Chemistry of otoliths from juvenile menhaden *Brevoortia patronus*: evaluating strontium, strontium:calcium and strontium isotope ratios as environmental indicators

Edward J. Chesney1,*, Brent M. McKee2, Thomas Blanchard3, Lui-Heung Chan3

1Louisiana Universities Marine Consortium, 8124 Hwy 56, Chauvin, Louisiana 70344, USA
2Tulane University, Department of Geology, 208 Dinwiddie Hall, New Orleans, Louisiana 70118, USA
3Louisiana State University, Dept of Geology and Geophysics, Baton Rouge, Louisiana 70803, USA

ABSTRACT. Laboratory studies were conducted to establish the utility of otolith chemistry for tracing gulf menhaden *Brevoortia patronus* environmental histories. Menhaden larvae were hatched from eggs and reared to juveniles under constant conditions at 3 temperatures (18, 22, 26°C) and 3 salinities (20, 26, 33.4). Whole otoliths from experimental fish along with their rearing waters were analyzed by ICPMS (inductively coupled plasma mass spectrometry) and TIMS (thermal ionization mass spectrometry) to determine the effects of temperature and salinity on Sr concentration, Sr/Ca and 87Sr/86Sr. A comparison of elemental composition by otolith type revealed significantly lower Sr concentrations and lower Sr/Ca ratios in the asteriscus versus the sagitta and lapillus. X-ray diffraction analyses for each otolith type determined that menhaden sagittae and lapilli are aragonite while their asterisci are vaterite. This suggests the type of calcium carbonate matrix deposited can affect the concentrations of trace elements incorporated into otoliths. In laboratory reared menhaden no significant relationships between Sr concentration or Sr/Ca ratios and temperature, salinity or menhaden growth rate were detected (ANOVA, p = 0.05). We found no indication that 87Sr/86Sr ratios of menhaden otoliths were affected by water temperature. 87Sr/86Sr did reflect the salinity of the rearing water; however, the relationship between Sr isotope ratios and salinity limits the utility of Sr isotope ratios as a precise indicator of salinity to low salinity environments (<20) or over wide ranges of salinity. A comparison between Sr isotope ratios of menhaden otoliths and menhaden vertebrae, along with an analysis of a vertebra from a reproductive adult bay anchovy *Anchoa mitchilli*, showed there was no significant difference between otolith and vertebra 87Sr/86Sr ratios, indicating that other bony structures might be suitable for strontium isotope analyses in small individual fish.

KEY WORDS: Menhaden - Otoliths - Environmental history - Strontium - Calcium - Strontium isotopes - ICPMS - TIMS

INTRODUCTION

The chemical analysis of biogenic calcium carbonate has been applied in a number of scientific disciplines, including fisheries, and to a wide variety of scientific problems. The fundamental premise of these analyses is that a variety of environmental and physiological information is incorporated into calcium carbonate structures and can be discerned with appropriate analytical methods (Radtke & Shafer 1992). Elemental analyses of fish hard parts have included studies of otoliths, vertebrae and scales. Previous fisheries applications encompass studies of spawning location, migrations associated with diadromy (Casselman 1982, Kalish 1989, Secor 1992, Coutant & Chen 1993, Rieman et al. 1994, Halden et al. 1995, Limburg 1995, Secor et al. 1995, Tzeng 1995, Tzeng et al. 1997), stock discrimination (Edmonds et al. 1989, 1991, 1992, 1995, Gunn et al. 1992), differentiation of spawning stocks (Kalish 1990, Campana & Gagne 1995), overwintering distrib-

Otolith chemistry has considerable potential to advance our understanding of fisheries oceanography, and the prospects for additional development of these techniques are promising because of recent and continued advances in elemental analysis technology (Coutant & Chen 1993, Fowler et al. 1995a, b, Thorrold et al. 1997). However, there is a need to better understand the numerous factors which can affect the incorporation of inorganic constituents into otoliths and other fish hard parts and be able to predict how these factors interact to complicate the interpretation of elemental signatures (Kalish 1989, 1991a, Sadovy & Severin 1992, Hoff & Fuiman 1993, 1995. Tzeng 1994, 1995, Fowler et al. 1995a, b, Gallahar & Kingford 1996). This is an increasing problem given the sophisticated analytical instruments and chemical techniques now being applied. The newer techniques have allowed researchers to expand the range of elements being analyzed and work at lower elemental concentrations where significant analytical and contamination problems can arise (Thresher et al. 1994, Thresher 1995, Secor & Chesney 1997). Although these techniques have been widely applied and are advancing rapidly, the underlying assumptions of the approach are often not adequately tested or calibrated prior to their application in the field.

