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[Article]

Comparison of Solution-Based versus Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Analysis of Larval Fish Otolith Microelemental Composition

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Abstract.—Otolith microchemistry has become a widely used tool for fisheries-based research in marine systems. However, its application to systems without well-defined juvenile nursery areas in which distinct otolith elemental signatures can develop (i.e., most freshwater systems) remains limited. In large part, this deficiency is due to unsuitable protocols for reliably processing and analyzing small otoliths of larvae. Herein, we evaluate the abilities of solution-based (SO) and laser ablation (LA) inductively coupled plasma mass spectrometry (ICPMS) to quantify the otolith elemental composition of larval yellow perch *Perca flavescens* captured in three distinct spawning locations in Lake Erie (USA–Canada). Analysis of otolith pairs by each technique demonstrated that both SO- and LA-ICPMS could be used to reliably quantify the more abundant elements, such as Sr and Ba. Magnesium and zinc, analyzed by use of both SO- and LA-ICPMS, also met the criteria for inclusion in our analyses (i.e., the coefficients of variation of standards were $<10.5\%$, and over 90% of samples were above detection limits at a single location). Upon closer inspection of the data, however, we found that estimates of Mg and Zn were only reliable for LA-ICPMS. Estimates of these two elements using SO-ICPMS were unrealistically high, probably owing to contamination during the otolith dissolution and handling phases. We also found that LA-ICPMS provided more precise estimates than did SO-ICPMS for nearly all elements explored, but LA-ICPMS was somewhat limited by high limits of detection for some elements. Despite these differences, both techniques could accurately discriminate among larvae produced in different Lake Erie spawning locations, primarily because of the significant variation in Sr among larval otoliths. Ultimately, although both methods are appropriate for analysis of otoliths from larvae, we recommend the use of LA-ICPMS in future otolith microchemical applications involving larvae.

Otoliths are permanent, metabolically inert (acellular) entities that are not altered or resorbed during periods of stress (unlike scales and bones) and that also grow incrementally throughout the life of a fish (Campana and Neilson 1985; Mugiya et al. 1991; Campana and Thorrold 2001). More recently, research has shown that otoliths also incorporate elements from the surrounding water (e.g., Mugiya and Tanaka 1995; Farrell and Campana 1996; Bath et al. 2000; Gillanders and Kingsford 2000; Milton and Chenery 2001b), which allows them to serve as a permanent record of the chemical environments experienced by fish (Campana 1999; Thresher 1999; Campana and Thorrold 2001). Capitalizing on this property, researchers have used otoliths as a natural environmental tag for a variety of purposes, including to determine migration histories (e.g., Rieman et al. 1994; Limburg 1995; Kafemann et al. 2000; Kennedy et al. 2002; Milton and Chenery 2003), to identify timing of metamorphosis (e.g., Cheng and Tzeng 1996; Arai et al. 1997, 2000; Marui et al. 2001), to discriminate among local spawning populations (e.g., Campana et al. 1995; Begg et al. 1998; de Pontual et al. 1999; Edmonds et al. 1999; Milton and Chenery 2001a), and to identify sources of recruits that survive to the fishery (Gillanders and Kingsford 1996; Thorrold et al. 1998, 2001).

Owing to the potential value of otolith microchemistry to fisheries ecology and management, numerous analytical techniques have been adapted to quantify the elemental concentrations of otoliths (Fowler et al. 1995a, 1995b; Campana et al. 1997; Campana 1999; Thresher 1999). One technique of growing importance has been inductively coupled plasma mass spectrometry (ICPMS). Its current, widespread use is probably related to its extremely

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low detection limits (parts per quadrillion to parts per trillion), which allow for a wide range of elements to be precisely and accurately quantified (Fowler et al. 1995a, 1995b; Campana et al. 1997; Campana 1999; Thresher 1999). Despite the recent use of both solution-based (SO) and laser ablation (LA) ICPMS, many uncertainties remain with regard to the use of this technique in otolith microelemental studies. For example, because of elevated detection limits that might arise from small otolith mass or contamination during storage and handling, our understanding of whether SO- versus LA-ICPMS is more suitable for processing larval otoliths remains limited (Townsend et al. 1989; Campana 1999; Thresher 1999; but see Brophy et al. 2003 and Martin et al. 2004).

Knowledge of whether microelemental analytical techniques such as SO- and LA-ICPMS could be satisfactorily conducted on otoliths from larvae is useful for stock discrimination investigations in marine systems (e.g., Townsend et al. 1995; DiBacco and Chadwick 2001; Brophy et al. 2003; Fitzgerald et al. 2004). This understanding, however, seems especially critical in freshwater systems like the Laurentian Great Lakes, where mixing of locally produced populations can occur early in life. In Lake Erie, for example, the mixing of local spawning populations (potential stocks) of important recreational and commercial fishes (e.g., yellow perch *Perca flavescens* and walleye *Sander vitreus*) typically occurs early during the larval stage because of small distances between local spawning grounds (Goodyear et al. 1982) and a lack of distinct, large nursery areas (i.e., wetlands, estuaries, or bays) where juveniles can reside and develop strong, site-specific elemental signatures in their otoliths (but see Brazner et al. 2004 for Lake Superior). In such instances, otoliths from small, newly hatched individuals that have not yet left their natal sites would be needed for analyses; this is problematic because small otoliths from larvae will inherently have less mass (i.e., less $CaCO_3$ that can incorporate water-borne elements) than those from juveniles or adults and would have a higher potential for significant contamination. Thus, for small, newly hatched larvae, limited otolith material might make it difficult to achieve sufficiently low detection limits, even when ICPMS is used (Campana 1999). Indeed, Brophy et al. (2003), in a study of larval fish (Atlantic herring *Clupea harengus*) otoliths analyzed by LA-ICPMS, suggested that small otolith size and subsequent contamination limited the use of all elements except Sr and highlighted the need for proper (i.e., clean) otolith preparation techniques.

Herein, using yellow perch captured in Lake Erie (USA–Canada), we compare the potential use of both SO- and LA-ICPMS to quantify the microelemental composition of freshwater fish larval otoliths, which are more than 20-fold smaller (range in mass from 0.1 to 27.5 μg) than those presented in most previously published studies focusing on marine fishes. In addition to identifying whether there are advantages associated with the use of SO- versus LA-ICPMS, we present a much-needed protocol (Thresher 1999; Brophy et al. 2003) for cleanly processing larval otoliths for microchemical work.

Methods

Field collections.—Yellow perch larvae used in the comparison of SO- versus LA-ICPMS were collected from three yellow perch production areas (Cooper et al. 1981a, 1981b, 1983; Goodyear et al. 1982; Ludsin et al. 1997) in Ohio waters of western and central Lake Erie during June 1994 and 1995. Larvae were collected near the mouths of two west-basin tributaries, the Maumee and Sandusky rivers (1994 and 1995), as well as near the mouth of the Grand River, located in central Lake Erie (1995) (Table 1; Figure 1). All larvae were collected during daylight hours by use of a surface-towed neuston net (2-m × 1-m mouth) with 500-μm mesh. Upon collection, larvae were immediately preserved in 95% ethanol and were transferred into glass shell vials containing FisherBrand trace-metal clean (high-performance liquid chromatography [HPLC] grade) 95% ethanol during summer 2001. At the time of transfer, total lengths (TL; nearest 0.1 mm) were recorded after larvae were re-hydrated in water for 30 s. Previous work has demonstrated that ethanol (both American Chemical Society and HPLC grade) has no measurable effect on the concentrations of the elements explored herein (Milton and Chenery 1998; Proctor and Thresher 1998; Hedges et al. 2004).

