**LA-ICP-MS chemical analysis of archaeological otoliths as a tool for
seasonality and site catchment studies**

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A B S T R A C T

Analysis of the trace element chemistry of otoliths via Laser Ablation-Inductively Coupled
Plasma-Mass Spectrometry (LA-ICP-MS) has become common in fisheries-related work,
allowing biologists to trace connectivity between habitats over the life cycles of fish. For
archaeological specimens, elements from the outer edges of archaeological otoliths have the
potential to provide information on site seasonality complementary to oxygen isotope data; they

also may inform on place of capture of fish, thus elucidating exploited ranges and/or social and economic links between settlements. Adopting this method will require careful consideration of a number of complicating factors related to metabolic processes affecting otolith production, analytical procedures peculiar to LA-ICP-MS, and diagenesis, in addition to the usual complications of species identification and assessment of sample adequacy. Here, we review such factors as they affect the utility of the method for sourcing and seasonality research with archaeological otoliths, using the results of a pilot study of specimens from two Woodland-period sites in coastal Alabama, southeastern USA, to illustrate the potential and the current limitations of the method for archaeological research.

Keywords: otoliths, LA-ICP-MS, trace elements, oxygen isotopes, site catchment, seasonality

1. Introduction

The last quarter-century has seen an explosion of fisheries-related work using otolith (fish “earstone”) chemistry (e.g., Hamilton and Warner 2009; López-Duarte et al. 2012; Ramsay et al. 2011; Thorrold et al. 1998; Weber et al. 2002). Such work is predicated upon several useful properties of otoliths relative to other biological structures: 1) otoliths begin growing during the larval stage and growth continues throughout the life of the fish; 2) growth is expressed in incremental bands representing short-term (even daily) periods; 3) otoliths are acellular and metabolically inert, meaning that, once formed, they are not resorbed or otherwise chemically altered via biogenic processes (e.g., Ramsay et al. 2011); and 4) otolith chemistry represents, to some degree, the chemical composition of the waters in which fish live over the course of their

lives. These characteristics combine to make otoliths particularly useful structures for determining fish natal habitats and connectivity, the exchange of individuals over habitat patches over time (López-Duarte et al. 2012), and such information lends itself to range-scale management practices.

For archaeology, information on otolith chemistry can be applied in two ways: 1) as a method of determining site seasonality that is complementary to O isotope and other traditional forms of seasonality assessment (Andrews et al. 2003; Saunders et al. 2005); and 2) establishing catchment, or the geographic range over which fish were harvested in the past (Disspain, Ulm, and Gillanders 2015). Before either of these applications can be realized, however, the many variables affecting otolith chemistry and those arising from different analytical methods must be recognized (Disspain, Ulm, and Gillanders 2015) and formal procedures established to control for those variables as much as possible. In this paper we discuss such variables, using data from a pilot study of otoliths from two coastal sites in the southeastern USA to illustrate both the promise of otolith chemical data for archaeology and the complications that analysts will face as they try to obtain such data and put them to use.

2. Sources of Variation in Otolith Chemistry

The basis for both modern and archaeological applications of otolith chemistry is the assumption that environmental differences over space are reflected in different elemental concentrations in water bodies, which in turn are reflected in otoliths, yielding information on spawning locales, point of capture, and any seasonal movement between. In a word, chemical “fingerprinting” of individual fish and/or species. In practice, there are a large number of factors that influence the incorporation of major (1 wt. % or more), minor (> 100 ppm) and trace (< 100

ppm) elements in otoliths, so the relationship between water chemistry and otolith chemical composition is not a direct one. Disspain, Ulm, and Gillanders (2015) have recently published a valuable overview related to this topic; we refer readers to their article for additional coverage of this topic and some interesting site-specific examples.

Otoliths are structures within the inner ear of vertebrates that function to help maintain balance (motion and orientation to gravity). In bony fishes, otoliths “float” behind the brain, at the back of the fish’s cranium. They are surrounded by a fluid called the endolymph. Elements are incorporated into otoliths as they crystallize. There are three kinds of otolith: the sagitta, the lapillus, and the asteriscus. Sagittae are preferentially used for time-dependent environmental tracking and paleoclimatic work due to larger size and formation at an early life stage (e.g., Hoff et al. 1997). All otoliths are calcium carbonate; asteriscii are vateritic, while the other two kinds are aragonitic. An important constituent is protein, which comprises from 1 – 8% of otoliths depending on species, with around 3 – 4% being a common amount (Campana 1999:265). Water-insoluble proteins (a.k.a. “otolin” – Degens et al. 1969) make up about half the protein present and provide the “structural framework” for calcification, while calcification rate is partly regulated by the remaining, water-soluble proteins (Campana 1999:265) and by temperature. The full effects of temperature on otolith formation are incompletely understood, but elements known to be affected include Mg, U, Na, K, Mn, Zn and Fe (Campana 1999). While some authors have seemingly detected a correlation between temperature and Sr incorporation in otoliths (e.g., Bath et al. 2000), Campana (1999:273) reviewed a large number of case studies and found no consistent relationship in this regard, except in very cold ($< 10^{\circ}\text{C}$) water. Sr concentrations are, however, affected by salinity (Campana 1999:267, 274; see also Kalish 1989).

