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Chemistry of tuna otoliths: assessment of base composition and postmortem handling effects

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Abstract Protocols used to collect and prepare otoliths for chemical analysis may result in either contamination or loss of elements, thus biasing population studies in unknown ways. We evaluated precision and bias associated with collection and cleaning procedures for three Atlantic tuna species: Atlantic bluefin tuna (Thunnus thynnus), yellowfin tuna (T. albacares), and blackfin tuna (T. atlanticus). Elemental concentrations were measured using solution-based inductively coupled plasma mass spectrometry and atomic absorption spectrophotometry. Seven elements were present above detection limits in all samples (Na, Mg, K, Ca, Mn, Sr, Ba). Mean concentrations of all seven elements were statistically indistinguishable in fresh pairs of otoliths of T. thynnus (mean error 5%, range 2–8%) and T. albacares (mean error 5%, range 3–7%); no indication of a left versus right otolith effect was observed. Otolith elemental concentrations were size dependent and significant inverse relationships were observed for Mg, Na, and K. Deliberate contamination of previously cleaned samples using a 10-ppm solution of a mixture of elements demonstrated that otoliths easily acquire surface contamination. Recleaning contaminated otoliths restored elemental concentrations to levels similar to control otoliths. Precision of paired comparisons between cleaned otoliths and those exposed to contamination and recleaned was high (mean error 6%). The effects of storage at two temperatures (7 days at −20°C, 3 days at 1°C) were investigated. For K, Ca, Sr, Mn, and Ba, variation between control (removed immediately) and treatment otoliths (in situ freezing or chilling) was similar to variation observed within fresh otolith pairs (mean error: fresh vs frozen 5%, fresh vs iced 5%). Statistically significant but small (<10%) postmortem storage effects were observed for Na and Mg. Estimates of error indexed to natural ranges in otolith chemistry of T. thynnus and T. albacares from different geographic regions in the Atlantic and Pacific showed that error values of several elements (Mg, Mn, Ba, Na, K) accounted for a small proportion of the natural range, suggesting levels of precision achieved in this study are suitable for the purpose of stock delineation.

Introduction

Recent advances in otolith chemistry have afforded researchers a powerful tool to investigate population structure and stock relationships of teleosts. The technique relies on the assumption that certain elements present in the otoliths are related to the physical and chemical environment, and resorption or remobilization of these elements during ontogeny is minimal (see reviews by Campana 1999; Thresher 1999). Thus, otolith elemental concentrations represent natural tags or fingerprints that reflect differences in the chemical composition of the individuals’ 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 (e.g. Edmunds et al. 1989, 1991, 1992; Kalish 1990; Campana et al. 1994, 1995, 1999; Thresher et al. 1994; Proctor et al. 1995; Secor et al. 2001). Further, these natural tags have
been used to identify nursery origin and appear effective in assessing the productivity of geographically distinct nurseries (Gillanders and Kingsford 1996; Thorrold et al. 1997, 1998; Secor and Zdanowicz 1998).

An important and largely untested assumption in past applications of otolith chemistry is that the concentration of measured elements is a true representation of the otolith’s in situ composition (Thresher 1999). Certain elements may be more labile within otoliths than others. In particular, those associated with the organic matrix (Dove et al. 1996) rather than those that substitute for elements within the lattice may be more prone to contamination from, or loss to, surrounding solutions. Postmortem handling procedures can cause changes in elemental concentrations within both the endolymph and otolith (Kalish 1991; Milton and Chenery 1998; Proctor and Thresher 1998; Thresher 1999). Consequently, the widely accepted tenet of otoliths as essentially stable biomarkers may not be valid and merits additional investigation. Specifically, the magnitude of errors introduced during handling needs to be thoroughly investigated to assess the credibility of elemental signatures used for stock delineation (Thresher 1999).