Inductively coupled plasma mass spectrometry (ICPMS) is a sensitive analytical technique that has been applied to various problems in fisheries via elemental analysis of fish otoliths and scales (Perkins et al. 1991, Coutant & Chen 1993. Fowler et al. 1995a, b, Thorrold et al. 1997). Like any analytical technique, ICPMS has its strengths and limitations. For example, ICPMS is very good at quantifying a suite of elements in solution with very good precision, but certain elements are difficult to analyze because of the formation of oxides that can cause spectral interference (Olesik 1991). While ICPMS is good at quantifying elemental concentrations, it is generally not suitable for quantifying isotopes or their ratios because of spectral overlap. Thermal ionization mass spectrometry (TIMS) requires much larger sample sizes than ICPMS and has not been adapted as a probing or ablation technique, but has the advantage that it can very precisely quantify isotope ratios of elements such as strontium $^{87}\text{Sr}/^{86}\text{Sr}$.

The goal of our research was to investigate the responses of chemical signatures within otoliths to environmental variables (temperature and salinity) believed to influence their chemical deposition. Our approach was to investigate the utility of otolith chemistry as a tracer of environmental history in menhaden under experimental conditions. Our objective was to validate the technique for applications to fishes residing in the marine coastal and estuarine environment of the Gulf of Mexico. The environmental conditions selected were designed to simulate conditions larval and juvenal menhaden might encounter in the local estuary as they migrate from offshore to the estuarine nursery environments.

**MATERIALS AND METHODS**

**Menhaden rearing.** Gulf menhaden Brevoortia patronus was selected for these studies because it has significant commercial value and large catches (500 million kg for 1990 in the Gulf of Mexico; National Marine Fisheries Service. Fisheries Statistics and Economics Division, pers. comm.), it can be reared in the laboratory (Hettler 1983), its larvae have been aged using standard otolith techniques (Warren 1988), and its early life history stages are exposed to several coastal environments in a relatively short time (coastal to nearshore to estuarine), making it a suitable candidate in which to follow environmental histories.

Laboratory populations of menhaden larvae were established from field collected eggs. Eggs were hatched at the ambient temperature of collection (14°C) and a salinity of 26. Larvae were stocked (200 per tank) in replicate tanks (76 l) in 9 treatments for a total of 18 tanks. Larvae were acclimated to experimental salinities (20, 26, 33.4) and temperatures (18, 22, 26°C) at rates of 1 part per thousand (ppt) d$^{-1}$ and 1°C d$^{-1}$, respectively.

Rotifers (Brachionus sp.) were cultured and fed to the first feeding menhaden larvae. In addition wild zooplankton were collected and size sorted (53 to 183 μm) to feed the larvae until they reached a size where they could ingest Artemia sp. nauplii. A liter of phytoplankton (Isochrysis galbana) was added to each tank every day, and juvenile menhaden might encounter in the local estuary as they migrate from offshore to the estuarine nursery environments.

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Plexiglas covers on each tank minimized evaporation between water changes. Once the menhaden reached a size appropriate to meet analytical requirements for otolith material they were anesthetized, weighed to estimate instantaneous growth rate, measured (TL in mm), and individually sealed in polyethylene bags and frozen.

**Otolith analyses.** Protocols were developed for proper trace element clean techniques. These included clean handling of otoliths and preparation of trace-metal free acids, water and hypochlorite. The criteria for final protocols was that the blanks had to be at or below the minimum detection limits of the ICPMS for acceptance of a sample. Otoliths were extracted and processed within portable laminar flow hoods which were constructed of polypropylene, fitted with HEPA filters and housed in a clean laboratory.

Once we established the appropriate clean procedures, a series of otoliths from field collected menhaden were prepared for preliminary analysis of elemental concentrations using ICPMS. Otic capsules from all specimens were removed and then individual otoliths were separated, cleaned and stored dry until analyzed. Otoliths were then weighed (to the nearest 0.1 µg) and prepared for solution based analysis using ICPMS or TIMS.

