

## Validation of Sr isotopes in otoliths by laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS): opening avenues in fisheries science applications

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**Abstract:** Advances in probe-based mass spectrometry allow for high spatial resolution of elemental and isotopic signatures in fish otoliths that can be used to address fundamental questions in fisheries ecology. Analyses of Chinook salmon (*Oncorhynchus tshawytscha*) otoliths from two river populations yield identical  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios using laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS) and thermal ionization mass spectrometry (TIMS). Results were obtained from freshwater otoliths with low Sr concentrations (300–800 ppm) using high spatial resolution (50  $\mu\text{m}$ ) corresponding to temporal histories of ~12 days fish growth. Low natural variation in  $^{87}\text{Sr}/^{86}\text{Sr}$  among otoliths from the same rivers allows for conservative estimates of external precision of techniques. Thus, we demonstrate that Sr isotope ratios obtained by LA-MC-ICPMS can be accurate and precise, bypassing the time-intensive sample preparation required by microdrilling and TIMS. This technique opens the use of Sr isotopes for broader ecological questions requiring large sample sizes to characterize nursery habitats, metapopulation dynamics, and stock discrimination similar to studies that focus on elemental concentrations, thereby providing a more robust tool for some freshwater and diadromous fishes.

**Résumé :** Les améliorations de la spectrométrie de masse par sondes permettent d'obtenir une très haute résolution spatiale des signatures d'éléments et d'isotopes dans les otolithes des poissons, qui peuvent alors servir à résoudre des problèmes fondamentaux en écologie des pêches. Nous obtenons des rapports  $^{87}\text{Sr}/^{86}\text{Sr}$  identiques dans des analyses d'otolithes de saumons quinnat (*Oncorhynchus tshawytscha*) de deux populations de rivière faites par spectrométrie de masse à plasma inductif avec multicollecteur après ablation au laser (LA-MC-ICPMS) et par spectrométrie de masse par ionisation thermique (TIMS). Ces résultats provenant d'otolithes d'eau douce à faible teneur de Sr (300–800 ppm) ont une haute résolution spatiale (50  $\mu\text{m}$ ) qui correspond à un déroulement temporel de ~12 jours de croissance chez les poissons. La faible variation naturelle de  $^{87}\text{Sr}/^{86}\text{Sr}$  parmi les otolithes d'une même rivière permet de faire des estimations conservatrices de la précision externe de la méthodologie. Nous démontrons ainsi que les rapports des isotopes Sr obtenus par LA-MC-ICPMS peuvent être exacts et précis, permettant de passer outre à la longue préparation des échantillons requise par le microforage et par TIMS. Cette technique ouvre la porte à l'utilisation des isotopes de Sr pour l'étude de problèmes écologiques plus vastes qui nécessitent de grands échantillons, comme la caractérisation des habitats de nursery, la dynamique des métapopulations et la discrimination des stocks; il en va de même pour les études qui se basent sur les concentrations d'éléments. Il s'agit donc d'outils plus robustes pour l'étude de certains poissons d'eau douce et de certains migrateurs diadromes.

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## Introduction

Advances in analytical techniques have led to the development of innovative tools for assessing a wide variety of fish population attributes. For example, chemical compositions of otoliths have proven useful as natural population markers to identify nursery habitats, natal homing, life history variation, and the scale of connectivity among fish populations (Campana and Thorrold (2001) and references therein). Use of natural chemical markers, such as multi-element concentrations (e.g., Sr, Ba, Mg) in otoliths, has the added benefit that chronometric properties of daily growth are permanently recorded (Campana and Thorrold 2001). For multi-element concentration determinations, development of new probe-based methodologies using laser ablation (LA) sampling in conjunction with inductively coupled plasma mass spectrometry (ICPMS) has greatly expanded their use, as additional research questions can be pursued by targeting specific growth regions in otoliths (Thorrold and Shuttleworth 2000). In contrast, application of Sr isotopes ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) remains in its infancy. Validation of LA sampling for measuring Sr isotopes in otoliths would similarly allow for more extensive use of Sr isotopes in fisheries science.