Specimen contamination can also lead to spurious results. Several elements useful in stock discrimination are commonly present at trace levels (<10 ppm). These elements (predominately transition metals) can be relatively abundant in field and laboratory environments, thereby increasing the probability of contamination. Specimen contamination is an important source of methodological error and appears to have compromised results of past studies, including work on scombrids (Calaprice 1986; Thresher et al. 1994). Researchers readily acknowledge that contamination is a critical issue, and field and laboratory handling procedures have been modified to minimize the effect; however, the efficiency of procedures used to clean (i.e. decontaminate) otoliths has not been adequately investigated.

In this study, we evaluate field and laboratory procedures used to collect and prepare tuna (Thunnus spp.) otoliths for elemental analysis. Two questions related to natural variation in otolith composition were addressed. First, otolith pairs from fresh tuna were compared to assess natural variation within pairs (i.e. asymmetric composition between right and left otoliths) and also to provide an estimate of “true” precision within pairs, which is required to evaluate experimental protocols. Second, ontogenetic (size) effects on otolith elemental concentrations were examined. Protocol tests were conducted to determine (1) the effectiveness of a rigorous cleaning procedure (5-min soak in dilute nitric acid) in removing surface contamination, and (2) the effects of postmortem storage procedures (freezing or icing) on otolith composition. Overall, four hypotheses were tested: $H_{0.1}$: chemistry of paired otoliths is identical; $H_{0.2}$: ontogeny (size) does not affect otolith chemistry; $H_{0.3}$: a rigorous cleaning procedure will provide surface decontamination without causing measurable changes in otolith chemistry; $H_{0.4}$: otolith chemistry of tuna subjected to different postmortem storage methods is similar. In addition, the resolving power of the approach was assessed by indexing estimates of precision to the total range of observed values for different stocks of Atlantic and Pacific tuna.

### Materials and methods

#### Specimen collection

Sagittal otoliths used in protocol trials were obtained from Atlantic bluefin tuna (Thunnus thynnus), yellowfin tuna (T. albacares), and blackfin tuna (T. atlanticus). Bluefin tuna and yellowfin tuna were collected from several sites in the Mid-Atlantic Bight in 1997 and 1998, respectively. Blackfin tuna were collected from the northwest Gulf of Mexico in 1999. Specimens of all three congeners were juveniles (age 0 to 3+) and sizes ranged accordingly: T. thynnus [101–102 cm fork length (FL)], T. albacares (56–120 cm FL), T. atlanticus (58–70 cm FL).

#### Elemental analysis

All reagents 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$_2$O). Before analysis, otoliths were carefully decontaminated. First, they were soaked in DDIH$_2$O to hydrate biological residue adhering to the surface of the sample; this residue was removed using fine-tipped forceps. Next, 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$_2$O 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 mass was reduced by approximately 4% as a result of the decontamination procedure (before and after dilute acid cleaning). In preparation for instrumental analysis, each otolith was weighed to the 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 digestes were proportional to sample weights to insure that all resulting solutions were of similar composition to minimize possible matrix effects that might complicate instrumental analysis. The digestes were diluted with DDIH$_2$O to a final acid concentration of 1% HNO$_3$. 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 magnesium (Mg), manganese (Mn), and barium (Ba) were quantified using the method of standard additions; concentrations of calcium (Ca) and strontium (Sr) were determined using external calibration procedures. Sodium (Na) and potassium (K) concentrations were measured by atomic absorption spectrophotometry (AAS) using a Perkin-Elmer Model 3300 AA spectrophotometer. 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. The SRM was NIST 915a (Calcium Carbonate Clinical Standard), 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 noncertified values are available for a few elements. Percent recovery of relevant values are ppm (μg g$^{-1}$) dry weight (Ca in %): Mg, 1.0; Ca, 40.0; and Mn, 0.6. Our results [average ppm dry weight ± 1 standard deviation (SD), $n = 5$] were Mg, 1.04 ± 0.05; Ca, 39.6 ± 0.6; and Mn, 0.62 ± 0.06. Samples of an otolith certified reference material (CRM) (Yoshinaga et al. 2000) produced at the National Institute of Environmental Studies (NIES) of Japan have been analyzed on several
occasions, although not concurrently with these samples, using this method. Certified values for the CRM are (ppm dry weight; Ca in %) Na, 2,230±100; Mg, 21 ±1; K, 282 ±8; Ca, 38.8 ±0.5; Sr, 2,360 ±50; and Ba, 2.89 ±0.09. Our results were (average ppm dry weight ± 1 SD, n = 18) Na, 2,380 ±103; Mg, 21.1 ±2.5; K, 334 ±21; Ca, 37.6 ±1.8; Sr, 2,240 ±124; and Ba, 2.84 ±0.53.