Otolith preparation.—All otolith processing occurred in a Class-100 clean room. Otoliths were removed and cleaned of tissue by use of glass probes while immersed in a drop of ultrapure Milli-Q water (MQW) on a glass microscope slide. Afterwards, otoliths were rinsed with MQW and were then transferred with a clean glass probe into a drop of purified-grade 6% NaOCl located within a covered petri dish. After otoliths from five fish were processed (~3 min/fish), individual petri dishes (still covered) were floated on top of a MQW bath located within an ULTRAsonik cleaner (model 57X; Ney Dental, Inc., Bloomfield, Connecticut), where they were sonicated for 2.5 min. Subsequently, a new glass probe was used to transfer otoliths from the NaOCl into a drop of MQW positioned on the opposite end of the same petri dish. Otoliths were sonicated again for 2.5 min to clean them of remnant NaOCl. Using otolith pairs from 10 yellow perch larvae, we found that cleaning otoliths

TABLE 1.—Details of collections used to compare otolith elemental concentrations of yellow perch larvae collected at three spawning locations in western (Maumee and Sandusky rivers) and central (Grand River) Lake Erie, Ohio (see Figure 1). Elemental concentrations from otoliths (left–right comparisons) were quantified by use of solution-based (SO) versus laser ablation (LA) inductively coupled plasma mass spectrometry (ICPMS). Included are collection dates, the number of otolith pairs analyzed (*N*), the number of collection sites within a location (sites), the number of replicate tows within a collection site (reps/site), the mean TL (±SD; mm) of larvae, and the average mass of individual otoliths (±SD; μg), as determined by SO-ICPMS. The last column indicates the number of independent larvae used to test the accuracy of the classification functions derived from the other samples (only one otolith per larvae was analyzed via either SO- or LA-ICPMS).

							Test otoliths	
Location	Collection date	N	Sites	Reps/site	TL	Otolith mass	SO	LA
Maumee River	2 Jun 1994	20[a]	2	2	15.6 ± 1.6	16.3 ± 6.5	10	
Sandusky River	1 Jun 1994	20[a]	3	2	15.7 ± 1.6	17.7 ± 7.9	10	
Maumee River	17 Jun 1995	20[a]	3	2	14.3 ± 2.0	5.7 ± 2.0		
Sandusky River	18 Jun 1995	6	1	2	13.3 ± 1.1	8.7 ± 3.5	24	12
Grand River	22 Jun 1995	12	2	1	17.1 ± 1.3	21.7 ± 6.4	18	

[a]One outlier was removed from this sample.

with NaOCl did not alter the concentrations of any of the elements analyzed, including Li, Mg, Sr, and Ba (paired *t*-tests: all $|t| < 2.08$, all $P > 0.05$). Thus, the NaOCl cleaning step may be unnecessary before processing with LA-ICPMS. Finally, otoliths were rinsed three times on the petri dish with MQW and then were placed, by means of a clean glass probe, either into a 10-mL high-density polyethylene auto-sampler vial (one otolith per vial; for SO-ICPMS analysis) or onto Scotch double-sided tape (number 665; 3M, St. Paul, Minnesota) mounted onto a petrographic microscope slide (for LA-ICPMS analysis). Each slide could hold up to approximately 180 pairs of larval otoliths.

All glassware (e.g., microscope slides, probes) and plasticware (e.g., petri dishes, auto-sampler vials) that came into contact with otoliths were acid-washed before use. The acid-wash consisted of an initial cleaning with Nitrox soap, a 24-h immersion in 13% nitric acid, a 24-h immersion in MQW, and three final rinses with MQW. All materials were dried for 24–48 h in a Class-100 clean room under a laminar-flow hood.

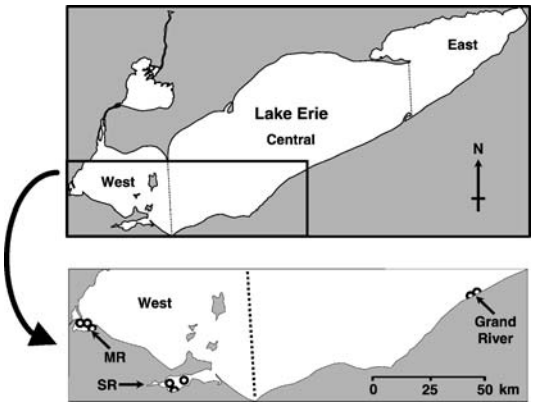

FIGURE 1.—Collection sites of larval yellow perch in western and central Lake Erie (Ohio waters). Larvae were collected with neuston nets near the mouths of the Maumee (MR) and Sandusky (SR) rivers ($n = 3$ sites) and at two locations near the Grand River mouth during June 1994 and 1995.

SO-ICPMS analysis.—Otoliths were dissolved in auto-sampler vials with a few drops of nonboiling Teflon-distilled 50% HNO_3 on a hotplate (65°C) positioned under a Class-100 laminar-flow hood, where they remained until the fluid evaporated (~90 min). Otoliths were then brought back into solution by adding 6.50 ± 0.01 g of ultrapure 1% HNO_3 spiked with Be, In, and Tl at concentrations of 5.0, 0.5, and 1.0 μg/g, respectively. These elements were used as internal standards to correct for drifts in sensitivity and mass-based bias during subsequent ICPMS analysis. Weights of individual vials were measured to the nearest 0.01 g before and after solution and otoliths were added; this allowed us to account for any differences in dilution volumes. Six procedural blanks were prepared in the same way, only without otoliths added. All vials were capped and stored until ICPMS analysis.

All SO analyses were conducted with a Thermo Elemental PQ3 ICPMS. Otoliths were analyzed in three runs, and samples from different locations were arranged randomly throughout the run to guard against potential confounding effects of instrumental drift. Multielement and single-element calibration standards, spiked with our internal standard elements and our internal standard solution (1% HNO_3 with Be, Th, and In), were placed at the beginning, middle, and end of sample runs to calibrate the ICPMS and to quantify instrument drift during the course of the run. Additionally, because all samples, including the pro-

TABLE 2.—Limits of detection (LOD [μg/g]), coefficient of variation (CV), and maximum percentages of otoliths above LODs (% >LOD) at a location for solution-based (SO) and laser ablation (LA) inductively coupled plasma mass spectrometry (ICPMS) conducted on Lake Erie larval yellow perch otoliths collected during 1994–1995. Ratios of SO-ICPMS to LA-ICPMS LODs also are provided. Values in bold italics indicate isotopic attributes that met our selection criteria for potential inclusion in analyses.