Elemental impurities in the otolith matrix can occur “within the crystal lattice as a [ionic] substitute for calcium, as an inclusion in the interstitial spaces, or in association with the proteinaceous matrix” (Campana 1999:266). Precipitation of other carbonates, such as SrCO_3 , is a less likely source of elemental inclusion than is ion substitution, which favors Sr, Mg, and Ba ions that have the same charge as Ca (2+) and ionic radii deviating from Ca by less than 25% (Campana 1999:266; Ramsay et al. 2011:831; Gaetani and Cohen 2006: 4617) and that are consequently bound into the crystal lattice. Among these elements, Sr has the most similar radius to Ca, while Mg and Ba are smaller and larger, respectively. On the other hand, elemental inclusions in interstitial spaces such as the “micro-channels” typical of otolith architecture are relatively poorly bound, so that elements such as Na, Cl, Zn and K are easily leached out (Campana 1999:266).

A major consideration in understanding the relative availability of elements for inclusion in otoliths is bioregulation (Bath et al. 2000:1705; Campana et al. 1994:1949; Kalish 1991). Fish take up elements via the gills; these are then passed into the blood plasma, from there into the endolymph, and from there into the crystallizing otolith (Campana 1999:266-267). There are various complicating factors in this process. For one, blood plasma chemistry can vary seasonally with temperature (Kalish 1991), a factor that likely is species-specific. Another complicating factor is osmoregulation, the maintenance of water levels in an organism. In fish, osmoregulatory processes “level out” the concentrations of elements such as Ca, Na, K, Mg, Cl, P, Cu, and S, even between fresh and saltwater environments (Campana 1999:268-269). Accordingly, “it is clearly unrealistic to expect the otolith content of physiologically regulated elements to reflect environmental abundance” (Campana 1999:269). Elements found in otoliths that are relatively unregulated, and therefore expected to more closely reflect environmental

loads, include Sr, Zn, Pb, Mn, Ba and Fe (Elsdon and Gillanders 2003; Campana 1999:269; Hamilton and Warner 2009; see also Disspain, Wilson, and Gillanders 2012). Campana (1999:269-270) also mentions Li, Cd, Ni and the “less abundant” elements as possibly falling into this category, although he notes that, with the exception of Sr, element:calcium ratios in blood plasma will be higher than in the otolith, “indicating that the otolith composition is not merely a passive reflection of plasma composition, even if correlated.” Some elements are then barred or taken up selectively from the blood plasma into the endolymph, “resulting in an endolymph concentration which is depleted in all major ions other than K” (Campana 1999:267). The otolith thus reflects the chemical composition of the endolymph more so than that of the aquatic environment.

Beyond uptake via the gills, some elemental contributions to otoliths come from drinking water (especially marine fish, which drink continually) and from food, although this contribution appears to be relatively low (e.g., ca. 10 – 20% of Ca and Sr for freshwater fishes – Campana 1999:267). Obviously, this can vary by species, and may vary seasonally by sex; for example, Mendoza-Carranza (2003) found that during the rainy season, male gafftopsail catfish (*Bagre marinus*) in near-shore waters consumed significantly more prey fish than did females.

Sr is recognized as the best marker for source (e.g., Ludsine et al. 2006; Pontual et al. 2000), especially for anadromous fish (Campana 1999; see also Disspain, Ulm, and Gillanders 2015), because: 1) it is the least-regulated element; 2) there is a purported lack of a temperature effect on Sr uptake (Campana 1999); and 3) differences in concentration between fresh and salt water (lower Sr:Ca in the former – Hale and Swearer 2008; Pontual et al. 2000) can be quite abrupt, (Hamilton et al. 2009), reflecting the 30 – 35% difference in salinity between the two environments. However, differences in Sr:Ca ratios between species, even those habitually

occupying the same areas, may be expected, as will be an increase in Sr:Ca with the age of the fish (Campana 1999; Hamilton et al. 2009). This latter effect probably is related to the rate of protein synthesis (Kalish 1989), which in turn affects the rate of crystallization of the otolith (Campana 1999:2740), which generally slows with age (Chang et al. 2012). Ramsay et al. (2011) were able to correct for the effects of otolith size via regression analysis, with data showing linear relationships; however, Thorrold et al. (1998:258) found “lower levels of most elemental and isotopic variables with increasing otolith weight,” an age-related effect that was not systematic enough to allow for mathematical correction. Such differences between studies likely reflect species-level variability. Elemental differences also can be expected with major ontogenetic phase shifts, such as the period of larval metamorphosis into juvenile stage (Hamilton et al. 2009:242; Mugiya and Satoh 1995; Swearer 2000).