Assessment of base composition

Otoliths from freshly collected T. thynnos (n = 6) and T. albacares (n = 22) tuna were processed using the protocols described above to compare elemental concentrations between right and left otoliths, blocking for individual fish effects. Precision of measurements was evaluated by estimating percent error:

\[
\text{Error} \, (\%) = \frac{\text{abs}(O_L - O_R)}{\left(\frac{1}{2}(O_L + O_R)\right)} \times 100,
\]

where \(O_L\) and \(O_R\) are the elemental concentration of the left and right sagittae, respectively. Size effects were examined for three size classes of T. albacares (55–65, 81–100, 101–120 cm FL). To minimize potential bias due to spatial and temporal variability, all individuals used for this trial were collected from Nags Head, North Carolina during May and June of 1998. However, it should be noted that we did not constrain environmental conditions and, as a result, it is not known whether changes are related to ontogenetic shifts in habitat (environmental exposure) or physiology.

Empirical testing of cleaning and storage effects

Assessment of the cleaning procedure was conducted using pairs of sagittal otoliths from 32 freshly caught T. albacares. Otolith pairs were cleaned using the previously described decontamination protocol (DDH2O water and nitric acid) and stored in plastic vials. One sagitta from each pair (based on random assignment) was designated the control otolith. The second sagitta (treatment otolith) was immersed in a 10-ppm solution of a mixture of elements for 2 min, then dried overnight. Since the primary aim of the exercise was to create contaminated otoliths to test cleaning protocols, immersion in the 10-ppm solution was a viable means producing consistent contamination. Because it is difficult to identify natural sources of contaminants in the field, our exercise did not attempt to mimic field conditions; nevertheless, our contamination scenario is similar in some respects to field conditions. It is difficult to wash and dry otoliths in the field and otoliths are often “wet” (coated with endolymph and residual tissue) when stored. As a result, solutes and other particulate matter dry on the surface of the otolith. Because a 10-ppm solution was used for this trial, we evaluated only those elements present naturally in otoliths at similar concentrations: Mg, Mn, and Ba (~0–20 ppm). Half of the treatment otoliths (16) were analyzed versus their respective controls to determine the degree of contamination for each element. The remaining half of the treated group was reclaned using the described protocol. Reclaned sagittae (treatment otoliths) were compared to their respective controls to evaluate the effectiveness of our decontamination procedure. Analyses were conducted during a single instrumental analytical session.

We conducted preliminary trials to assess our cleaning protocol and results indicate that differences between cleaned (acid rinse) and untreated otoliths of striped bass (Morone saxatilis) are negligible (Secor et al. 2001). Paired differences suggest minor but highly consistent losses in concentrations for Mg, Mn, and Zn. Na showed a moderate difference (12%), but difference was consistent among tested pairs. Ca, Sr, and Ba showed trivial differences between cleaned and untreated otoliths (2–6%), particularly considering that control pairs of otoliths (both untreated) showed 2–5%.

The effects of freezing and cooling on otolith composition were assessed by first removing one of the sagittal otoliths from freshly caught T. atlanticus. Selection of the otolith for extraction (i.e. left vs right) was based on random assignment. Otoliths were extracted by making a small (5 cm) dorso-ventral transverse cut beginning at the posterior section of the occipital bone. Next, a cranial-caudal frontal cut was made through the neurocranium to the initial cut and the block of tissue was removed, exposing the top of the brain. One of the two otoliths (designated the control otolith) was removed, and the remaining otolith was left intact in the saccular vestibule. Brain tissue removed during the extraction of the control otolith was replaced and the block of tissue was reattached in an attempt to preserve any exchange of fluids in the brain cavity and vestibular apparatus. Following removal of the control otolith, T. atlanticus carcasses were placed either into a freezer (–20°C) for 7 days or on ice in a storage chest (1°C) for 3 days. T. atlanticus carcasses were then thawed (ca. 2–4 h) and the second sagitta (treatment otolith) was removed. Preparation of paired otoliths (n = 12 for each treatment) for elemental analysis was conducted using the procedure described above.