	SO-ICPMS			LA-ICPMS			LOD ratio
Isotope	LOD	CV	% >LOD	LOD	CV	% >LOD	(SO:LA)
^{25}Mg	6.154	***10.3***	***100***	1.096	***6.0***	***100***	5.6
^{55}Mn	7.482	23.9	***95***	34.902	61.5	12	0.2
$^{62/60}$Ni[a]	2.015	82.4	74	1.035	***4.7***	6	1.9
$^{63/65}$Cu[a]	1.280	114.9	***95***	0.301	11.3	53	4.3
^{66}Zn	1.244	***8.2***	***100***	3.444	***7.6***	***91***	0.4
^{85}Rb	0.130	22.7	50	0.032	***4.7***	40	4.1
^{86}Sr	1.974	***3.6***	***100***	2.446	***4.7***	***100***	0.8
^{88}Sr	0.102	***2.6***	***100***	1.782	***3.4***	***100***	0.1
^{138}Ba	0.043	***9.7***	***100***	0.251	***4.1***	***100***	0.2
^{140}Ce	0.014	81.4	16	0.003	***2.6***	2	4.2
^{208}Pb	0.407	46.2	58	0.446	***5.7***	47	0.9
^{238}U	0.020	47.0	0	1.960	24.5	0	0.0

[a]The two numbers are the isotopes measured by SO- and LA-ICPMS, respectively.

cedural blanks, were spiked with known concentrations of Be, T1, and In, we could correct for drift or matrix effects on an individual sample basis. Because Ca variability within and between samples is insignificant, as is the sum of all metals that are likely to be found in otoliths (<<1% by weight), we also used Ca as an internal standard based on the stoichiometric concentration of Ca in pure $CaCO_3$ (40.08% by weight). The mass of individual otoliths was determined via SO-ICPMS by using the measured Ca concentrations and calculating the mass of $CaCO_3$ from the concentration represented in the original otolith.

Three laboratory reference materials, created from otoliths of adult walleyes collected in Lake Erie's western basin, were randomly positioned among our unknown samples ($n = 3$ replicates). These reference materials allowed us to estimate measurement precision for all elements. To correct for measurement error associated with contamination during sample processing and cleaning, we subtracted the mean elemental concentrations of the six procedural blanks from the raw elemental concentrations of the otoliths.

Using SO-ICPMS, we measured 28 isotopes and quantified 22 elements (Table 2). Multiple isotopes were quantified for several of the elements, particularly the transition elements (e.g., Ni, Cu, and Zn), which can be difficult to determine in solution because of Ca molecular ions (CaO^+ and $CaOH^+$). If interferences were measured, they were corrected by utilizing the known isotopic abundances of Ca, O, and H. These interferences are orders of magnitude lower for LA-ICPMS than for SO-ICPMS because of the lack of the H_2O matrix for the former. Herein, however, we only present information on elements that also were quantified with LA-ICPMS. Further, because of elevated detection limits and imprecise quantification, not all elements were suitable for postprocessing analysis. For an element to be included in our analyses, it had to be precisely measured; specifically, the average coefficient of variation (CV = [SD/mean] × 100) for at least one measured isotope, as determined from our walleye standards, had to be less than 10.5% (Gillanders and Kingsford 1996). In addition, 90% or more of the samples had to be above the limits of detection (LOD) at a minimum of one location. The LOD is equal to $3 \cdot SD_{X\text{blank}} + X_{\text{blank}}$, where X_{blank} is the mean concentration of an element in the six procedural blanks and $SD_{X\text{blank}}$ is the associated standard deviation of the mean (Thorrold et al. 1998).

LA-ICPMS analysis.—For the LA analysis, we used a Continuum Surelite I solid-state Nd-doped yttrium aluminum garnet laser (wavelength = 266 nm; maximum power = 20 mJ; pulse rate = 20 Hz; pulse width = 4–6 ns) coupled to a Thermo Elemental X7 ICPMS. Since SO-ICPMS is a "bulk" method that integrates across the entire otolith, we traversed the diameter of the otolith with the laser (average dwell time per otolith = 45 s) to make the fairest comparison between SO- and LA-ICPMS techniques. To account for different otolith thicknesses, we adjusted the power and spot size of the laser (range = 6–15 μm in diameter) to avoid "burning" through the otolith and picking up contamination from the underlying tape and glass. By beginning and ending each laser transect on the double-sided tape, we could easily determine where the otolith began and ended in our analysis because mass 120, representative of a C molecular ion (instrumentally measured as ^{120}Sn) would spike noticeably when the laser hit the tape (S.A.L., personal observation). This "Sn" peak also occurred when the

laser burned through the otolith and into the tape or glass. To correct for ablation yield differences that resulted from varying the laser spot size, we used Ca as the internal standard, given that this element comprises a relatively large, constant proportion of the otolith (>99% Campana 1999; Thresher 1999).

Because a tradeoff exists in LA-ICPMS between the number of elements (measured isotopes) that can be scanned in a given time period and otolith size, we quantified a smaller subset of elements for the LA work than for the SO work (see Table 2). We chose this suite of elements for the LA-ICPMS work primarily because they were found to be useful discriminators in other studies or were likely to be present in the $CaCO_3$ crystal structure (aragonite) and therefore not subject to handling or storage effects (Milton and Chenery 1998; Proctor and Thresher 1998; Campana 1999; Thresher 1999; Hedges et al. 2004).

A glass reference standard (National Institute of Standards and Technology [NIST] 610 or 612) with known concentrations of elements was analyzed before and after every 16 samples ($n = 2$ replicates before and after), which allowed for quantification and correction of instrumental drift. This same standard also was used to determine precision (CV) in estimating elemental concentrations. The Ar–N carrier gas (i.e., background) was analyzed for 60 s before every sample, which allowed LODs to be calculated for individual samples based on the following formula:

$$\mathrm{LOD} = \frac{3 \cdot \sigma_{\mathrm{bgd}}}{S \cdot Y} \cdot \sqrt{\frac{1}{N_{\mathrm{bgd}}} + \frac{1}{N_{\mathrm{pk}}}}$$

where σ_{bgd} = the SD of the preablation determination of the background; N_{bgd} and N_{pk} = replicate determinations used in the integration of the background and ablation signal, respectively; S = mean sensitivity (counts/s per unit concentration) for the NIST reference standard; and Y = ablation yield relative to the NIST reference standard, determined from the measured count rates and known concentrations of the internal standard (Longerich et al. 1996; Jackson 2001). Essentially, an element's concentration needs to be greater than three SDs above background levels (after correcting for ablation yield, instrument, drift, and sensitivity) in order to be above the LOD. As with SO-ICPMS, for an element to be used in our analysis its concentration had to be above the LOD for 90% of the samples at a location and it had to be measured precisely (CV < 10.5%).