Sr isotopes are powerful markers that can be used to identify natal freshwater habitats (Ingram and Weber 1999; Kennedy et al. 2000, 2002) and track small-scale movement patterns (Kennedy et al. 2000) and migration between marine and freshwater environments (Outridge et al. 2002; Bacon et al. 2004; Woodhead et al. 2005) and have use in determining spatial structure and natal source contribution for anadromous fishes (Barnett-Johnson 2004). In contrast to trace element concentrations, the utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  is driven by the following characteristics: (i) Sr readily substitutes for calcium in otoliths (Campana and Thorrold 2001); (ii) Sr isotopes in otoliths and water are not altered by biotic (trophic dynamics) or abiotic mechanisms (temperature, salinity) (Kennedy et al. (2000) and references therein); (iii) Sr isotopes are controlled by the age and composition of rocks comprising individual watersheds and are stable across ecological time (Kennedy et al. 2000); and (iv) globally invariant Sr isotope ratios of ocean water ( $\sim 0.70917$ ) provide a constant baseline for detecting fish movement between freshwater and marine environments (Outridge et al. 2002; Woodhead et al. 2005).

Previous research highlights variations in Sr isotopes that hold promise for critical ecological and fisheries questions, but to date no research has applied these markers (Ingram and Weber 1999; Kennedy et al. 2000, 2002). This results largely from laborious sample acquisition (e.g., micromilling), preparation (e.g., chromatographic purification), and slow analytical processing using thermal ionization mass spectrometry (TIMS), making use of Sr isotopes prohibitive for addressing many ecological questions that require large sample sizes (i.e.,  $>100$ ). In addition, limited spatial resolution of micromilling (typically  $>40\ \mu\text{m}$ ) prevents applications that track fish movement on smaller spatial and temporal scales that are attainable using laser sampling ( $\leq 5\ \mu\text{m}$ ; Woodhead et al. 2005).

Recent research (Outridge et al. 2002; Woodhead et al. 2005) demonstrates the potential of measuring Sr isotopes in otoliths by laser ablation multicollector inductively coupled

plasma mass spectrometry (LA-MC-ICPMS), but many assumptions have not been validated. To date, no study has demonstrated that Sr isotopes can be measured accurately with high within-run and external precisions for typical otoliths with low Sr concentrations (300–800 ppm). Instead, repeated measurements of marine carbonate references (corals or mollusks) with  $\geq 1200$  ppm Sr have been used as proxies that can overestimate within-run and external precisions and underestimate the impact of interfering elements in otoliths (Outridge et al. 2002; Woodhead et al. 2005).

For Sr isotopes acquired by LA-MC-ICPMS to be useful and valid for otolith studies, we evaluated the following criteria using otoliths from juvenile Chinook salmon (*Oncorhynchus tshawytscha*) over a range of Sr concentrations: (i) within-run measurements must be precise (i.e., in-run errors of single analyses must be less than the range of Sr isotopes in target otolith groups); (ii)  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios must be accurate (i.e., results must be similar to values determined by TIMS, the standard for accurate and precise Sr isotope measurements); and (iii) the technique must produce high external precisions (i.e., small variation in distribution of  $^{87}\text{Sr}/^{86}\text{Sr}$  for a single sample measured repeatedly or, more conservatively, in multiple samples from the same population). These are primary factors in resolving potentially small differences in Sr isotopes common in ecological systems. Results to date do not provide a clear answer as to whether LA-MC-ICPMS can generate accurate results with high within-run and external precisions for freshwater otoliths, much less under what conditions this technique can be used over micromilling and TIMS.