Resolving power of technique for natural stocks

An additional metric was estimated to assess the capability of otolith chemistry as a means of delineating natural stocks of tunas. Estimates of baseline precision (error) derived from left versus right comparisons of T. thynnos and T. albacares otoliths were expressed as a percent of the total range observed in natural stocks of Pacific bluefin tuna, T. orientalis (15–54 cm FL), T. thynnos (28–75 cm FL), and T. albacares (56–120 cm FL). Otoliths used for range estimates were collected over a 5-year period: T. orientalis (1995–1997), T. thynnos (1998–1999), T. albacares (1997–1998). Both species of bluefin tuna were collected from several regions: T. orientalis (East China Sea, Sea of Japan, Pacific Ocean off Shimok, T. thynnos (Mid-Atlantic Bight, Alboran Sea, Ionian Sea, Tyrrhenian Sea, Bay of Biscay). All T. albacares were collected in the Mid-Atlantic Bight. All values within ± 2 SD of the mean were used to approximate the range of elemental concentrations for each of the three stocks. Error estimates from left versus right comparisons were similar to levels of precision observed in cleaning and storage trials. Therefore, only error terms derived from left versus right comparisons were indexed to population range.

Data analysis

Mean elemental concentrations among paired tests (control vs treatment otoliths) were compared using a paired (dependent) t-test. One-way analysis of variance (ANOVA) was applied to size-specific elemental data. This analysis was performed separately on each element. Tukey’s HSD test was used to find a posteriori differences (x = 0.05) among sample means. Prior to statistical testing, residuals were examined for normality and homogeneity among factor levels. Due to small samples sizes, the assumption of normality was not met in some cases; however, t-test and ANOVA are robust to most types and magnitudes of departure from normality and, therefore, non-normality is unlikely to compromise results (Underwood 1981).

Results

“Base composition” of tuna otoliths

Mean concentrations of elements in Thunnus thynnos ranged widely: Na, 2,640 ±61; Mg, 14.3 ±1; K, 327 ±27; Ca, 38.2 ±1.0; Mn, 0.29 ±0.06; Sr, 1,610 ±96; and Ba, 1.29 ±0.24; concentrations are given in ppm dry weight ±1 SD (Ca in %). Trace element composition of juvenile T. thynnos otoliths was similar to whole otolith assays of T. albacares: Na, 2,940 ±103; Mg, 19.3 ±3.4; K, 341 ±41; Ca, 38.3 ±1.8; Mn, 0.92 ±0.29; Sr, 1,610 ±96; and Ba 1.49 ±0.31.
Univariate contrasts of these elements within pairs of otoliths from *T. thynnus* and *T. albacares* indicated the extent of agreement between measurements was high, and elemental incorporation between left and right sagittae was symmetrical. Concentrations of all seven elements were statistically indistinguishable (*t*-test, *P* > 0.05) within fresh pairs of *T. thynnus* otoliths (mean error 5%, range 2–8%) (Fig. 1). Except for Mg, no indication of a left versus right otolith effect was observed within pairs of *T. albacares* otoliths (mean error 5%, range 3–7%); mean concentration of Mg was 5% higher in right sagittal otoliths of *T. albacares* (Fig. 1).

Levels of Na, Mg, and K were significantly different among size classes of *T. albacares* (*P* < 0.05). Concentrations of all three elements were inversely related to size (Fig. 2). Tukey’s HSD test indicated elemental concentrations of Na, Mg, and K of the largest size class (101–120 cm FL) were significantly lower than the smallest size class (56–65 cm FL). Levels of Ca, Mn, Sr, and Ba were variable but did not differ significantly among size classes investigated (*P* > 0.05).