Otolith cleaning consisted of soaking in NaOCl to remove organic tissues, followed by successive washings with distilled water. Otoliths for ICPMS were dissolved in doubled distilled Vycor HNO₃ (40 µl), then diluted with distilled water (4 ml) for a total sample volume of 4.04 ml. Samples were run on a Fisons™ model PQ2+ equipped with a high performance interface. Calibration procedures for the ICPMS consisted of running 4 prepared calibration standards followed by a 5th calibration check with NIST (National Institute of Standards and Technology) traceable standards. If the calibration check did not match the original the calibration procedure was repeated. During each run of the ICPMS a QC (quality control) standard was run every tenth sample to check for drift. If the instrument drifted significantly during a run (>10%), the run was suspended while the initial calibration procedure was repeated. For each analytical series a set of procedural blanks were run on the clean water, acids and digestion containers to identify and correct for any sources of contamination.

Samples of otoliths from field collected menhaden were analyzed to compare the chemistry of their sagittae, asterisci and lapilli and to initially determine which elements offered the most promising elemental signals. Menhaden otoliths from each treatment of the experiment were analyzed to determine the composition of a 10-element suite using ICPMS and Sr isotopes using TIMS. We also analyzed samples of the experimental water from each tank twice, once taken in the middle and once at the end of the rearing experiment, and each time both before and after a water change, to verify that the chemistry of our rearing waters was stable. All water samples were filtered (0.45 µm), acidified (1%), and sealed in acid cleaned polyethylene vials. Because of the high relative concentrations of calcium in otoliths and to avoid interference with analyses of other elements, Ca was analyzed using AES (flame atomic emission spectrometry). Calcium matrices for each otolith type were determined using metal free acids, water and hypochlorite. The criteria for final protocols was that the blanks had to be at or below the minimum detection limits of the ICPMS for acceptance of a sample. Otoliths were then weighed (to the nearest 0.1 µg) and prepared for solution based analysis using ICPMS or TIMS.

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Otoliths for TIMS were prepared by weighing pairs of sagittae (1 to 3 mg). They were then washed with distilled water and dissolved in 2 N double distilled HCl. Sr was separated from the sample solutions by a standard ion chromatography technique, using AG50W × 8 (Biorad) cation exchange resin. The eluted Sr fraction was mixed with 20 µl of 0.025 M H₃PO₄ and evaporated to dryness. The residue was taken up in ~3 µl of 1 N HNO₃ for loading on a pre-baked Rhenium (Re) filament. Isotopic measurements were made on a 90° sector thermal ionization mass spectrometer, Finnigan MAT 262, using double Re filaments. Data were acquired using simultaneous collection with multi-Faraday cups. The ⁸⁷Sr/⁸⁶Sr ratios were normalized to ⁶⁰Sr/⁶⁰Sr = 0.1194. One hundred ratios were collected for replicate analyses of the standard NBS 987 yielded an average value of ⁸⁷Sr/⁸⁶Sr = 0.710262 ± 0.000007 (n = 12, 2σm). Results from replicate analyses of the sea water were ⁸⁷Sr/⁸⁶Sr = 0.709177 ± 0.000005 (n = 7, 2σm).

Elemental data were analyzed by either 1-way or 2-way ANOVA. Data were transformed to normalize them prior to analysis (logₓ(x)).

**RESULTS**

Initial ICPMS screenings established that it was possible for us to analyze all otolith pairs (sagitta, lapillus, asteriscus) from fish as small as 30 mm TL (total length) and much smaller if only the largest otolith (sagitta) was analyzed. The smallest otoliths analyzed by our solution based methods were ~10 µg dry weight. Weights of otoliths pairs were very consistent within a fish and by type of otolith, indicating symmetry in the process of otolith formation of juvenile menhaden. Results for the elemental data comparing pairs of otoliths from the same fish showed that the chemistry was also consistent within pairs of the same type of otolith from the same fish. However, significant varia-
The analysis of the calcium carbonate matrix using XRD determined that menhaden asterisci are vaterite while their sagittae and lapilli are aragonite.

Analysis of rearing waters showed salinity to have a significant positive relationship versus Sr, Ca, and Sr/Ca ratio and an inverse relationship versus $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (ANOVA, $p = 0.05$) (Table 1, Figs. 2 & 3). A comparison of Sr and Sr/Ba ratios in our rearing waters showed that there was some depletion of Sr in the rearing waters due to rearing but that the slight depletion did not significantly affect the differences between the treatments or the ratios between trace elements such as Ba and Sr (Fig. 4).

