the Sea of Japan and East China Sea nurseries are influenced by freshwater input and likely to have lower salinity levels. Mean annual salinity values in East China Sea and Sea of Japan are variable and annually 1 to 3 ‰ lower than in the Pacific Ocean nursery off Shikoku (Boyer et al. 1998). Sr levels of particulate matter from marginal seas of the western Pacific Ocean show similar patterns of regional distribution (Arakaki et al. 1994). As such, we believe consistent oceanic conditions at the nursery off Shikoku produced an elevated Sr signature, resulting in small but significant differences between marginal sea and offshore nurseries.

Although differences in ambient chemistry appear to play a primary role in determining the otolith composition of *Thunnus orientalis*, other contributing factors may influence trace element incorporation, including temperature, diet, physiological stress, ontogeny and genetics (Campana 1999, Thresher 1999). The effect of temperature on otolith composition has been studied more than any other variable, and elements incorporated into aragonitic structures (corals, otoliths) appear to vary as a function of temperature. Substantial temperature effects have been reported for several elements, most notably Sr (e.g., Kalish 1989, Townsend 1992, Hoff & Fuiman 1993). However, results to date on the relation between temperature and element:Ca ratios are inconsistent and indicate that temperature and element:Ca ratios are not generally related (Campana 1999). In this study, seawater temperatures differed greatly among nursery grounds used by *T. orientalis*. Annual differences in East China Sea and Pacific Ocean nurseries were minimal; however, both locations are markedly warmer (ca 5 to 10°C) than the Sea of Japan (Antonov et al. 1998). Salient differences in elemental profiles of *T. orientalis* were observed for East China Sea and Pacific Ocean nurseries even

though thermal conditions were relatively similar, suggesting that factors other than temperature are responsible for distinct differences observed in this study. Direct evidence documenting the importance of factors other than temperature on otolith composition is limited, and results are equivocal for several of the proposed factors such as diet (Farrell & Campana 1996). Still, elemental signatures may be modified by these factors, and detailed evaluations of genetic or physiological mechanisms that determine otolith composition are needed to assess the reliability of the application.

The utility of otoliths as tracers of environmental history is determined to an extent by the temporal stability of chemical signatures. Several studies have examined the issue of temporal stability and results indicate that stock-specific signatures vary among years. Patterson et al. (1999) found that elemental concentrations of Nassau grouper *Epinephelus striatus* differed between 2 consecutive years. Similarly, Edmonds et al. (1992) found that otolith composition of yellow-eye mullet (*Aldrichetta forsteri*) collected in different years varied; however, patterns of separation were similar, suggesting a stable pattern of composition. More recently, Campana et al. (2000) found that the long-term stability (≥4 yr) of the otolith fingerprints of Atlantic cod (*Gadus morhua*) was not evident but suggested that fingerprints differed relatively little over 2 years, indicating that such fingerprints may serve only as short-term natural tags. Earlier work by Campana et al. (1995) indicated that site-specific differences among locations on the Nova Scotian Shelf explained most of the variability in otolith composition, despite significant interannual trends. Here, concentrations of individual elements in the otoliths of juvenile *Thunnus orientalis* varied among 3 annual cohorts collected in East China Sea. More specifically, concentrations of 3 elements (Mg, Mn and Ba) differed among years, with Mg showing a conflicting trend. Unfortunately, data on elemental concentrations in the western Pacific Ocean over the 3 yr study period are not available to evaluate the relation between ambient concentrations and otolith chemistry. Although interannual trends were evident, differences in otolith chemistry among nurseries appear to be greater than temporal variability within a nursery, suggesting that these natural markers were relatively stable over several year classes of juvenile *T. orientalis* in the North Pacific Ocean. Nevertheless, our design was unbalanced and did not allow for rigorous testing of temporal stability of signatures from all 3 nurseries.