Data analysis.—Linear regression was used to generalize differences between SO- and LA-ICPMS techniques across all samples. To determine whether the ICPMS technique used influenced our ability to discriminate among larvae produced in different locations within each year, we first used multivariate analysis of variance (MANOVA)—with ICPMS and location as the main factors—to quantify the effect of these factors on otolith elemental concentrations. Posthoc contrasts of elemental concentrations were subsequently made by use of Tukey's honestly significant difference (HSD) tests for unequal sample sizes (Statistica version 6.0, StatSoft, Inc.). Afterwards, we ran a forward stepwise linear discriminant function analysis (LDFA; Statistica) for each year; separate analyses of the LA data alone, the SO data alone, and the combined data sets for each year were performed to explore whether our classification ability changed with the measurement technique. For an element to be included in the final model, its F-value had to exceed 1.0 and its tolerance had to be greater than 0.01. To test the ability of our classification functions to accurately discriminate fish of known origin, we classified otoliths from an independent set of yellow perch larvae collected from the same dates and locations (see Table 1). Unlike larvae used to develop the classification functions, for which both sagittal otoliths were analyzed, only one otolith from each test larva was analyzed with either SO- or LA-ICPMS.

All data were $\log_{10}$ transformed to achieve normality (Kolmogorov–Smirnov normality test; all $P \geq 0.10$). In addition, because outliers can unduly influence the results of multivariate analyses (Tabachnick and Fidell 2001), we removed highly significant multivariate outliers (>3.0 SD) based on a relative Euclidian distance measure (McCune and Grace 2002). A total of three outliers were removed from our analyses (see Table 1).

Results

Usable Elements

Both techniques demonstrated the ability to precisely quantify otolith elemental composition. Of the 11 elements explored with both techniques, only four (Mg, Zn, Sr, and Ba) satisfactorily met our criteria regarding detection limits and precision for both SO- and LA-ICPMS (Table 2). Despite these similarities, LA-ICPMS measured elemental concentrations more precisely than did SO-ICPMS. In fact, all but one element quantified with LA-ICPMS had CVs less than 25% (the other was ~62% owing to carrier gas interference), whereas 5 of 11 elements quantified with SO-ICPMS had CVs exceeding 25% (ranging as high as 115%) (Table 2). The number of elements satisfactorily above detection limits at a location (i.e., >90%), however, was greater for SO-ICPMS (six elements) than for LA-ICPMS (four elements) (Table 2). Thus, LA-ICPMS was somewhat limited by an

ability to overcome high LODs; if Ni, Rb, Ce, and Pb had possessed a greater percentage of samples above LODs, these elements would have met our criteria because they were measured precisely (CV $<$ 10.5% Table 2). By contrast, an ability to precisely measure elements limited the SO approach; Mn and Cu met our detection limits criteria but were not measured precisely (Table 2).

Based on these results, comparisons between ICPMS techniques are only possible for Mg, Zn, Sr, and Ba. For both SO- and LA-ICPMS, the two isotopes used to quantify Sr concentrations (^{86}Sr, ^{88}Sr) were highly correlated (both correlation coefficients $r \geq 0.98$, both $P < 0.00001$); thus, Sr concentrations were estimated only from ^{86}Sr.

Comparisons of Elements between ICPMS Techniques

Overall, both SO- and LA-ICPMS produced nearly identical estimates of Sr. Strontium concentrations from one technique could be accurately predicted with estimates from the other (coefficient of determination $R^2 = 0.97$; $P < 0.00001$), and the slope of the regression line between techniques did not differ from a 1:1 line (slope $\pm$ SE $= 1.005 \pm 0.002$; intercept $=$ 0 in this model; Figure 2a).

Estimates of Ba concentrations also were strongly, positively related between ICPMS techniques (linear regression: $R^2 = 0.69$; $P < 0.00001$; Figure 2b). However, the slope of the relationship between SO- and LA-ICPMS for Ba was less than 1.0 (slope $\pm$ SE $=$ 0.913 ± 0.012; intercept $= 0$ in this model; Figure 2b), indicating that Ba concentrations determined with SO-ICPMS were slightly higher than those determined by use of LA-ICPMS.

In contrast to our analyses of Sr and Ba, little correspondence between techniques was seen for Mg (Figure 2c) and Zn (Figure 2d). In fact, for both elements, no relationship was evident between otolith pairs (both $R^2 \leq 0.01$, both $P \geq 0.36$).

Differences in Elemental Signatures among Sites

Otolith mass was strongly, positively related to fish size, even for small yellow perch larvae ($r = 0.82$, $P <$ 0.001). Thus, because more otolith material was processed in analysis of large versus small otoliths regardless of technique, we needed to consider the potential influence of otolith mass in our analyses. For 1994 analyses, otolith mass was not a concern because mean TLs and otolith masses of yellow perch larvae did not differ between locations (both $P \geq 0.38$, df $=$ 74 for both; see Table 1 for means). The fact that Sr, Ba, Mg, and Zn concentrations were not strongly related to otolith mass across locations (all $R^2 \leq 0.08$) also alleviated concerns about the confounding effects of otolith size. Thus, for 1994, we did not need to account for the effects of otolith mass in our MANOVA. During 1995, however, both fish length and otolith mass were greater for Grand River yellow perch than for Maumee River and Sandusky River fish (one-way ANOVA, Tukey's HSD for unequal sample sizes: both $F > 29.0$, both $P < 0.0001$; see Table 1). To remove the potential influence of otolith mass in our exploration of site differences, otolith mass (OTO_-MASS) was used as a covariate in our MANOVA during 1995.

During 1994, both main effects (location and ICPMS) were highly significant in our MANOVA, whereas the interaction term was not (Tables 3, left columns). Essentially, Sr concentrations were higher in larvae from the Sandusky River than in those from the Maumee River (i.e., location effect) and were not influenced by ICPMS technique (Table 4; Figure 3a). Barium levels also were higher in the Sandusky River than in the Maumee River regardless of ICPMS technique (Figure 3b). However, average Ba concentrations were estimated to be higher for SO-ICPMS than for LA-ICPMS, thus accounting for the significant ICPMS effect (Table 4; Figure 3b). These results are consistent with previous regression results (Figure 2b). Although no differences were detected between locations for Mg and Zn (Table 4), a strong ICPMS effect was found for both elements. Similar to the regression analysis, SO-ICPMS provided higher estimates for both elements than did LA-ICPMS (Table 4; Figure 3c and d, respectively).

During 1995, both main effects (ICPMS and location) were again significant, as was their interaction (Table 3; right columns). As in 1994, Sr was not influenced by ICPMS technique (Table 4) and was higher in the Sandusky River otoliths than in the Maumee River otoliths (Figure 3e). Further, both of these western basin sites had higher otolith Sr concentrations than did the Grand River (Figure 3e). Barium concentrations also differed among locations (Figure 3f). Similar to 1994, Ba levels in 1995 were higher in Sandusky River otoliths than in Maumee River otoliths. In addition, Grand River Ba levels were higher than Maumee River levels but did not consistently differ from Sandusky River levels. The lack of difference between Grand River and Sandusky River Ba concentrations was due to SO-ICPMS (but not LA-ICPMS) producing high Grand River Ba levels (Figure 3f). This difference, in turn, caused the ICPMS $\times$ location interaction term to be significant (Table 4).