**Experiment 1: cleaning effects**

Concentrations of Mg, Mn, and Ba were significantly higher (*P* < 0.01) in deliberately contaminated otoliths than in control otoliths. Error values were in the range of approximately 20–150%, indicating that the treatment effectively contaminated otoliths (Fig. 3). Recleaning contaminated otoliths with our decontamination procedure restored elemental concentrations to control levels. Estimated error values of cleaned sagittae (control) and sagittae exposed to cleaning, contamination, and recleaning (treatment) were low; mean error ranged from 4% to 6% (Fig. 3). Although errors were equivalent to estimates of natural variation in paired otoliths, small but statistically significant differences were observed for Mg and Ba (*P* < 0.05). Elemental concentrations of Mg in recleaned otoliths were 4% lower than control otoliths. In contrast, levels of Ba were 5% higher in control otoliths.

**Experiment 2: postmortem storage effects**

Postmortem effects due to storage treatment were not significant for K, Ca, Sr, Mn, or Ba (Fig. 4). Levels of Na were significantly lower in otoliths exposed to in situ freezing (*P* = 0.006) than control otoliths (mean concentration: 2.720 and 2.790 ppm, respectively). Despite the difference, error values between paired otoliths were low (~2.5%). Mg levels in otoliths exposed to in situ cooling were significantly higher (*P* = 0.016) than control otoliths (mean concentration: 13.1 and 11.6 ppm, respectively). Variability of Mg between pairs was also relatively high (error 12.4%) compared to the overall error estimates of all elements combined (control vs frozen 4.7%, control vs iced 5.2%).

**Evaluation of natural stocks**

Otolith chemistry of *Thunnus* spp. collected from different geographic regions appears relatively similar in bulk composition; however, salient patterns were present for certain elements (Table 1). Range estimates of several elements were wider for groups collected from several regions (*T. thynnus thynnus*, *T. thynnus orientalis*) than for those from a single locale (*T. albacares*). Baseline precision (error) derived from paired comparisons (*H* *H* *H* *H* 0.1) of *T. thynnus* and *T. albacares* otoliths were expressed as a percent of the total range observed within a particular region or stock (Table 1), and results indicated that error estimates of several elements represent a small proportion of the total range. For both species of bluefin tuna, error estimates of Mg, Mn, Ba, Na, and K accounted for less than 10% of the observed range, with many values around 1–3%. Similar results were observed for *T. albacares*; however, the percent values were slightly higher. In contrast, differences were markedly higher for Ca and Sr across species and/or subspecies (29.6–44.6% and 17.1–37.1%, respectively).
Fig. 2 Otolith elemental concentrations of seven elements measured among three size classes of yellowfin tuna (*T. albacares*). Values are means based on all individuals within a size class ± SE. Concentrations given in μg/g dry weight (ppm) ± 1 standard deviation (SD) except for Ca (%).

<table>
<thead>
<tr>
<th>Size Class</th>
<th>Thunnus albacares</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56-65 cm FL</td>
</tr>
<tr>
<td></td>
<td>N = 8</td>
</tr>
<tr>
<td>2</td>
<td>80-100 cm FL</td>
</tr>
<tr>
<td></td>
<td>N = 7</td>
</tr>
<tr>
<td>3</td>
<td>101-120 cm FL</td>
</tr>
<tr>
<td></td>
<td>N = 7</td>
</tr>
</tbody>
</table>