In summary, we showed that juvenile *Thunnus orientalis* originating from Pacific Ocean and marginal sea nurseries can be discriminated using otolith elemental fingerprints. Moreover, elemental signatures show some degree of temporal persistence, with most of the elemental variability explained by the location of the nursery. Consequently, trace element signatures of *T. orientalis* appear promising and may be used to assign individual fishes accurately to nursery grounds. Determination of nursery origin or stock structure will rely on procedures that isolate and quantify otolith chemistry in the juvenile portion of the otolith (laser ablation ICPMS, microsculpting coupled with solution-based ICPMS). Such information will provide a means of identifying the source of sub-adult and adult *T. orientalis* and will be used to assess the relative contribution of different nursery grounds. Accordingly, fundamental questions regarding the stock association and mixing rates of *T. orientalis* and other pelagic species may be resolved in future investigations.

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LITERATURE CITED

Antonov J, Levitus S, Boyer TP, Conkright K, O'Brien T, Stephens C (1998) World ocean atlas 1998, Vol 2. Temperature of the Pacific Ocean. NOAA Atlas NESDIS 28. US Government Printing Office, Washington, DC

Arakaki T, Dokiya Y, Kodama Y, Ohyama JI, Ogawa K, Sagi T (1994) Chemical characterization of sediments from the East China Sea and the Yellow Sea. Geochem J 28:31–46

Bayliff WH (1994) A review of the biology and fisheries for northern bluefin tuna, *Thunnus thynnus*, in the Pacific Ocean. FAO Fish Tech Pap 336(2):244–295

Bayliff WH, Ishizuka Y, Deriso RB (1991) Growth, movement, and attrition of northern bluefin tuna, *Thunnus thynnus*, in the Pacific Ocean, as determined by tagging. Bull IATTC 20:1–94

Boyer TP Levitus S, Antonov J, Conkright K, O'Brien T, Stephens C (1998) World ocean atlas 1998 Vol 5: salinity of the Pacific Ocean. NOAA Atlas NESDIS 31. US Government Printing Office, Washington, DC

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188:263–297

Campana SE, Gagne JA (1995) Cod stock discrimination using ICPMS elemental assays of otoliths. In: Secor DH, Campana SE, Dean JM (eds) Recent developments in fish otolith research. Belle W Baruch Library in Marine Sciences No. 19. University of South Carolina Press, Columbia, p 671–691

Campana SE, Gagne JA, McLaren JW (1995) Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar Ecol Prog Ser 122:115–120

Campana SE, Chouinard GA, Hanson JM, Fréchet A (1999) Mixing and migration of overwintering Atlantic cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence. Can J Fish Aquat Sci 56:1873–1881

Campana SE, Chouinard GA, Hanson JM, Fréchet A (2000) Otolith elemental fingerprints as biological tracers of fish stocks. Fish Res 46:343–357

Collette BB (1999) Mackerels, molecules, and morphology. Proc 5th Indo Pac Fish Conf Soc Fr Ichthyol 1999:149–164

Collette BB, Nauen CE (1983) FAO species catalogue, Vol 2. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO Fish Symp 125(2):90–92

Edmonds JS, Lenanton RCJ, Caputi N, Morita M (1992) Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. Fish Res 13:39–51

Edmonds JS, Caputi N, Moran MJ, Fletcher WJ, Morita M (1995) Population discrimination by variation in concentrations of minor and trace elements in sagittae of two western Australian teleosts. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. University of South Carolina Press, Columbia, p 665–670

Farrell J, Campana SE (1996) Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comp Biochem Physiol 115A: 103–109

Gillanders BM, Kingsford MJ (1996) Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar Ecol Prog Ser 141:13–20

Hoff GR, Fuiman LA (1993) Morphometry and composition of red drum otoliths: changes associated with temperature, somatic growth rate, and age. Comp Biochem Physiol 106A:209–219

Hung JJ, Chan CL (1998) Distribution and enrichment of particulate trace metals in the southern East China Sea. Geochem J 32:189–203