No differences in Mg and Zn were detected among locations sampled in 1995, although ICPMS effects were detected. As with previous regression results, Mg and Zn concentrations were higher for SO-ICPMS than

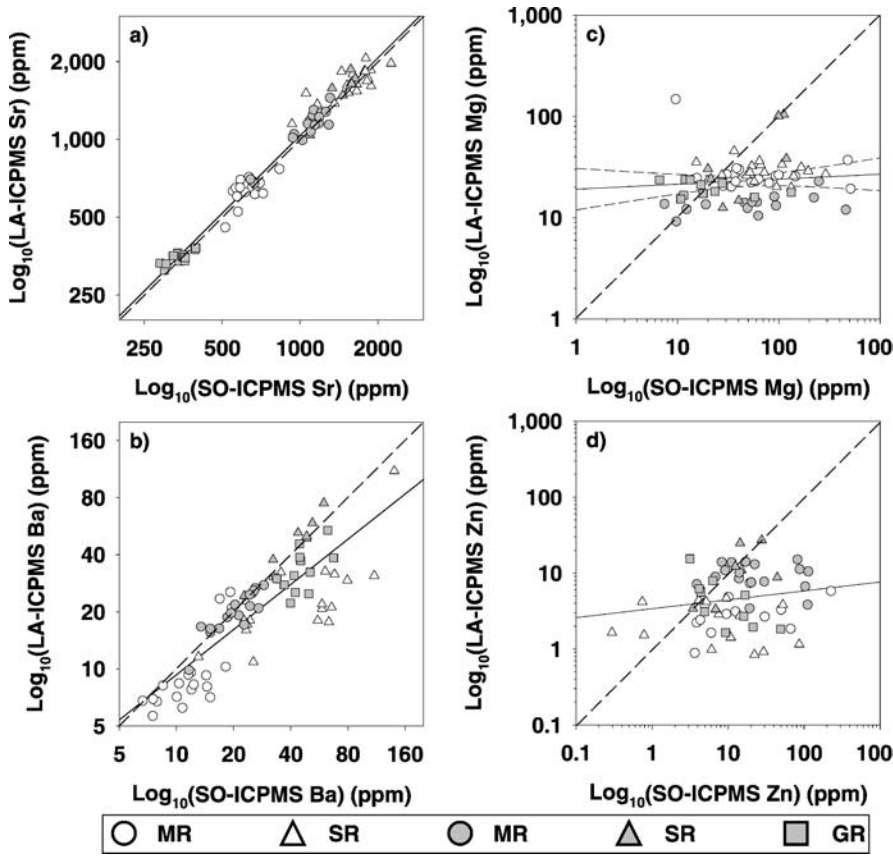

FIGURE 2.—Plots of **(a)** Sr, **(b)** Ba, **(c)** Mg, and **(d)** Zn concentrations (ppm [μg/g]) in otoliths of larval yellow perch, as quantified by solution-based (SO) and laser ablation (LA) inductively coupled plasma mass spectrometry (ICPMS). Larvae were collected in Ohio waters of Lake Erie near the mouths of the Maumee River (MR), Sandusky River (SR), and Grand River (GR) during 1994 (unshaded symbols) and 1995 (shaded symbols). Regression lines (solid lines) and expected one-to-one lines (dashed lines) are provided.

for LA-ICPMS (Table 4; Figure 3g and h, respectively). In addition, an ICPMS × location interaction was detected for Mg, which was due to Maumee River Mg being higher for SO-ICPMS than for LA-ICPMS (Figure 3g).

Ability to Discriminate Using SO- versus LA-ICPMS

Our ability to discriminate between the Maumee and Sandusky rivers was perfect (i.e., 100% classification accuracy) for 1994 data, regardless of whether SO- and LA-ICPMS data were analyzed independently or together. For each of the three LDFAs conducted, all 20 independent test larvae were classified correctly. The final models for each of the three LDFAs contained two or three elements; Sr was the only element common to all of them (Table 5). Given the large *F*-value, highly significant *P*-value, and the strong correlation between Sr and LDFA axis 1 for each of the 1994 analyses (Table 5), Sr was clearly the most important discriminatory element between locations. Tight clustering among otoliths processed with SO- and LA-ICPMS in LDFA canonical analysis clearly demonstrates the lack of influence of ICPMS technique when characterizing site-specific otolith signatures (Figure 4a), as does our excellent classification ability when otoliths from both techniques are used to discriminate between sites (100%).

During 1995, we were again able to discriminate among sites extremely well regardless of the ICPMS technique used (Figure 4b). As occurred in 1994, Sr was the most important discriminator among sites and was most highly correlated with LDFA root 1 in all analyses (Table 5). This axis was most useful for discriminating the western basin sites (Maumee and

TABLE 3.—Results from multivariate analyses of variance used to explore inductively coupled plasma mass spectrometry (ICPMS) technique (solution-based or laser ablation) and location (Maumee, Sandusky, and Grand rivers, Ohio) effects on micro-elemental composition (Sr, Ba, Mg, and Zn) of larval yellow perch otoliths collected in Lake Erie during 1994 and 1995. Otolith mass was used as a covariate in the 1995 analysis because average otolith masses differed among locations. Significant values ($\alpha = 0.05$) are shown in bold italics.

	1994					1995				
Factor	Effect df	Error df	Wilk's lambda	*F*	*P*	Effect df	Error df	Wilk's lambda	*F*	*P*
Otolith mass						4	58	0.900	1.6	0.1818
ICPMS	4	56	0.576	9.6	***<0.0001***	4	58	0.773	4.3	***0.0043***
Location	4	56	0.105	293.6	***<0.0001***	8	116	0.035	3.5	***<0.0001***
ICPMS × location	4	56	0.907	1.9	0.2326	8	116	0.700	2.8	***0.0066***

Sandusky rivers) from the Grand River site (Figure 4b). Residual variation (~4–12%) during 1995 was mostly explained by Ba, which was the element most highly correlated with LDFA root 2 in all analyses (Table 5). This element was most useful for discriminating between the Maumee and Sandusky rivers during 1995 (Figure 4b). In turn, when all otoliths from both SO- and LA-ICPMS analyses were combined for 1995, our ability to accurately classify our independent test larvae was solid (average across sites = 85%). For Grand River test larvae, we had 100% classification accuracy for each LDFA, whereas Sandusky River test larvae were classified correctly to a lesser extent (range = 69–72% for all three LDFAs). Specifically, nine (SO-only LDFA) or ten (LA-only and combined LDFAs) Sandusky River fish were incorrectly classified as Maumee River fish, and one larva was classified as a Grand River fish in all three LDFAs.

Discussion

Previous work has clearly demonstrated the value of SO- and LA-ICPMS for microelemental analysis of juvenile and adult otoliths. However, our understanding as to whether ICPMS approaches, particularly LA-ICPMS, could be used to quantify the elemental composition of otoliths from larvae remains somewhat

TABLE 4.—Results by individual elements from multivariate analyses of variance used to explore inductively coupled plasma mass spectrometry (ICPMS) technique (solution-based or laser ablation) and location (Maumee, Sandusky, and Grand rivers, Ohio) effects on microelemental composition (Sr, Ba, Mg, and Zn) of larval yellow perch otoliths collected in Lake Erie during 1994 and 1995. Otolith mass was used as a covariate in the 1995 analysis because average otolith masses differed among locations. Significant values ($\alpha = 0.05$) are shown in bold italics.