No significant relationship of strontium or Sr/Ca ratios to temperature, salinity or menhaden instantaneous growth rate was detected for menhaden otoliths (ANOVA, $p = 0.05$) (Figs. 5 to 8), nor was a significant interaction between temperature and salinity detected (2-way ANOVA, $p = 0.05$). We did not detect a significant difference in strontium isotopes in the otoliths of our salinity treatments (ANOVA, $p = 0.05$) across the salinity range tested in our experiments (Fig. 9). Nevertheless, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were higher on average in the lower salinity samples and were significantly higher in our field collected specimens than in our laboratory reared fish (Mann-Whitney, $p = 0.01$) reflecting a different range of environmental signals than tested in our experiments (Fig. 10).

We also analyzed the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the vertebrae of a menhaden reared at a salinity of 20 and 26°C and for comparison the vertebra from an adult bay anchovy *Anchoa mitchilli* collected as a small juvenile in low salinity waters (<10) but reared

![](image)

**Fig. 1.** *Brevoortia patronus*. Strontium concentrations of menhaden otoliths compared by otolith type. Each bar represents an individual sagitta, asteriscus or lapillus.

<table>
<thead>
<tr>
<th>Otolith Type</th>
<th>Sagitta</th>
<th>Asteriscus</th>
<th>Lapillus</th>
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<tbody>
<tr>
<td>Strontium (ng·µg⁻¹)</td>
<td>14.5</td>
<td>18.6</td>
<td>19.2</td>
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</table>

**Table 1.** Strontium and calcium concentrations in rearing waters plotted against salinity. Samples are rearing waters prior to exposure to the fish. Strontium was analyzed by ICPMS and calcium by AES.
Strontium as an environmental indicator

Elemental chemistry has been used to infer the environmental history of a number of aquatic organisms, including fish, corals, mollusks and foraminifera, with strontium deposition often serving as the key environmental indicator (Smith et al. 1979, Schneider & Smith 1982, Hess et al. 1986, Beck et al. 1992, Ingram & Depalo 1993, Weinbauer & Velmirov 1995). It has been documented that fish otoliths can be marked by exposing the fish to elevated concentrations of strontium (100 to 10,000× ambient) in a bath (Sauer & Watabe 1988, Brown & Harris 1995). The result is extremely elevated Sr concentrations in the daily rings formed during exposure to increased strontium in solution. This suggests that deposition of Sr in otoliths can be influenced by Sr concentration in a fish's aquatic environment under certain circumstances.

Salinity and strontium

Worldwide patterns of strontium distribution in rivers and oceans are well established (Palmer & Edmond 1989, 1992). Strontium concentrations vary substantially among drainage basins due to the different Sr composition in source rocks and different weathering patterns (Palmer & Edmond 1992). Strontium concentrations are typically low in freshwater relative to marine systems and increase in direct proportion to salinity (Palmer & Edmond 1989), with some exceptions. Strontium isotope ratios typically change over a salinity gradient, with higher ⁸⁷Sr/⁸⁶Sr ratios generally occurring at lower salinity in most, but not all, estuaries (Palmer & Edmond 1989). Based on these principles, strontium and strontium isotope incorporation into otoliths should provide an environmental indicator of salinity if strontium and its isotopes are deposited in relation to their abundance in aquatic environments, especially across estuarine gradients. Salinity related signatures based on strontium deposition in otoliths have been reported for anadromous and catadromous species.

DISCUSSION

Fig. 3. Strontium isotope ratios of rearing waters plotted against salinity. Error bars are ±2 standard errors. Curve is calculated based on a 2-end-member mixing formula. Data for salinity of 35 are open Gulf of Mexico water plotted for reference (L. Zhang & L.-H. Chan unpubl. data)

for an additional year in a constant environment with a salinity of 20 at 27°C. We found no significant difference in the ⁸⁷Sr/⁸⁶Sr ratios of the vertebra from the menhaden, their otoliths or their rearing water (Table 2). For the bay anchovy vertebra we found a ⁸⁷Sr/⁸⁶Sr ratio consistent with the observed salinity history of that individual, namely a salinity signal of 20 diluted somewhat by an initial growth period at a lower salinity (Table 2).