Environmental variability, while the very basis for how otoliths can be used in sourcing studies, is at the same time another area of concern. Major differences between marine and freshwater systems are primarily found in the common marine salts (Campana 1999:271). However, Sr (or any other chemical) loads are not consistent in stream discharge or in ocean basins over time due to “upwelling, volcanic activity, pollution, biological activity, and inter-annual differences” that can “act to confound any expected [chemical] relationship” between source water and otolith chemistry on a one-to-one basis” (Campana 1999:271). Water depth is another factor influencing chemical availability, with some elements (e.g., Ba, Zn, Cd) showing enrichment at greater depth in the ocean (Campana 1999:267).

Other factors that can influence elemental uptake include pH and dissolved O concentration of water (Campana 1999:267), side of the fish (Campana 1999:283; Thresher et al. 1994; but see Andrus and Crowe 2002; Campana et al. 1994; Proctor and Thresher 1998), and

part of the otolith sampled (e.g., Pontual et al. 2000). Given all the above caveats, Campana's (1999:271) cautionary observation, "Do the elemental concentrations in the otolith reflect the elemental concentrations of the ambient water? The answer is an unequivocal 'sometimes'," is certainly warranted, especially given the additional complications arising from sampling choices, analytical protocols, and the vagaries of archaeological material. We discuss these below within the context of a pilot study designed to explore seasonality and chemical fingerprinting using archaeological otoliths.

3. A Pilot Study from the Northern Coast of the Gulf of Mexico: Sites and Expectations

To explore the potential for trace elemental analysis of archaeological specimens to contribute to studies of site seasonality and catchment area, we analyzed twelve sea catfish otoliths from pit features at two Woodland-period sites on the coast of Alabama. Plash Island (1BA134) is primarily a late Middle Woodland-period site (A.D. 325-640) with minor later occupation. The Bayou St. John, or Orange Beach site, is a primarily Late Woodland-period site (A.D. 650-1040) with minor earlier and later occupation (Reitz et al. 2013). Reitz et al. (2013) provide details about the sites, contexts, and specimens. Although the sites are only about 25 km distant from one another as the crow flies, different water chemistry can be expected due to the drainages represented. Plash Island is located on the northern edge of Bon Secour Bay, an eastward extension of Mobile Bay. The Alabama River feeds the bay, representing a very large drainage incorporating several different physiographic provinces and associated geological substrates and soil types. The Bayou St. John site is located on the southwest part of Perdido Bay (Figure 1), which receives water from the Conecuh River, a much smaller stream geographically restricted to the Coastal Plain.

In fisheries research, otoliths often are analyzed via whole-sample, solution-based methods. While this is particularly useful for the chemical analysis of larval otoliths to characterize natal areas, it sacrifices the time-dependent chemical series represented in growth bands developed later in life (Campana 1999:282-283; Chang et al. 2012; Jones and Chen 2003). An increasingly popular set of methods involves the use of beams, especially lasers, to obtain chemical data from different parts of the otolith. Chief among these methods is Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) (e.g., Barnett-Johnson et al. 2005; Campana et al. 1994; Chang et al. 2012; Ludsin et al. 2006; Pontual et al. 2000). LA-ICP-MS has seen increasing use in archaeological research over the last several years (e.g., Speakman and Neff 2005), including work on zooarchaeological remains (e.g., Peacock et al. 2010). There are good reasons for the popularity of the method, including minimal sample preparation, high sensitivity, rapid acquisition of multi-elemental data, isotopic discrimination, and targeted data acquisition due to high spatial resolution (typically 20 – 40 microns for carbonate minerals) (Resano et al. 2009:1). We used LA-ICP-MS to address questions of source water chemistry and seasonality, drawing upon the appropriate fisheries literature to set our analytical parameters.