	1994					1995				
Factor	df	Sum of squares	Mean square	*F*	*P*	df	Sum of squares	Mean square	*F*	*P*
Strontium										
Otolith mass						1	0.002	0.002	0.4	0.5124
ICPMS	1	0.004	0.004	0.8	0.3876	1	0.003	0.003	0.8	0.3900
Location	1	2.564	2.567	486.4	***<0.0001***	2	1.458	0.729	192.9	***<0.0001***
ICPMS × location	1	0.004	0.004	0.7	0.4153	2	0.002	0.001	0.3	0.7388
Error	59	0.311	0.005			61	0.230	0.004		
Barium										
Otolith mass						1	0.033	0.033	3.0	0.0898
ICPMS	1	0.531	0.531	10.5	***0.0020***	1	0.033	0.033	3.0	0.0885
Location	1	3.896	3.896	76.9	***<0.0001***	2	1.281	0.640	58.1	***<0.0001***
ICPMS × location	1	0.179	0.179	3.5	0.0648	2	0.104	0.052	4.7	***0.0125***
Error	59	2.989	0.051			61	0.672	0.011		
Magnesium										
Otolith mass						1	0.004	0.004	0.0	0.8676
ICPMS	1	2.462	2.462	29.9	***<0.0001***	1	0.987	0.987	7.3	***0.0091***
Location	1	0.013	0.013	0.2	0.6908	2	0.609	0.305	2.2	0.1154
ICPMS × location	1	0.000	0.000	0.0	0.9934	2	1.467	0.733	5.4	***0.0070***
Error	59	4.857	0.082			61	8.304	0.136		
Zinc										
Otolith mass						1	0.018	0.018	0.1	0.7138
ICPMS	1	4.904	4.904	19.05	***<0.0001***	1	1.106	1.106	8.2	***0.0056***
Location	1	0.245	0.245	1.0	0.3332	2	0.314	0.157	1.2	0.3176
ICPMS × location	1	0.000	0.000	0.0	0.9954	2	0.140	0.070	0.5	0.5966
Error	59	15.187	0.257			61	8.189	0.134		

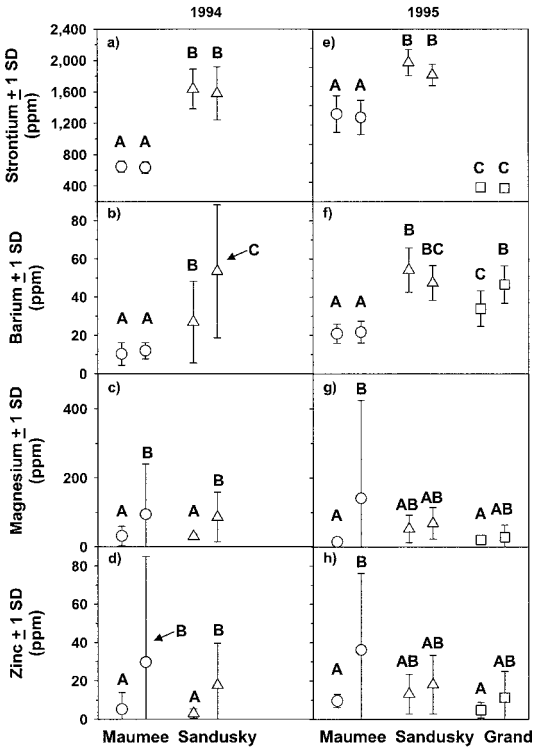

FIGURE 3.—Concentrations (ppm [μg/g]) of **(a, e)** Sr, **(b, f)** Ba, **(c, g)** Mg, and **(d, h)** Zn in otoliths of Lake Erie larval yellow perch collected near three spawning locations (Maumee, Sandusky, and Grand rivers, Ohio) during 1994 and 1995. Otolith elemental concentrations were quantified with solution-based (SO; shaded symbols) and laser ablation (LA; unshaded symbols) inductively coupled plasma mass spectrometry (ICPMS). Within a given panel, means with similar letters do not differ (Tukey's honestly significant difference test; $\alpha = 0.05$).

limited. For example, Brophy et al. (2003) attempted to explore the application of LA-ICPMS for quantifying the elemental composition of otoliths from Atlantic herring larvae. Those authors found that only Sr was reliably quantified in otoliths, whereas other element concentrations (Ba, Mg, Zn, and Pb) appeared to be artificially high, due either to contamination during storage or handling. Given the potential for stock mixing early in life (i.e., during the larval stage) in freshwater systems and hence the need for quantifying elemental concentrations of larval otoliths, we have explored the value of both SO- and LA-ICPMS approaches. The need to explore both approaches also stems from the fact that the analytical technique used to quantify otolith microchemistry has been found to influence results (e.g., Campana et al. 1997; de Pontual et al. 2000; Secor et al. 2002). As such, Campana et al. (1997) recommended that care must be taken when comparing results that were produced from different laboratories or analytical approaches.

In our study of larval yellow perch otoliths, Sr concentrations did not differ between analytical approaches for any of our sites. This result is critical given this element's importance for discriminating among larval yellow perch from different local spawning populations. Our results confirm those from other investigations of Sr levels in otoliths from juvenile and adult fish. For example, Thorrold et al. (1997) found strong, positive one-to-one relationships between LA-ICPMS and isotope dilution (ID) ICPMS for Sr in otoliths of juvenile Atlantic croakers *Micropogonias undulatus*. De Pontual et al. (1999), in a study of otoliths from juvenile soles *Solea solea*, found that SO- and LA-ICPMS had similar classification abilities owing to the consistent estimation of elements between techniques. This robustness in estimating Sr levels is probably due to the high abundance of this element relative to other trace elements in both water and otoliths (Campana et al. 1997; Campana 1999; Thresher 1999).

By contrast, Ba concentrations appeared somewhat dependent on the analytical technique used. Initially, we were concerned that Ba concentrations might be higher for LA- than SO-ICPMS, because of the potential for the "burning" of the laser through the otolith and into the Ba-rich mounting glass substrate. This hypothesis was not supported. Similarly, if the higher Ba concentrations found in the SO-ICPMS analyses were due to Ba contamination during solution preparation or analysis, then the disparity between techniques should be consistent across otolith masses. This was not the case, as the locations with the smallest otoliths (Sandusky and Maumee rivers in 1995) showed no differences between the two analytical techniques.