**Discussion**

Otolith chemical composition is not a simple representation of ambient water chemistry. Recent studies suggest that otolith composition is determined by complex interactions between environmental conditions, genetics, and physiological processes (Campana 1999; Thresher 1999). Further, analytical and handling procedures may affect what is measured in the otolith sample. Campana (1999) suggested that analytical and handling biases could be reduced through use of careful protocols employing decontaminated instruments and environments. However, such conditions may be impractical or impossible to achieve in field collections of cosmopolitan species such as tunas. The imposition of such controls would also preclude examination of historical samples. Moreover, handling protocols used to date have not been rigorously tested between paired otoliths, blocking for individual differences among fish. Proctor and Thresher (1998) observed that the magnitude of error due to handling and analytical effects was similar to differences used to define population structure in past applications and suggested three options to isolate this error: (1) include handling as a separate statistical factor; (2) exclude elements most prone to handling effects; and (3) rigorously acid wash otoliths and examine “residual elements.” Thresher (1999) concluded that the first option was impractical for most studies and the second option would constrain analysis to a relatively few
have demonstrated that fluid migration is potentially a viable mechanism for mobilizing elements loosely bound in the otolith matrix (Gauldie et al. 1998; Milton and Chenery 1998, Proctor and Thresher 1998).

Similar to contamination, postmortem handling-induced artifacts may change otolith composition and potentially compromise the credibility of elemental data. Recent observations suggest that routine specimen handling influences trace element concentrations (Milton and Chenery 1998, Proctor and Thresher 1998) and thus the assumption that otoliths are essentially closed systems may be flawed, at least for certain elements. Postmortem handling procedures examined for bulk chemical analysis of T. atrilattus otoliths appear to have nominal effects on composition. Of the seven elements examined in the freezing and icing trials, only one element in each trial showed a difference (Na and Mg, respectively). Although statistically significant, these differences were small in magnitude and unlikely to compromise assessments of stock structure. The effect of freezing on otolith composition was recently examined for tropical shad (Ternua latea tola) from eastern Malaysia (Milton and Chenery 1998).

Elements such as alkali metals (e.g., Li, Na, K, Rb) and halogens (e.g., Cl, Br) are present as single-valent inclusions and are thought by some to be extremely labile (Proctor and Thresher 1998). Thus, the usefulness of these elements as biological markers may be limited. In contrast, the alkaline-earth metals (Mg, Sr, Ba) are divalent ions and are thought to substitute for Ca in the otolith matrix. These elements are more likely to be firmly bound to the crystal lattice and in situ concentrations appear unaffected by different handling and cleaning protocols. Similarly, certain transition metals (e.g., Mn, Co, Cu, Zn) occur as divalent ions and may be reliable biological markers (e.g., Milton and Chenery 1998). The stability of alkaline-earth metals and transition metals make them good candidates for future studies on tunas. Further, the majority of elements reported multiple times in the literature as stock discriminators fall into one of these two categories of metals (Campana 1999; Thresher 1999).

Figure 3 Mean percent error of three elements measured between paired sagittal otoliths of yellowfin tuna (T. albacares). A Plot compares control otoliths (cleaned) to treatment otoliths (contaminated). B Plot compares control otoliths (cleaned) to treatment otoliths (contaminated and re-cleaned). Arrows indicate direction of change from control otoliths. Dashed line shown in both plots is given at an error of 5%
Fig. 4 Otolith elemental concentration of seven elements measured between paired sagittal otoliths of blackfin tuna (*T. atlanticus*) from experiments examining the effect of post-mortem storage. Closed circles on plots compare control (fresh) to treatment (frozen) otoliths. Open circles compare control (fresh) to treatment (iced) otoliths. Concentrations given in µg/g dry weight (ppm) ± 1 SD except for Ca (%). Probability values from paired (dependent) *t*-tests are given. Asterisks denote significant results.

Natural variability between pairs of tuna otoliths was remarkably small, suggesting a high level of precision (reproducibility of repeated measures) of bulk chemical analysis by ICPMS and AAS. For all seven elements reported, estimates of precision between paired otoliths, which include both natural and analytical sources of variability, were low (~10%). Based on our estimates of analytical precision of CRM comparisons, it appears
Table 1 Concentrations of elements present in the sagittal otoliths of juvenile *Thunnus orientalis*, *T. thynnus*, and *T. albacares* from several regions in the Atlantic Ocean, Pacific Ocean, and associated seas. Standard deviations (SD) and range estimates are shown for each species (subspecies). Error estimates from paired trials were expressed as a percent of the total range (difference between maximum and minimum values for all points within 2 SD of the mean) for each species. Concentrations given in µg/g dry weight (ppm)±1 SD except for Ca (%).