Fig. 4. Strontium:barium and strontium:calcium (×1000) ratios of the rearing waters plotted against salinity. Data are for rearing waters sampled just before and after water changes in each replicate rearing aquaria. Samples were taken near the end of the experiment. Also plotted are rearing waters prior to exposure to the fish. Strontium and barium were analyzed by ICPMS and calcium by AES.
Temperature and strontium

The concentration of Sr in calcified biogenic structures has also been applied as a temperature indicator for a variety of marine organisms including fish (Gauldie et al. 1986, Radtke 1989, Townsend et al. 1989, 1992, 1995, Radtke et al. 1990). The basis for interpretation of temperature in relation to strontium concentrations in aragonite otoliths is that the co-precipitation of Sr within aragonite in seawater is temperature dependent (Kinsman 1969). Our experiments were designed to establish the degree of temperature and/or salinity sensitivity of Sr

such as eels, striped bass Morone saxatilis, and salmonids (Casselman 1982, Kalish 1990, Secor 1992, Coutant & Chen 1993, Limburg 1995, Secor et al. 1995, Tzeng 1995). However, we did not detect a significant effect of salinity on Sr concentration or Sr/Ca ratios in menhaden otoliths in our experiments. This suggests that other factors may be complicating the interpretation of a salinity signal based on Sr concentration under certain conditions. Another possibility is that, for menhaden, deposition of Sr in otoliths is not proportional to Sr concentration in the environment, with deposition changing very little once some threshold concentration of salts or ions is reached. Other experimental work on Sr deposition in fish otoliths suggests that Sr deposition is related to the salinity of the water, although the relationship may not always be proportional across a salinity gradient. Several recently published experimental studies reported either no response of Sr concentration to salinity or a complex response to a combination of salinity, temperature and other factors (Fowler et al. 1995a, Hoff & Fuiman 1995, Tzeng 1995). In an experimental study of temperature and salinity effects on Sr/Ca ratios in striped bass (Secor et al. 1995) a strong relationship to salinity was demonstrated between 0 and 12 ppt and a predictive resolution of 5 ppt was estimated. However, the relationship was not linear, indicating changes in the depositional processes of Sr and Ca between high and low salinity environments. A further complication to interpreting strontium in relation to calcium as an indicator of salinity is that Sr/Ca ratio is not constant over a salinity gradient (Fig. 4). Both Sr and Ca increase with salinity, but, because Sr typically increases more rapidly than Ca, Sr/Ca ratios typically increase with salinity of estuarine waters (Rieman et al. 1994, Tzeng 1995; Fig. 4)

![Sr/Ca ratios](image)
The environmental conditions in a fish’s aquatic environment is likely to be difficult to interpret in spite of the apparently simple univariate correlations that have been reported. Although the technique has been applied widely and some degree of environmental response to salinity or temperature has been reported in otoliths for numerous species, there is a growing body of evidence that suggests that these relationships are limited in their application as precise predictors of temperature or salinity in the field because of the strong potential for confoundedness among factors that can potentially affect the chemical signature. At this point we understand the factors controlling the process of Sr deposition in otoliths well enough to limit the utility of Sr or Sr/Ca ratios to studies to which they are especially well suited, such as studies of diadromous fish migrations (Casselman 1982, Kalish 1989, Secor 1992, Coutant & Chen 1993, Riemann et al. 1994, Halder et al. 1995, Limburg 1995, Secor et al. 1995, Tzeng 1995, Secor & Piccoli 1996, Tzeng et al. 1997), during which the strong contrast in the chemistry of the environment and the changes in osmoregulation a fish encounters combine to produce a clear demarcation in otolith chemistry in spite of other factors that might be affecting the process of biomineralization. Future efforts may need to focus on alternative approaches and analytical techniques which offer greater promise as more reliable predictors of environmental history (see Kalish 1991b, Gauldie et al. 1995, Romanek & Gauldie 1996).
We speculate that this was because the specimens from the field had resided in low salinity environments typically encountered by menhaden in coastal Louisiana, USA, marshes. Menhaden are commonly found in the low salinity and freshwater environments where the menhaden with unknown history were collected. The strontium isotope ratios for the 3 unknowns suggested they had resided at low salinities, nominally 0.5, 8.5 and 13 (Fig. 10). These results provide strong evidence that Sr isotopes can be a precise and useful indicator of salinity in fishes, especially when salinity differences are extreme or at lower salinity ranges where changes in \( \frac{^{87}\text{Sr}}{^{86}\text{Sr}} \) are likely to be greatest (Fig. 10).