Another possible explanation for the discrepancy may relate to effects of heterogeneity in Ba concentrations in otoliths at some sites and an inability to analyze the extreme edges of larval otoliths with LA-ICPMS. For example, otoliths at some sites demonstrate strongly zoned Ba concentrations that are lower near the core (signified by a large Mn spike) than near the edge (Figure 5a). During laser sampling, the extreme outer edge (5–10 μm depending on otolith size) of otoliths typically cannot be reliably used because of the possibility of laser burning through the thin otolith and into the mounting material (signified by elevated levels of Sn in our ICPMS output). If that outer edge, which can be a significant proportion of the volume (mass) of a larval otolith, has a high Ba content (e.g., Grand River site, Figure 5a), then the average

TABLE 5.—Results from linear discriminant function analyses (LDFAs) used to quantify whether inductively coupled plasma mass spectrometry (ICPMS) technique (solution-based [SO] and laser ablation [LA]) influences the ability to differentiate larval yellow perch from three Lake Erie spawning locations. Analyses were conducted for otoliths from both 1994 and 1995. Included are individual and combined (1) statistics for overall models (model) and the individual elements (Sr, Ba, Mg, and Zn) included in each model, (2) Pearson's correlation coefficients (r) describing the relationship between each LDFA root (axis) and each included element, and (3) eigenvalues (λ) and proportion of overall variance explained by each root (R^2). Sample sizes are given in Table 1. Significant P values ($P \leq 0.05$) are shown in bold italics, as is the r-value most strongly correlated with each LDFA root.

Year	ICPMS method	Effect	Wilk's lambda	F	P	LDFA root 1	LDFA root 2
1994	SO only	Model	0.124	123.7	***<0.0001***		
		Sr	0.982	242.2	***<0.0001***	***r = 0.95***	
		Mg	0.137	3.6	0.066	$r = 0.05$	
		λ, R^2				7.07, 100%	
	LA only	Model	0.071	149.0	***<0.0001***		
		Sr	0.476	195.0	***<0.0001***	***r = 0.93***	
		Ba	0.077	3.1	0.087	$r = 0.27$	
		Zn	0.076	2.8	0.106	$r = 0.02$	
		λ, R^2				13.15, 100%	
	SO and LA combined	Model	0.104	313.3	***<0.0001***		
		Sr	0.989	618.9	***<0.0001***	***r = 0.97***	
		Mg	0.110	3.9	0.051	$r = 0.04$	
		λ, R^2				**8.58, 100%**	
1995	SO only	Model	0.012	87.7	***<0.0001***		
		Sr	0.241	312.2	***<0.0001***	***r = 0.68***	$r = -0.69$
		Ba	0.049	51.1	***<0.0001***	$r = 0.21$	***r = −0.97***
		Zn	0.013	2.3	0.113	$r = -0.07$	$r = -0.12$
		λ, R^2				32.80, 96%	1.52, 4%
	LA only	Model	0.006	94.0	***<0.0001***		
		Sr	0.120	306.2	***<0.0001***	***r = 0.76***	$r = -0.14$
		Ba	0.017	28.7	***<0.0001***	$r = -0.04$	***r = −0.69***
		Mg	0.012	16.5	***<0.0001***	$r = 0.01$	$r = -0.51$
		Zn	0.007	4.0	***0.023***	$r = 0.13$	$r = 0.03$
		λ, R^2				30.93, 88%	4.40, 12%
	SO and LA combined	Model	0.143	169.3	***<0.0001***		
		Sr	0.274	627.4	***<0.0001***	***r = 0.78***	$r = -0.62$
		Ba	0.053	93.0	***<0.0001***	$r = -0.14$	***r = −0.98***
		Zn	0.016	3.2	***0.047***	$r = 0.10$	$r = 0.07$
		λ, R^2				26.14, 94%	1.58, 6%

concentration of Ba (or any similarly zoned element) determined by LA-ICPMS would be lower than that determined by SO-ICPMS analysis, in which the entire otolith is analyzed.

Despite differences in Ba at a minority of sites, the relationship between Ba levels estimated by SO- and LA-ICPMS was both positive and strong, indicating that both techniques can be useful for analyzing otoliths from larvae. In support of this notion, Thorrold et al. (1997) and Secor et al. (2002) documented strong, positive one-to-one relationships for ID- and LA-ICPMS approaches in juvenile Atlantic croaker otoliths. Furthermore, the fact that our ability to discriminate among individuals captured in different Lake Erie spawning locations during both years was not influenced by ICPMS technique supports the use of either technique for estimating Ba concentration.

Unlike Sr and Ba, little correspondence was found between SO- and LA-ICPMS with regard to estimating Mg and Zn concentrations in larval otoliths. Specifically, we found that SO-ICPMS tended to produce higher estimates of both elements than did LA-ICPMS. Although Thorrold et al. (1997) documented a similar result in their analysis of Zn in Atlantic croaker otoliths when comparing ID-ICPMS with LA-ICPMS, they did find a strong positive relationship between techniques.

Previous published concentrations of Mg and Zn in juvenile and adult otoliths of marine and freshwater species are typically less than 50 ppm (Campana 1999; Thresher 1999; Secor et al. 2001, 2002). Magnesium concentrations, however, exceeded this level in 47% of the otoliths analyzed with SO-ICPMS, whereas only 3 of 75 otoliths (4%) measured by LA-ICPMS had Mg concentrations over 50 ppm. Similarly, Zn concentrations exceeded 50 ppm in 13% of our otoliths analyzed using SO-ICPMS, whereas none of the otoliths analyzed using LA-ICPMS exceeded this level. Given these relationships, we suggest that the SO-ICPMS estimates caused the lack of correspondence between techniques, and that these unrealistically high estimates of Mg and Zn were due to

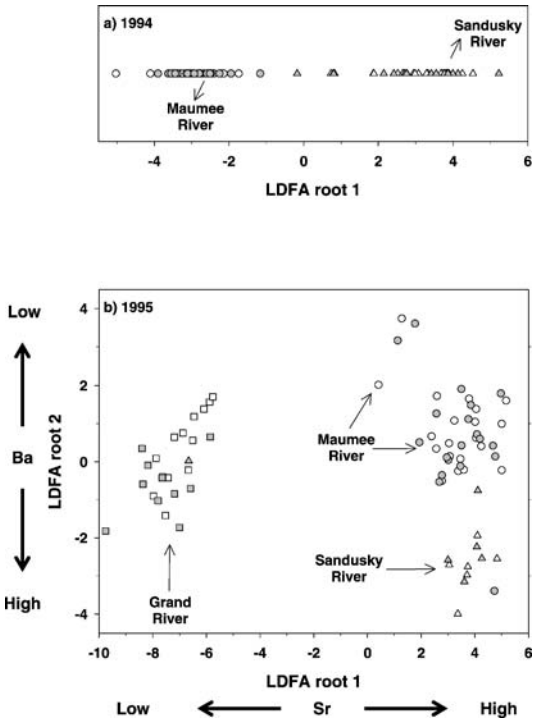

FIGURE 4.—Results of a linear discriminant function analyses (LDFAs) conducted on otoliths from yellow perch captured in Ohio waters of Lake Erie near the mouth of the Maumee (circles), Sandusky (triangles), and Grand (squares) rivers during **(a)** 1994 and **(b)** 1995. Otolith elemental concentrations (Ba and Sr) were quantified with solution-based (shaded symbols) or laser-ablation (unshaded symbols) inductively coupled plasma mass spectrometry. Elements that were most highly correlated with each LDFA root are indicated to show differences among locations.