Any chemical differences between the two sets of specimens hypothetically represent different chemistry of the source waters. For example, Reitz et al. (2013) found, on average, more negative $\delta^{18}\text{O}$ values in shells and otoliths from the Plash Island site as compared to specimens from Bayou St. John, a result probably due to the greater amount of freshwater entering Mobile Bay. For this question, we restrict our discussion to data obtained from the first laser raster line (i.e., from near the outer edge) on each otolith. We expected to see differences between sample sites via an ordination approach using elements that fell above detection limits. In terms of seasonality, deriving expectations from the literature for most trace elements is not

straightforward. Ba, for example, should increase near river sources (Hamilton et al. 2008, and references therein), yet also follows a nutrient-style profile in that it increases with water depth (Campana 1999; Hamilton et al. 2009). Thus, although Ba can readily substitute for Ca in the crystalline matrix of otoliths, it can be obtained from different habitats at different times of the year, depending on the habits of the fish species involved. The same caveat applies to Sn and Cd (Campana 1999:271). As noted above, Na, Cl, Zn and K are easily leached (Campana 1999:266), making them less-than-desirable elements for archaeological specimens. Ba and Zn often show low detection and poor precision in LA-ICP-MS testing of otolith lab standards (e.g., Thorrold et al. 1998) but sometimes work well (e.g., Ludsins et al. 2006); the causes of such variability currently are poorly known. For both areas of inquiry, the most reasonable expectation concerns Sr, which should decrease with freshwater input (e.g., Pontual et al. 2000:499). Sr levels revealed via LA-ICP-MS should mirror the seasonal rise-and-fall patterns noted in $\delta^{18}\text{O}$ values in otoliths (Reitz et al. 2013).

We stress here that our work is exploratory in nature: we did not try to address all of the complicating issues discussed earlier, many of which also are discussed by Disspain, Ulm, and Gillanders (2015). We merely wished to investigate whether, given a set of protocols for LA-ICP-MS analysis derived from the fisheries literature, correspondences between sites and otolith chemistry and/or between trace elements and light isotopes from the same specimens existed in our study area. For other work involving season and place of capture as derived from LA-ICP-MS analysis of archaeological otoliths, see Disspain, Wilson, and Gillanders (2012).

4. Materials and Methods

One problem with archaeological specimens is identification, which will be more or less difficult depending on species (e.g., Chen et al. 2011; see also Disspain, Ulm, and Gillanders 2015) and preservation state. Two species of sea catfish, the gafftopsail catfish *Bagre marinus* and the hardhead catfish *Ariopsis felis*, are confidently represented in the twelve otoliths we analyzed for trace elements, but some specimens could be one or the other, and these were accordingly identified only to the family level (Ariidae) (Table 1). Both *B. marinus* and *A. felis* spawn in inshore waters in the summer months and move to deeper water in the winter, although some fish may be found near-shore year-round (e.g., Mendoza-Carranza 2003).

Table 1. Otoliths used in both LA-ICP-MS elemental analysis and oxygen isotope analysis.		
Georgia Museum of Natural History Specimen #	Archaeological Site	Species
2600469.A	Plash Island	<i>Ariopsis felis</i>
2600470.A	Plash Island	<i>Bagre marinus</i>
2600019.B	Plash Island	<i>Bagre marinus</i>
2600021.A	Plash Island	Ariidae
2600020.B	Plash Island	<i>Ariopsis felis</i>
2600418.A	Plash Island	<i>Ariopsis felis</i>
2600517.A	Plash Island	Ariidae
2600017.B	Plash Island	<i>Ariopsis felis</i>
2600018.A	Plash Island	<i>Bagre marinus</i>
2560147.A	Bayou St. John	<i>Bagre marinus</i>
2560217.B	Bayou St. John	Ariidae

2560215.A	Bayou St. John	<i>Ariopsis felis</i>
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Another problem is post-depositional chemical alteration (Andrus and Crowe 2002; Disspain, Ulm, and Gillanders 2015). While chemical change may be detected via microscopy or cathodoluminescence showing conversion of the unstable polymorph aragonite to calcite over time (Campana 1999:265; Collins 2012), this may not aid in the detection of contamination in the interstitial spaces provided by the microchannel architecture of otoliths. Similar to chemical contamination, the effects of leaching will be hard to determine. In addition, there may well be significant pre-depositional alteration of otoliths due to burning (Andrus and Crowe 2002), smoking, or other preservation/preparation practices. Visually sorting out burned or otherwise obviously modified otoliths (Andrus and Crowe 2002; Disspain, Ulm, and Gillanders 2015) is necessary. Our specimens appeared relatively young and well-preserved (e.g., did not appear "chalky," burned, or discolored).