A potential limitation of this method for application to fish otoliths is the sample size required for TIMS. As much as a few milligrams of otolith could be required, depending on the Sr concentration of the sample. This would eliminate the possibility of analyzing otoliths of small juveniles and larvae. Chemical analyses of fish hard parts are typically done on otoliths because they have been shown to more reliably retain their chemical integrity throughout the life history of the fish, while calcium can be mobilized from other bony structures under certain circumstances (Mugiya & Watabe 1977, Carragher & Summer 1991, Mugiya & Tanaka 1995). It is reason-
Table 2. Brevoortia patronus. Comparison of \(^{87}\text{Sr}/^{86}\text{Sr}\) of rearing water with a salinity of 20 with otoliths and vertebra from menhaden reared at the same salinity. Also included are results for a bay anchovy Anchoa mitchilli adult reared primarily at a salinity of 20 for comparison (see text for details).

<table>
<thead>
<tr>
<th>Sample</th>
<th>(^{87}\text{Sr}/^{86}\text{Sr})</th>
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<tbody>
<tr>
<td>Rearing water (n = 1)</td>
<td>0.709188 ± 0.000007</td>
</tr>
<tr>
<td>Menhaden otolith (n = 6)</td>
<td>0.709189 ± 0.000008</td>
</tr>
<tr>
<td>Menhaden vertebrae (n = 1)</td>
<td>0.709198 ± 0.000013</td>
</tr>
<tr>
<td>Anchovy vertebrae (n = 1)</td>
<td>0.709210 ± 0.000009</td>
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able to believe that under most circumstances Sr isotope ratios in vertebrae may be insensitive to Ca mobilization effects. We tested this supposition by analyzing vertebrae of 2 fish species.

We found no difference in the \(^{87}\text{Sr}/^{86}\text{Sr}\) ratios of the vertebra from the juvenile menhaden, their otoliths or their rearing water (Table 2). A bay anchovy Anchoa mitchilli provided a more rigorous test of the suitability of vertebra because it was an adult from a continuously spawning population held in the laboratory for more than a year. If the integrity of the strontium isotope ratios could be affected by life history stresses then a serial spawning adult would provide the most rigorous test. The bay anchovy vertebra showed a \(^{87}\text{Sr}/^{86}\text{Sr}\) ratio consistent with the individual’s observed salinity history, namely a salinity of 20 diluted somewhat by an initial growth period at a lower salinity (Table 2).

The mobilization of calcium from bony structures of fishes is a complex process. Mobilization of calcium from scales is known to be significant during periods of vitellogenesis and as a result of other stresses, yet in the same studies in which substantial calcium mobilization occurred in scales, no mobilization was detected in vertebrae (Mugiya & Watabe 1977, Carragher & Sumpter 1991). Other experiments have induced some mobilization in scales and ribs as well as effects on incorporation of strontium into otoliths with injection of reproductive hormones (Mugiya & Tanaka 1995). Our results and studies of strontium isotope ratios in salmon (Koch et al. 1992) suggest that, in vertebrae, strontium isotope ratios may be stable under some or most conditions, either because Ca and Sr are not mobilized or, if they are, the strontium isotope ratios are unaffected. This assumption needs further study in the future before it can apply. A major advantage of being able to use vertebrae for strontium isotope analyses is that it would allow smaller individual fish, including late larval stages, to be analyzed by TIMS. The ability to utilize vertebrae for TIMS would also leave the otoliths for aging and other chemical analyses. Although this technique has not been applied to fisheries problems before, analysis of fish hard parts by TIMS has been applied in archeological and paleontological studies, in which the analyses were done with both vertebrae and teeth for a variety of fishes with apparent success (Smits et al. 1991, Koch et al. 1992).

One final limitation to the applicability of strontium isotope ratios as a salinity indicator should be noted. As was pointed out earlier, the high concentrations of Sr in seawater and low concentrations in freshwater generate a curvilinear relationship of strontium isotope ratios with changes in salinity. Changes in strontium isotope ratios are proportionately greater at low salinity (Fig. 10), thus strontium isotopes are a more sensitive indicator at low salinity. Another factor to consider in the application of Sr isotopes as a salinity indicator is that strontium isotope ratios vary significantly for different river drainage systems and may vary seasonally depending on runoff patterns (Palmer & Edmond 1989, 1992). In some cases differences in Sr isotope ratios will be greater between estuarine and coastal systems than within a particular system, while in other systems the technique will be useless as a salinity indicator if the freshwater input to the estuary has a Sr isotope ratio close to that of seawater.