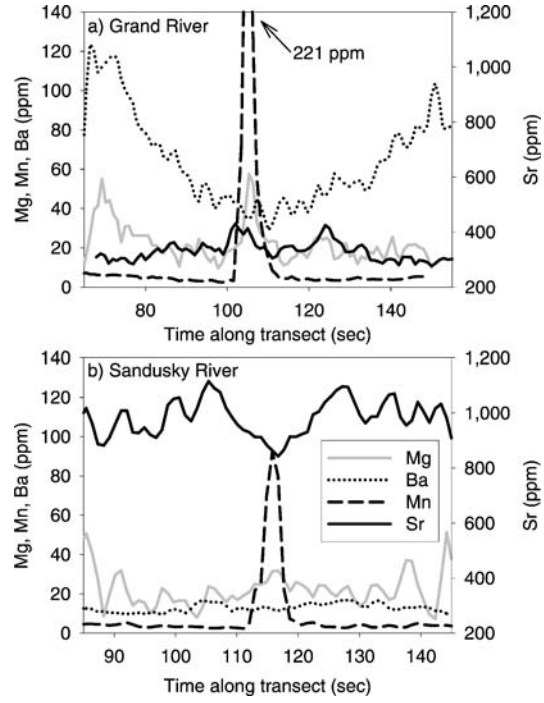

FIGURE 5.—Time series elemental data (Mg, Mn, Sr, and Ba; ppm [μg/g]) from otoliths of Lake Erie larval yellow perch captured near the mouths of **(a)** the Grand River (larval TL = 15.4 mm; otolith mass = 10.5 μg; average Mg = 26.1 ppm; average Mn = 12.2 ppm; average Sr = 332.7 ppm; average Ba = 52.7 ppm) and **(b)** the Sandusky River, Ohio (larval TL = 11.0 mm; otolith mass = 7.1 μg; average Mg = 21.1 ppm; average Mn = 9.5 ppm; average Sr = 987.3 ppm; average Ba = 13.1 ppm). A strong Mn peak signifies the otolith core. Background levels were quantified before and after the times shown here, and extreme edges of the otolith are not displayed due to laser burning through the otolith.

contamination in the preparation and analysis of the solutions.

As with our investigation, Brophy et al. (2003) demonstrated that LA-ICPMS was suitable for quantifying Sr in otoliths of larvae. In addition, even though LA-ICPMS was used in that study, problems with contamination were evident in estimates of Ba, Mg, and Zn. Mean concentrations of Mg and Zn in Brophy et al. (2003) exceeded 500 and 100 ppm, respectively, which are far beyond expected levels. Brophy et al. (2003) attributed their source of contamination to the effects of freezing; however, they did not ultrasonically clean their larval Atlantic herring otoliths prior to analysis. Thus, it is possible that residual surface contamination was responsible for their high measured Mg and Zn concentrations.

In addition to issues associated with contamination, LA-ICPMS was superior to SO-ICPMS in terms of precisely measuring elemental concentrations. Specifically, all but one element measured with LA-ICPMS had a CV less than 25%, whereas 5 of 11 elements quantified by SO-ICPMS did not. In fact, with the exception of Sr and Mn, LA-ICPMS had lower CVs than did SO-ICPMS, indicating the general superiority of LA-ICPMS for analyzing very small otoliths. This result counters previously published studies that found SO-ICPMS provided more precise (i.e., less variable) measurements of otolith microconstituents than did LA-ICPMS (see Figure 12 in Campana 1999). This discrepancy is probably due to the fact that the amount of larval otolith material available for analysis was not sufficient to allow measurement of solutions at the optimum dilution range for SO-ICPMS (dilution factors of 1,000–10,000) given that minimum solution volumes (a few hundred μL) are still needed for analysis.

Our findings also countered those of previous

studies in that SO-ICPMS did not consistently provide lower LODs than LA-ICPMS. De Pontual et al. (2000), in their comparison of SO- and LA-ICPMS techniques for analyzing juvenile sole otoliths, found that SO-ICPMS LODs were generally one to three orders of magnitude lower than LA-ICPMS LODs, thereby allowing the authors to include 10 more elements in the SO-ICPMS analysis than in the LA-ICPMS analysis. They attributed their low LODs to the use of a micronebulizer, which reduced the amount of solution needed to dilute the otoliths. While SO-ICPMS analyses of larval otoliths might benefit from use of a micronebulizer or other small-volume solution introduction system, they will still be hindered by the lack of otolith mass sufficient for optimum solution analysis with the best detection limits. In contrast, for LA-ICPMS, the amount of material sampled by the laser need not vary significantly for large versus small otoliths, giving rise to more constant LODs for a given analytical system.

Although it is clear from this study that both the SO- and LA-ICPMS approaches are suitable for quantifying the elemental composition of otoliths from larvae, LA-ICPMS has several specific advantages. With appropriate cleaning protocols, it is much less susceptible to contamination than is SO-ICPMS, which requires additional dissolution and analytical steps. In addition, LA-ICPMS does not suffer the same degradation in LODs as otolith size decreases. Further, the ability of LA-ICPMS to determine the chemistry of the larval otoliths continuously from core to edge offers a potentially better means to discriminate among individuals. At present, most other studies (including this one) have relied simply on elemental signatures derived from average elemental concentrations. Conceivably, these data could be analyzed in a more sophisticated fashion (i.e., with time-series analyses) to help discriminate among individuals or perhaps to shed insight into migration histories, habitat use patterns, or developmental histories (e.g., timing of metamorphosis). Laser-ablation ICPMS analysis also may allow us to more fully understand how physiology influences the elemental composition of otoliths. This latter perspective seems especially critical for research that attempts to use core signatures to identify natal origins (i.e., most stock discrimination research). For example, despite being collected from two different locations and having different average concentrations, the larval otoliths displayed in Figure 5 have similar distribution patterns for Mn and Mg (large peaks in core) and Ba and Sr (low in core with peaks on either side of it). Given that we have found near-identical patterns for yellow perch larvae captured in other Lake Erie locations, as well as for other Lake Erie fishes (e.g., walleyes, white bass *Morone chrysops*, and freshwater drum *Aplodinotus grunniens*), it is likely that there is strong physiological regulation of elemental deposition in larval otoliths. In turn, researchers must be cautious when deciding which portion of the otolith to call the "core." Thus, LA-ICPMS offers the potential to use otoliths to address a wider range of questions (i.e., more than just stock discrimination) than are possible with SO-ICPMS. Finally, we recommend LA-ICPMS over SO-ICPMS for practical reasons; LA-ICPMS is faster and less expensive than SO-ICPMS because additional samples do not need to be analyzed independently and because the sample preparation is more complex for SO-ICPMS than for LA-ICPMS.

In summary, although further improvements can be anticipated for both SO-ICPMS (use of low-volume sample introduction systems) and LA-ICPMS techniques, the extremely limited mass of larval otoliths strongly suggests that LA-ICPMS techniques are superior for studies involving larval fish populations (e.g., freshwater systems). Given that we found little evidence of contamination in our otoliths processed with LA-ICPMS, we strongly suggest that all future otolith microchemical investigations involving larvae incorporate a sonication step into their otolith preparation protocols.

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