For light isotope analysis (originally reported in Reitz et al. 2013), whole specimens were mounted to petrographic slides using Crystalbond thermal adhesive, bi-sectioned through the core, and polished to reveal inner growth bands. Slide-mounted specimens were micromilled using a Merchantek/New Wave computer-controlled 3-D micromill. Between 17 and 22 sample lines were milled for each otolith; the first sample line was along the edge of the otolith, and subsequent lines were spaced at 50- μ m intervals following the growth geometry of the otolith. Samples were analyzed on a Thermo Gas Bench II coupled to a Thermo Delta Plus and/or a Thermo Delta V isotope ratio mass spectrometer (IRMS) at the University of Alabama Stable Isotope Lab. All samples were run with NBS-19 standard (in literature also referred to as TS-Limestone) so the final results could be expressed in parts per mil (‰) relative to the Vienna

Pee Dee Belemnite (VPDB) international standard. Precision, based on similar analyses in the past, is typically about 0.08‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{18}\text{O}$. The NBS-19 analyses also were used to assess and correct, if necessary, for drift and linearity on each sample run (Reitz et al. 2013:30).

LA-ICP-MS analysis was subsequently conducted using the same specimens. Protocols were derived from a number of papers originating in fisheries work (e.g., Barnett-Johnson et al. 2005; Campana 1999; Campana et al. 1994; Chang et al. 2012; Cook 2011; de Pontual et al. 2000; Hamilton and Warner 2009; Jones and Chen 2003; Ludsen et al. 2006; Ramsay et al. 2011; Sturm 2008; Thorrold et al. 1998). The instrument used was a New Wave Research UP-213 Laser Ablation unit coupled to a Perkin Elmer Elan DRC II mass spectrometer, housed in the Department of Chemistry at Mississippi State University.

Determination and availability of suitable matrix-matched standards for calibration has been described as the “Achilles heel” of LA-ICP-MS (Resano et al. 2009:13), and this problem has been noted specifically for the analysis of otoliths by many authors (e.g., Campana 1999:283-284; Jones and Chen 2003:433; Portual et al. 2000). Many researchers have relied upon commercially available NIST glasses (e.g., Hamilton and Warner 2009; Chang et al. 2012), while others have used chemically characterized, ground-and-pelleted otoliths (e.g., Chang et al. 2012) as standards. As Campana (1999:284) points out, the lack of a commonly-used standard material makes comparison of published data sets difficult. For our study, calcite reference materials were used to test the calibration curves of the instrument. Mg/Ca and Sr/Ca in those standards (NBS-19, Blue-CC, OKA) were determined independently by isotope dilution ICP-MS and published in Gabitov et al. (2013). Elemental ratios of Mg/Ca, Sr/Ca, and Ba/Ca were determined using Blue-CC standard, the best match for measured elemental ratios in otoliths.

284 LA-ICP-MS intensity ratios $^{24}\text{Mg}/^{44}\text{Ca}$, $^{88}\text{Sr}/^{44}\text{Ca}$ and $^{138}\text{Ba}/^{44}\text{Ca}$ were normalized to those where
285 $\text{Mg}/\text{Ca}=3.62$ mmol/mol (millimole of Mg per mole of Ca), $\text{Sr}/\text{Ca}=0.605$ mmol/mol, and
286 $\text{Ba}=1.17 \cdot 10^{-2}$ mmol/mol. Standard deviations between nine individual measurements on
287 carbonate reference material were 15, 16, and 28 % for Mg/Ca, Sr/Ca, and Ba/Ca respectively.
288 Those errors are significantly larger than those published in Gabitov et al. (2013), but smaller
289 than observed heterogeneity in otoliths. Due to the lack of a Na carbonate standard, $^{23}\text{Na}/^{44}\text{Ca}$
290 was used for comparison between different samples. Blanks were run before each otolith and
291 standards before and after each otolith. ^{44}Ca was used as an internal standard.

292 Cleaning of modern otoliths prior to ablation typically is a complicated affair involving a
293 series of washes, sonication, use of clean rooms, etc. (e.g., Campana 1999; Chang et al. 2012;
294 Hamilton and Warner 2009; Ludsing et al. 2006). These practices are primarily related to the
295 removal of any adhering biological material and/or contaminants from sample preparation, such
296 as sectioning or grinding. For our archaeological specimens, two pre-ablation passes were made
297 to remove surface contaminants that might have remained from micromilling or other external
298 sources.