## Methods

Juvenile Chinook salmon from the fall run were collected from the Feather ( $N = 5$ ) and Mokelumne ( $N = 8$ ) rivers in the California Central Valley in April 2002. Average age and size of juveniles was  $\sim 100$  days and  $78 \pm 10$  mm (standard deviation, SD) fork length. Sagittal otoliths were extracted, washed, and stored dry in vials prior to cleaning and mounting. Left otoliths were soaked for 6 h in 30% Suprapur<sup>®</sup>  $\text{H}_2\text{O}_2$  and rinsed using ultrafiltered water and  $0.01\ \text{mol}\ \text{HNO}_3\cdot\text{L}^{-1}$ .

### Laser analysis

Otoliths analyzed by LA-MC-ICPMS were mounted sulcus side up on microscope slides and polished using  $\text{Al}_2\text{O}_3$  lapping paper until daily increments were exposed. Otoliths were randomized by site and sampled using laser tracks ( $60\ \mu\text{m}$  wide  $\times$   $500\ \mu\text{m}$  long  $\times$   $80\ \mu\text{m}$  deep) in the ventral region along the longest axis parallel to daily increments ( $5\ \mu\text{m}\cdot\text{increment}^{-1}$ ;  $\sim 12$  days) where the plane of growth exhibits the least curvature (Fig. 1). Thirty ratios, each integrated for 8 s, were measured for each laser track and used to estimate within-run precision ( $2 \times$  standard error, 2SE). We used a New Wave UP213nm laser and ThermoFinnigan Neptune<sup>®</sup> MC-ICPMS with specific instrument, laser, and interference corrections described in Ramos et al. (2004).

The greatest challenge in obtaining accurate Sr isotope ratios using LA-MC-ICPMS lies in accounting for interfering elements or molecules that overlap with Sr isotope masses.

**Table 1.** Paired  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses using laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS) and microdrilling/thermal ionization mass spectrometry (TIMS) of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) otoliths, with summary statistics of two-way analysis of variance (ANOVA).

Sample	LA-MC-ICPMS		TIMS		
	$^{87}\text{Sr}/^{86}\text{Sr}$	Within-run error <sup>a</sup>	$^{87}\text{Sr}/^{86}\text{Sr}$	Within-run error <sup>b</sup>	Rb/Sr <sup>c</sup>
Feather River					
1	0.706161	0.000053	0.706204	0.000015	
2	0.706119	0.000041	0.706116	0.000014	
3	0.706116	0.000031	0.706135	0.000013	0.0009
4	0.706180	0.000038	0.706177	0.000013	0.0010
5	0.706174	0.000037	0.706140	0.000028	
Mokelumne River					
6	0.706852	0.000051	0.706873	0.000014	
7	0.706851	0.000059	0.706822	0.000010	
8	0.706881	0.000038	0.706848	0.000013	
9	0.706886	0.000023	0.706869	0.000014	
10	0.706892	0.000040	0.706883	0.000056	
11	0.706924	0.000034	0.706899	0.000011	
12	0.706899	0.000025	0.706844	0.000014	0.0008
13	0.706985	0.000031	0.706864	0.000015	
Marine carbonate					
14	0.709205	0.000036			
15	0.709166	0.000058			
16	0.709175	0.000041			
17	0.709153	0.000041			
18	0.709162	0.000034			
19	0.709140	0.000019			
20	0.709150	0.000025			
21	0.709168	0.000034			
22	0.709182	0.000042			
23	0.709190	0.000030			
24	0.709170	0.000021			
25	0.709168	0.000039			
26	0.709182	0.000040			
27	0.709148	0.000053			
28	0.709180	0.000041			
29	0.709176	0.000048			
ANOVA	df	Mean square	F	P	
Instrument type	1	0.000000001	0.590	0.583	
River (blocked)	1	0.000003255	0.003	<0.001	
Instrument × river	1	0.000000001	1.880	0.184	

<sup>a</sup>2 × standard error of 30 ratios, each integrated for 8 s using LA-MC-ICPMS.

<sup>b</sup>2 × standard error of ~100 ratios, each integrated for 5 s using TIMS (NBS987 = 0.710229, N = 5).