Hung JJ, Lin CS, Hung GW, Chung YC (1999) Lateral transport of lithogenic particles from the continental margin of the southern East China Sea. Estuar Coast Shelf Sci 49: 483–499

Ingram BL, Sloan D (1992) Strontium isotopic composition of estuarine sediments as paleosalinity-paleoclimate indicator. Science 255:68–72

Kalish JM (1989) Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J Exp Mar Biol Ecol 132:151–178

Kalish JM (1990) Use of otolith microchemistry to distinguish progeny of sympatric anadromous and non-anadromous salmonids. Fish Bull 88:657–666

Kitagawa Y, Nishikawa Y, Kuboto T, Okiyama M (1995) Distribution of ichthyoplankton in the Japan Sea during summer, 1984, with special reference to scombroid fishes. Bull Jpn Soc Fish Oceanogr 59:107–114

Limburg KE (1995) Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. Mar Ecol Prog Ser 119:25–35

Milton DA, Chenery SR, Farmer MG, Blaber SJM (1997) Identifying the spawning estuaries of the tropical shad, terubok *Tenualosa toli*, using otolith microchemistry. Mar Ecol Prog Ser 153:283–291

Okiyama M (1974) Occurrence of the postlarvae of bluefin tuna, *Thunnus thynnus*, in the Japan Sea. Jpn Sea Reg Fish Res Lab Bull 25:89–97

Okiyama M, Yamamoto G (1979) Successful spawning of some holepipelagic fishes in the Sea of Japan and zoogeographical implications. In: Proceedings of the seventh Japan-Soviet joint symposium on aquaculture. Tokai University Press, Tokyo, p 223–233

Patterson HM, Thorrold SR, Shenker JM (1999) Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from the Bahamas and Belize using solution-based ICP-MS. Coral Reefs 18:171–178

Proctor CH, Thresher RE, Gunn JS, Mills DJ, Harrowfield IR, Sie SH (1995) Stock structure of the southern bluefin tuna *Thunnus maccoyii*: an investigation based on probe microanalysis of otolith composition. Mar Biol 122:511–526

Rooker JR, Zdanowicz VS, Secor DH (2001) Chemistry of tuna otoliths: assessment of base composition and post-mortem handling effects. Mar Biol 139:35–43

Secor DH, Rooker JR (2000) Is otolith strontium a useful scalar of life cycles in estuarine fishes? Fish Res 46: 359–371

Secor DH, Zdanowicz V (1998) Otolith microconstituent analysis of juvenile bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea and Pacific Ocean. Fish Res 36: 251–256

Secor DH, Rooker JR, Zlokovitz E, Zdanowicz VS (2001) Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Mar Ecol Prog Ser 211:245–253

Thorrold SR, Jones CM, Campana SE (1997) Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnol Oceanogr 42:102–111

Thorrold SR, Jones CM, Campana SE, McLaren JW, Lam JWH (1998) Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnol Oceanogr 43:1826–1835

Thresher RE (1999) Elemental composition of otoliths as a stock delineator in fishes. Fish Res 43:165–204

Townsend DW, Radtke RL, Corwin S, Libby DA (1992) Strontium:calcium ratios in juvenile Atlantic herring *Clupea harengus* L. otoliths as a function of water temperature. J Exp Mar Biol Ecol 160:131–140

Wilkinson L, Blank G, Gruber C (1996) Desktop data analysis with SYSTAT. Prentice Hall, Englewood Cliffs, NJ

Yang ZS, Milliman JD, Fitzgerald MG (1983) Transfer of water and sediments from the Yangtze River to the East China Sea, June 1980. Can J Fish Aquat Sci 40(Suppl 1):72–82

Yonemori T (1988) Japanese systems for collecting and processing tuna catch and fishing effort of longline fishery. In: Collection of working documents, Vol 3. Indo-Pacific Tuna Development and Management Program, Colombo, p 412–414

Yoshinaga J, Nakama A, Morita M, Edmonds JS (2000) Fish otolith reference material for quality assurance of chemical analyses. Mar Chem 69:91–97

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