299 An omnipresent problem with LA-ICP-MS is instrumental drift (Campana et al. 1994). As
300 Jones and Chen (2003:433) put it, “lasers provide a transient signal that is not stable. Because of
301 this instability, relative standard deviation (RSD) can be as high as 59% depending on the
302 structure of the sample surface with up to 25% RSD using calibration standards.” Pontual et al.
303 (2000:505) noted “poor consistency between [analytical] sessions for some elements,” a
304 phenomenon “clearly related to the apparatus calibration procedure.” Campana et al. (1994) also
305 discuss the effects of operating conditions on analytical results, noting, for example, large, abrupt
306 shifts in elemental levels associated with shutting down of the plasma torch during breaks. They

caution that “It is important to note that the magnitude of the isotope concentration shifts associated with the [LA]ICPMS far exceeded the sample-to-sample or stock-to-stock variability” (Campana et al. 1994:1946). Results also are conditioned by a host of choices relating to analytical protocols, such as beam width, run time, laser frequency, pulse rate, spot or raster pattern, etc. (see also Campana 1999; Resano et al. 2009). While small beam widths (e.g., 10 μm) can yield data from shorter periods of growth (one to a few days), more elements will be below detection limits (e.g., Chang et al. 2012), so there is tradeoff between temporal precision and data acquisition. Chang et al. (2012) compared 10, 20, and 30 μm beam widths and, not surprisingly, obtained the best results with the largest width, which they estimated represented 30–45 days of growth for hake (*Merluccius merluccius*). (Interestingly, the effect varied by suites of elements in fish from different water bodies—Chang et al. 2012). Still other sources of analytical noise are the buildup of ablated material in the machine (e.g., Ca accumulation on the skimmer cones – Campana et al. 1994), which necessitates frequent cleaning, and isotopic interference of ^{40}Ca with ^{40}Ar in the carrier gas (e.g., Barnett-Johnson et al. 2005), which limits the choice of analytes.

For our study, the equipment was calibrated daily and sample order alternated between sites. Line interval was set at 50 μm , with the number of lines set to correspond with the micromilled part of the otolith previously excavated for light isotope analysis (Reitz et al. 2013). The laser raster was set to 500 μm -long lines running parallel with the micromilled lines. This correspondence is approximate, however, as magnification was too high to allow exact matching of raster lines and micromill channels to be made, a problem that may be correctable in future comparative work by staining of milled specimens or shading via directed light. Given that the fish were relatively young (Reitz et al. 2013), and therefore fast-growing, the initial (outer) raster

line should produce data representing chemical inputs from the waters where the fish were captured. Laser run time was 50 seconds. Scan speed was 10 $\mu\text{m sec}^{-1}$. Laser intensity was set at 50%. Beam width was 30 μm . Frequency was 5 Hz, pulse was 45 mJ, and argon flow rate was 1.5 L-min⁻¹. Level of detection was set at 3 standard deviations (s.d.) above background. Elements initially recorded were ²³⁸U, ²⁰⁸Pb, ¹³⁸Ba, ¹³⁷Ba, ²⁴Mg, ⁸⁷Sr, ⁸⁸Sr, ⁸⁶Sr, ⁵⁵Mn, ²³Na, K, ⁵⁷Fe, ⁵⁴Fe, ⁸⁵Rb, ¹¹⁸Sn, ⁶⁶Zn, ⁴²Ca, ⁴³Ca, ⁴⁸Ca, ⁷Li, ²⁸Si, ⁵⁹Co, and ⁶³Cu. Including standards, blanks, and individual raster lines on otoliths, 305 sample datasets were obtained. The full data set is available upon request to the second author.

5. Results

Only four elements (Sr, Na, Mg and Ba) were consistently noted in sufficient abundance to be used in multivariate analysis. For investigation of chemical makeup related to source area, the data were analyzed via ordination, which positions sites (or specimens) along axes of variation (gradients) drawn through multi-dimensional space. A number of ordination methods are routinely used for exploratory purposes in ecology and palaeoecology, for a variety of data types (including chemical data—see Legendre and Birks 2012). These include principal components analysis (PCA), correspondence analysis (CA), principal coordinates analysis (PCoA), non-metric multidimensional scaling (NMDS), and derivations thereof. Ordination also is used in archaeology, usually for archaeobotanical (e.g., Valamoti and Jones 2003) or zooarchaeological (e.g., Peacock 2002) data, although it is seeing increasing use in the display of artifactual data such as pottery types (e.g., Nance et al. 2013; Ramenofsky et al. 2009; Smith and Neiman 2007). Choosing the proper ordination techniques in ecological studies involves consideration of factors

for which there may be no clear analog in archaeological work, or for which the rationale for particular choices may not be apparent.