<table>
<thead>
<tr>
<th></th>
<th>Li</th>
<th>Mg</th>
<th>Mn</th>
<th>Ba</th>
<th>Ca</th>
<th>Sr</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thunnus orientalis</em>: Pacific Ocean 1995–1997 (<em>n</em> = 69)</td>
<td>0.210</td>
<td>40.50</td>
<td>1.260</td>
<td>1.061</td>
<td>37.3</td>
<td>1,266</td>
<td>3,892</td>
<td>356</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.085</td>
<td>14.72</td>
<td>0.464</td>
<td>0.247</td>
<td>1.1</td>
<td>72</td>
<td>666</td>
<td>41</td>
</tr>
<tr>
<td>Max</td>
<td>0.448</td>
<td>72.74</td>
<td>3.266</td>
<td>1.707</td>
<td>41.5</td>
<td>1,472</td>
<td>6,001</td>
<td>521</td>
</tr>
<tr>
<td>Min</td>
<td>0.060</td>
<td>12.93</td>
<td>0.486</td>
<td>0.599</td>
<td>34.3</td>
<td>1,136</td>
<td>2,881</td>
<td>268</td>
</tr>
<tr>
<td>Range of values within 2 SD of the mean</td>
<td>0.289</td>
<td>56.03</td>
<td>1.704</td>
<td>0.911</td>
<td>3.4</td>
<td>269</td>
<td>2,007</td>
<td>160</td>
</tr>
<tr>
<td>Percent of population range</td>
<td>NA</td>
<td>1.2</td>
<td>1.5</td>
<td>4.1</td>
<td>38.0</td>
<td>37.1</td>
<td>3.1</td>
<td>7.3</td>
</tr>
<tr>
<td><em>T. thynnus</em>: Atlantic Ocean 1998–1999 (<em>n</em> = 92)</td>
<td>0.228</td>
<td>31.67</td>
<td>0.998</td>
<td>0.879</td>
<td>37.8</td>
<td>1,180</td>
<td>3,668</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.072</td>
<td>7.61</td>
<td>0.380</td>
<td>0.333</td>
<td>1.3</td>
<td>151</td>
<td>348</td>
<td>167</td>
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<tr>
<td>Max</td>
<td>0.431</td>
<td>55.33</td>
<td>1.949</td>
<td>1.972</td>
<td>41.6</td>
<td>1,558</td>
<td>4,247</td>
<td>1,230</td>
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<tr>
<td>Min</td>
<td>0.102</td>
<td>17.06</td>
<td>0.312</td>
<td>0.518</td>
<td>35.3</td>
<td>827</td>
<td>2,871</td>
<td>434</td>
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<tr>
<td>Range of values within 2 SD of the mean</td>
<td>0.253</td>
<td>27.12</td>
<td>1.408</td>
<td>0.989</td>
<td>4.4</td>
<td>564</td>
<td>1,255</td>
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<tr>
<td>Percent of population range</td>
<td>NA</td>
<td>2.4</td>
<td>1.8</td>
<td>3.8</td>
<td>29.6</td>
<td>17.1</td>
<td>5.0</td>
<td>2.1</td>
</tr>
<tr>
<td><em>T. albacares</em>: Atlantic Ocean 1997–1998 (<em>n</em> = 56)</td>
<td>0.187</td>
<td>15.74</td>
<td>0.831</td>
<td>1.317</td>
<td>38.5</td>
<td>1,415</td>
<td>2,960</td>
<td>403</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.065</td>
<td>3.95</td>
<td>0.253</td>
<td>0.316</td>
<td>1.3</td>
<td>98</td>
<td>121</td>
<td>70</td>
</tr>
<tr>
<td>Max</td>
<td>0.330</td>
<td>26.13</td>
<td>1.620</td>
<td>2.446</td>
<td>42.4</td>
<td>1,697</td>
<td>3,172</td>
<td>572</td>
</tr>
<tr>
<td>Min</td>
<td>0.094</td>
<td>7.62</td>
<td>0.298</td>
<td>0.472</td>
<td>31.5</td>
<td>1,227</td>
<td>2,689</td>
<td>285</td>
</tr>
<tr>
<td>Range of values within 2 SD of the mean</td>
<td>0.221</td>
<td>13.36</td>
<td>0.848</td>
<td>1.015</td>
<td>3.9</td>
<td>350</td>
<td>433</td>
<td>241</td>
</tr>
<tr>
<td>Percent of population range</td>
<td>NA</td>
<td>10.1</td>
<td>8.5</td>
<td>6.9</td>
<td>44.6</td>
<td>18.5</td>
<td>19.8</td>
<td>5.8</td>
</tr>
</tbody>
</table>