Other factors affecting otolith chemistry

Matrix effects

ICPMS analyses detected a difference in the concentration of elements deposited in each of the 3 otolith types that was consistent in both laboratory reared and field collected menhaden. Concentrations of elements were similar in pattern but higher in the sagittae and lapilli than in asterisci (Fig. 1). We speculate that the difference in the chemistry is probably due to differences in the calcium carbonate matrix of the otolith pairs. Vaterite otoliths occur either as the principal otolith crystal in some fishes or as a partial or complete replacement morph in other fishes (Carlstrom 1963, Gaudie 1986). Recent unpublished research with several species of fishes reported that asterisci are typically vaterite rather than aragonite in many teleosts (Dunkelberger unpubl. abstract 1993). In menhaden, concentrations of calcium were similar in all 3 otolith pairs, thus the trace elements were incorporated into the vaterite matrix at a much lower concentration than into the aragonite matrix of the same individual. Work with goldfish Carassius auratus found a similar pattern of Sr incorporation into asterisci and lapilli, with Sr/Ca ratios 12 times higher in the lapilli (Mugiya & Tanaka 1995). This difference in trace element incorporation in otolith types has significant implications for the chemi-
cal analysis of asterisci and other biogenic structures composed of vaterite rather than aragonite. For example, some fishes, such as sturgeons and hagfishes, have vaterite rather than aragonite sagittal otoliths (Carlstrom 1963), and aberrant vaterite sagittae can be produced under certain conditions in salmon, striped bass and red drum (Radtker 1978, Gauldie et al. 1986, David et al. 1994). These may complicate the interpretation of results in studies of otolith chemistry if a vaterite matrix goes undetected. One potential advantage of asterisci being composed of vaterite is that the differences in chemistry could be studied and exploited to gain a better understanding of the effects of matrix structure on the process of ionic contamination in biogenic calcium carbonate (Gauldie 1986).

Confounding effects

It is impossible to state explicitly why no definitive signals were detected for either temperature or salinity based on Sr concentration and Sr/Ca in menhaden otoliths. Both temperature and salinity signals based on Sr concentration and Sr/Ca in otoliths have been reported for several fishes. Several plausible explanations are possible. It is possible that (1) menhaden otoliths do not conform to patterns seen in other species because of differences in their biology or physiology, (2) the signal was there but because several factors were influencing otolith chemistry simultaneously the pattern was unrecognizable, or (3) some of the previous research has provided an oversimplified understanding of otolith chemistry. We know that otolith chemistry and specifically Sr deposition can be influenced by several factors, including growth, developmental stage, temperature, salinity and physiology (Kalish 1989, 1991a, Sadovy & Severin 1995, Hoff & Fuiman 1993, 1995, Fowler et al. 1995a, Tzeng 1995).

By superimposing multiple environmental signals, where one factor increases as Sr deposition increases and another factor increases as Sr decreases, a pattern might be produced with no detectable net change over a significant range of environmental variables. For some fishes strontium incorporation in otoliths was reported to be inversely proportional to rearing temperature while strontium concentrations in the ocean are proportional to salinity. Sr deposition in otoliths should always increase with increasing salinity if deposition is always proportional to Sr concentration in seawater. If ontogenetic and growth rate differences also occur then a strong possibility of confoundedness among factors exists, which could be difficult or impossible to interpret in field collected specimens.