Because we are not employing environmental variables matched to the chemical data, ours is an indirect gradient analysis. Using the program PC-Ord (McCune and Mefford 2011), we chose to use Bray-Curtis (polar) ordination, a distance-based (rather than eigen-based) technique via which endpoints represent the extremes in the data, with all other samples defined relative to the endpoints (Rossi 2010). While both Jackson (1993:16) and Oksanen (2013) note that basically similar results may be obtainable with Euclidean and Bray-Curtis solutions, polar ordination is suitable for either qualitative or quantitative data, works for either linear (short, trending) or unimodal (long, Gaussian-curve) gradients, and the results are amongst the easiest to visualize (Rossi 2010). We use the Sørensen distance measurement to dampen the effects of single, large differences in the data (Jackson 1993:12; Oksanen 2013:5). No downweighting of rare (low abundance) elements or other adjustments were made.

Analysis was first run using all twelve specimens, using data from the outermost raster line of each otolith to approximate the environment of the capture locale. Although two of the three Bayou St. John specimens are negative along the two major axes of variation (Axis 1 = 88.48% of the variation, Axis 2 = 8.8% of the variation, cumulative value of 97.34%), no real patterns emerge in the data (Figure 2). While all elements are contributing to the distribution of specimens, Sr has the most effect along both Axis 1 ($r = .986$) and Axis 2 ($r = .601$). The other elements in order of contribution to the distribution are Na (.742% along Axis 1) and Mg ($r = .680$ along Axis 2). Ba had little effect along either axis.

The lack of any coherent pattern (e.g., clear separation of Plash Island vs. Bayou St. John) likely results from variability arising from a number of sources, including species differences,

seasonal variation, inter-annual variation, minor variations in equipment operation, and other sources of noise as discussed above. Accordingly, although sample size is quite small, we reran the analysis using just the specimens identified as *A. felis* (4 from Plash Island, 1 from Bayou St. John) (Figure 3). In this case, the one specimen from Bayou St. John was distinguishable from the Plash Island specimens, being the only one to be negative along Axis 2. The major contributor to this placement along Axis 2 is Na ($r = .600$). It is Axis 1 that accounts for most of the variation in the data set, however (95.61% of the total variance), so the relative placement of specimens along Axis 2 (3.72% of the variance, 99.33% cumulative) may be of little consequence. Again, the major contributor to Axis 1 distributions is Sr ($r = 1.000$), followed by Ba ($r = .922$), Mg ($r = .814$), and Na ($r = .874$). Three of the four Plash Island samples are relatively high in Na, while one Plash Island and the Bayou St. John otolith are low in Na. Sr and Mg show similar distributions across all specimens, while Ba varies inversely with those two elements.

Results were mixed in terms of seasonality, for which we used element:Ca profiles from the full set of raster lines in order to obtain a temporal sequence from each otolith. The O isotope data for each otolith were plotted in order of ontogeny; these are referred to as the isotope "profiles." Season-of-death was determined from the profiles following methods detailed by Colaninno (2012). In the graphs, the outer edge of the otolith is on the left; as noted above, the mill lines for O isotope samples and the laser raster lines are not precisely matched, so comparison of the curves is necessarily approximate. In some cases, what appear to be clear seasonal trends occur that are consistent with the O isotope data (Reitz et al. 2013). Two examples are given here, both of which represent *A. felis*. The first specimen, GMNH 2560215.A from Bayou St. John, shows a $\delta^{18}\text{O}$ profile typical of summer capture (Figure 4). As expected,

the Sr:Ca profile shows lower Sr concentrations at the time of capture. In this case, Mg also followed the same profile. Conversely, specimen GMNH 2600017.B from Plash Island shows an O profile indicating winter capture (Figure 5); again, strontium behaves as predicted, with a high value at the terminal edge of the otolith. As mentioned above, it is this strontium effect that is pulling the specimens apart on Axis 1 in Figure 2, suggesting that seasonality is reflected in the multivariate analysis as well. However, as is often the case with O isotopes, (Reitz et al. 2013), clear elemental seasonal profiles were not visible on the majority of specimens analyzed via LA-ICP-MS. Until more control over the many variables that can affect results are obtained, we will remain uncertain as to why this is the case.

6. Conclusions

While some apparently excellent results have been achieved in the chemical fingerprinting of both freshwater and saltwater fish otoliths using LA-ICP-MS, there is, in fact, great variability in success rates for source characterization reported in the literature, from 100% (e.g., Ludsin et al. 2006) to as low as 50% (e.g., Thorrold et al. 1998). The causes of this variability remain unclear, but it is likely that there are many contributing factors in any particular study. The caveat for archaeologists is that adoption of elemental analysis of otoliths via LA-ICP-MS (or by any other method) must proceed with extreme caution while work on modern samples helps clarify how data should best be obtained and interpreted and while sources of analytical noise peculiar to archaeological material are explored.