that most of the variability between pairs is due to analytical sources rather than natural variability (asymmetric composition between pairs). Although it was not possible to partition effectively the effects of analytical and natural variability, observed levels of precision were similar or lower than levels associated with other techniques commonly used in stock delineation studies (Waldman et al. 1997). In fact, indicator elements (significant factors in multivariate analysis) used in previous studies to differentiate stocks often vary by at least a factor of 2 (e.g. Secor and Zdanowicz 1998). Precision estimates expressed as a percent of the total range for tuna stocks (*T. thynnus*, *T. albacares*) in the Atlantic and Pacific demonstrated that error values of several elements (e.g. Mg, Mn, Ba, Na, and K) represent a small proportion of the natural range of concentrations and thus their potential discriminatory power is high. Rooker et al. (2001) correctly classified all juvenile *T. thynnus* from two regional nurseries in the western Pacific [Pacific Ocean vs marginal seas (Sea of Japan, East China Sea)] using otolith chemistry. Differences in Mn concentrations contributed most to their multivariate model and mean concentrations between stocks varied by approximately 0.8 ppm; error estimates observed in this study for Mn represent only 3% of the difference between mean concentrations of Mn observed in the two nurseries, suggesting levels of precision achieved in this study are adequate for the purpose of stock delineation.

Variation in elemental concentration along the otolith growth axis has been reported in several probe- and laser-based studies, and ontogenetic differences in trace element impurities are substantial for certain elements (e.g. Thresher et al. 1994; Fowler et al. 1995; Thorrold et al. 1997; Proctor and Thresher 1998). For example, differences in otolith Sr are pronounced for certain species (e.g. anadromous fishes) and have been used to reconstruct salinity and temperature histories (e.g. Radtke 1989; Townsend et al. 1989, 1992; Secor and Rooker 2000). Since trace element levels commonly vary along the growth axis, population assessments based on analytical results from pooled samples (i.e. several age classes or cohorts) are potentially biased. In *T. albacares*, significant differences among size classes were present for three elements (Na, Mg, K). Concentrations of all three elements decreased with increasing size. Spatial shifts that could influence environmental exposure to certain elements are likely to be one cause of these differences, although physiological changes related to ontogeny also may contribute. Ontogenetic shifts in growth rate, metabolic rate, diet, and condition are common, and likely to alter the chemistry of blood plasma, endolymph, and otoliths by way of changes in protein-mediated discrimination or metal-binding capacity (Kalish 1989, 1991). The effects of ontogeny on otolith composition can be statistically isolated in otolith composition studies by using size as a covariate (e.g. Secor and Zdanowicz 1998; Campana et al. 2000); however, precaution should be exercised when interpreting chemistry data from different size (age) classes since it is likely that these individuals have experienced different environmental conditions. Although individuals were collected from the same region in this study, we did not constrain environmental conditions and, as a result, it is not known whether changes are related to ontogenetic shifts in habitat (environmental exposure) or physiology.

In summary, based on high levels of precision for seven elements (Na, Mg, K, Ca, Mn, Sr, Ba), variability
Kalish JM (1990) Use of otolith microchemistry to distinguish progeny of sympatric anadromous and non-anadromous salmonids. Fish Bull 88:657–666
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

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References
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