Organismal effects

Inherent variability in the physiology, life history and biogeochemistry among different species of fishes is undoubtedly a significant part of the difficulty in interpreting chemical patterns in otoliths (Kalish 1989, 1991a). As with menhaden, no relationship of Sr or Sr/Ca ratios to temperature was found in Atlantic salmon and blue grenadier (Kalish 1989). The pattern of variability in Sr/Ca reported for the salmon and grenadier otoliths was very similar in pattern to what we observed in menhaden otoliths. For laboratory reared Fundulus heteroclitus and herring Clupea harengus larvae, strong inverse relationships between Sr/Ca ratio and temperature have been reported (Radtker 1989, Townsend et al. 1992). For herring the relationship was nonlinear with a strong effect on Sr at low temperature and little or no effect at higher temperatures (Townsend et al. 1992). In experiments with Atlantic croaker the relationship of Sr concentration with temperature was positive, while the Sr/Ca ratio remained constant among temperature treatments (Fowler et al. 1995a). In field collected white grunt, Sr/Ca in otoliths was more directly correlated with growth rate than temperature (Sadovy & Severin 1992). In red drum reared under constant conditions in the laboratory, Sr concentration were highly correlated with temperature; however, there was a high degree of variability within the otoliths in spite of rigorous environmental controls and significant effects of other factors (Hoff & Fuiman 1995). We could not detect any intra-otolith effects because solution based analyses integrated the results of effects that may have influenced changing patterns of elemental deposition in the menhaden otoliths. In Japanese eels a strong relationship of Sr/Ca with rearing salinity was detected for elvers captured in the field and then reared in the laboratory (Tzeng 1995). However, when the eels were returned to the salinity in which they were captured the Sr/Ca signal did not return to its former ratio. Tzeng (1995) concluded that there was a significant ontogenetic effect on Sr/Ca ratio in eels in addition to any salinity effect. Another experimental study of otolith chemistry examined both salinity and temperature effects on trace element chemistry of Atlantic croaker otoliths (Fowler et al. 1995a, b). In this study, it was concluded that temperature was the dominant factor affecting the Sr chemistry, with secondary effects of otolith size and salinity. In our experiments we reared the menhaden to similar sizes so that growth rate, not otolith size, was the principal variate and we detected no effect of growth rate on Sr or Sr/Ca ratio.

The entire process of calcium and strontium metabolism in fishes needs further research before a sound
basis for interpreting patterns of trace-element incorporation into biogenic calcium carbonate can be established. Numerous factors can affect biomineralization in fishes. Otoliths have been the focus of chemical analyses because they are believed to be most resistant to calcium mobilization in most circumstances (Mugiya & Watabe 1977, Carragher & Sumpter 1991), but even otoliths are not immune to physiological and life history influences (Kalish 1989, 1991a, Wright et al. 1992, Mugiya & Tanaka 1995). Other bony structures, such as vertebrae and spines, have been used successfully to age fishes when otoliths proved difficult, and their potential in hard part chemistry should be evaluated more closely in the future.

Experimental artifacts

Some of the confusion involved in the interpretation of otolith elemental patterns may be due to artifacts of the experimental designs used in laboratory rearing experiments. In batch culture experiments with periodic water changes, evaporation can concentrate elements within rearing waters, while uptake by the fish has the potential to reduce concentrations. Experiments testing temperature effects would be especially vulnerable to confoundedness if stocking densities of fish were high, evaporation not controlled, or water not exchanged regularly. Another potential experimental artifact of rearing experiments could be the source water used in the experiments and the procedure used to make up the treatment waters. We used filtered natural source waters from the local environment as our end members for mixing our experimental water so that our rearing water provided a profile of chemistry representative of the natural environment (i.e. Mississippi River estuary). Seawater diluted with freshwater that is different in origin from local freshwater runoff or artificially produced seawater (Secor et al. 1995) is likely to be significantly different in chemistry than natural water and may complicate interpretations of elemental signals in hard parts of wild fish based on fish reared in water with a significantly different chemistry. These problems cannot be detected or resolved if the chemistry of end members and the rearing waters are not analyzed, which has seldom been done in experimental studies of otolith chemistry.

Analytical artifacts

Another potential problem that needs more careful consideration in future studies of elemental chemistry of fish bony structures is the inherent variability in the process of estimating low concentrations of materials in very small samples sizes such as those produced by an ablation technique or when analyzing small otoliths, especially when the results are to be expressed as a ratio. Otolith strontium data are typically reported as a ratio of Sr to Ca. Although there are obvious reasons for this convention, such as standardization with probe based techniques, a drawback is that it incorporates the errors associated with the precision of measurement of both variables, significantly increasing the overall error (Sokal & Rohlf 1981). Because Sr is in much lower concentrations than Ca and closer to the limits of precision of some analytical techniques, overall precision is significantly reduced and the potential for spurious relationships increased (Gunn et al. 1992).

More sensitive techniques such as ICPMS can reduce this problem, especially with solution based analyses, and may in the future help provide a better understanding of the relationship between elemental signatures of otoliths and environmental signals.

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