The method does show promise as a way of assessing seasonality, but as relevant data can be obtained via O isotope analysis of otoliths and from other, more traditional, zooarchaeological methods (e.g., assemblage composition, size grading of fish), it is unclear that, even if seasonal signatures can be obtained from trace element analysis, anything of real additional worth can be obtained from the

application of LA-ICP-MS, except perhaps as a cross-check on existing methods. The seasonality question will not in any case ultimately be settled by site-by-site judgments, as sedentariness itself likely represents a shift in the scale of selection (Dunnell 1996) given that obtaining/processing/circulating resources doubtless required different mechanisms than were in place for mobile groups. As Reitz et al. (2013) discuss, we are at a point where variability in how, when and where such selection took place can be addressed and hopefully, from an evolutionary perspective, explained. Whether LA-ICP-MS analysis of otoliths can routinely contribute in this regard via seasonality determination has not yet been demonstrated with certainty.

A related promise, mostly unrealized at this point, is to obtain chemical data representing the place of capture. If local chemical signatures can be established (see below), then chemical “outliers” become important as likely representing different water bodies (e.g., estuaries fed by different rivers) where fish were captured. Such “imports” could represent either the actual catchment being exploited by Native fishers or foodstuffs brought to a site for communal gatherings, either situation being enabled by the use of watercraft for transport (Ames 2002). Although finding such patterns, if they exist, would entail analyzing large numbers of otoliths, choosing samples from particular kinds of archaeological contexts (e.g., midden vs. mound-side debris) would be an obvious tactic to employ. Establishing spatial links across settlement patterns via otolith chemistry or isotopic composition is one way to begin exploring variability in settlement patterns across space and through time. One way forward may be to focus less on divalent cations and more on stable isotopes and those elements that do not substitute for Ca in otoliths (e.g., Andrus and Thompson 2011). When linked with O isotope and morphometrics (Disspain 2010; Disspain, Ulm, and Gillanders 2015; Disspain, Wilson, and Gillanders 2012) such data may be used formally to establish site catchment, which would be a very powerful tool for exploring the evolution of sedentary settlement patterns, for example.

An interesting question is whether archaeological otoliths have applied value; e.g., to look for shifts in fish ranges or natal areas over “deep” time. A problem in this regard is in determining the expected “local” signature of the otolith core. In many, if not most, cases modern fish cannot be used to establish expectations for archaeological otolith chemistry due to water pollution and other changes in external conditions. Size grading of archaeological samples can indicate the life stage at which fish were captured, but if small (young) fish are assumed to be “local,” their use in chemical characterization becomes a circular argument, given that they could have been captured elsewhere and transported to the site. Also, growth rates between past and modern fish may have been significantly different (Andrews et al. 2003; Van Neer et al. 2002), so modern analogs must be approached with great caution. To provide such baseline data, it will be necessary to analyze large numbers of otoliths of target fish species that reasonably can be assumed to have been locally obtained at an early life stage based on size and habit, then removing statistical outliers to establish expected local elemental ranges. As is always the case in environmental archaeology, however, researchers must be aware of, and try to test for, complications arising from equifinality issues. And even with such work, the pollution problem still persists in reverse: that is, there can be no direct comparison of archaeological to modern specimens; rather, one would have to look at ranges via archaeological specimens and compare them to ranges established via modern specimens to look for change over time. This means, of course, that archaeological specimens representing off-shore seasonal signatures would have to be present in archaeological deposits as well. At a broad level, simple comparisons of archaeological vs. modern otoliths, either as whole-samples or via LA-ICP-MS, can at least be informative where the scale of modern pollution (e.g., Ba uptake) is concerned, and such

comparisons also may yield information of interest for paleoclimatologists (Andrus and Crowe 2002:292).

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Figure 1. Location of Plash Island and Bayou St. John sites. From Reitz et al. (2013).

Figure 2. Ordination of all otolith specimens tested from Plash Island and Bayou St. John sites using Sr, Na, Mg and Ba.

Figure 3. Ordination of specimens of *Ariopsis felis* from Plash Island and Bayou St. John sites using Sr, Na, Mg and Ba.

Figure 4. Oxygen isotope ($\delta^{18}\text{O}$, ‰), Mg/Ca, and Sr/Ca curves from otolith GMNH 2560215.A from the Bayou St. John site, representing summer capture. The X-axis represents successive micromill tracks/laser rasters beginning (L) at 50 μm from the edge of the otolith.

Figure 5. Oxygen isotope, Mg/Ca, and Sr/Ca curves from otolith GMNH 2600017.B from the Plash Island site, indicating winter capture. The X-axis represents successive micromill tracks/laser rasters beginning (L) at 50 μm from the edge of the otolith.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5