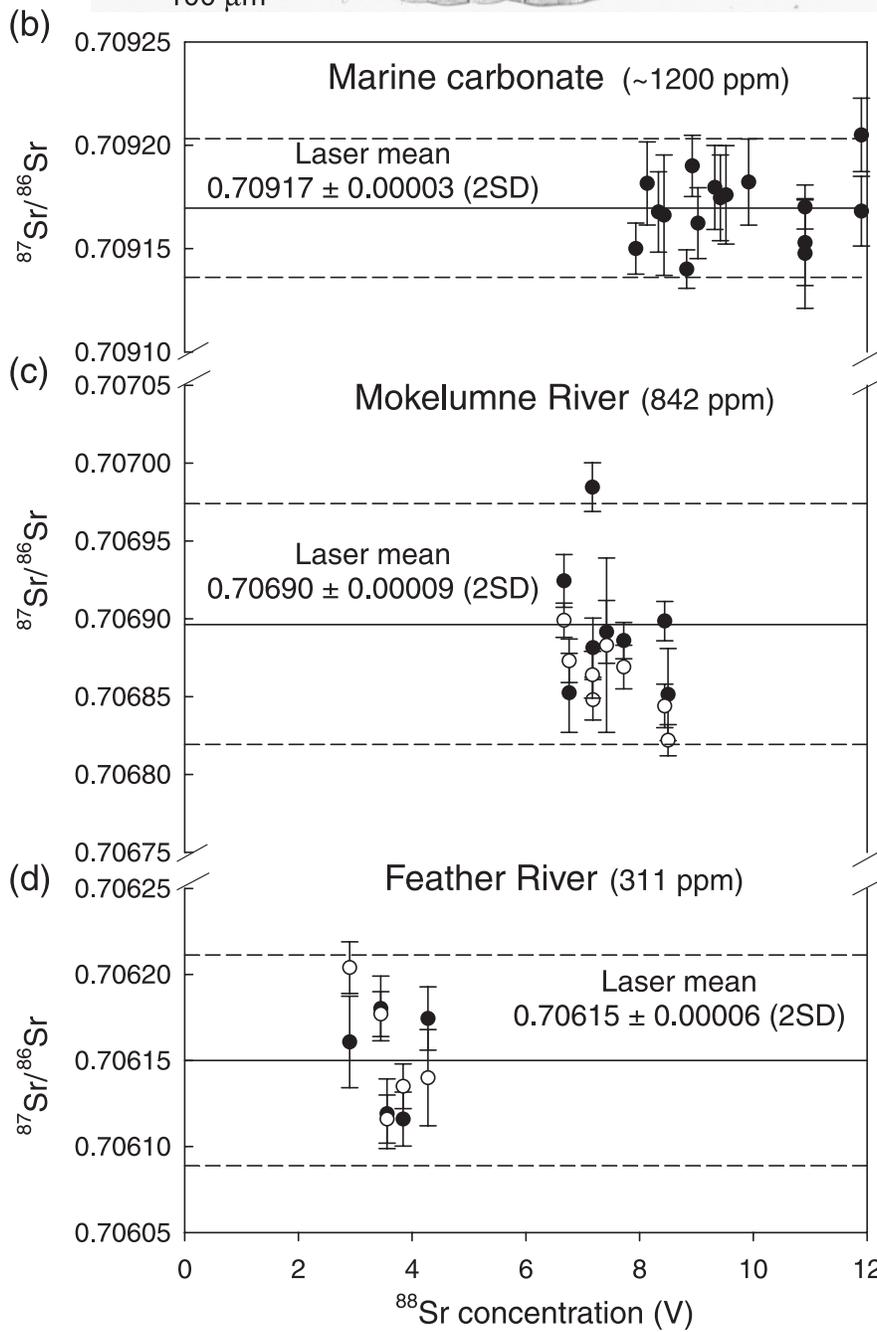
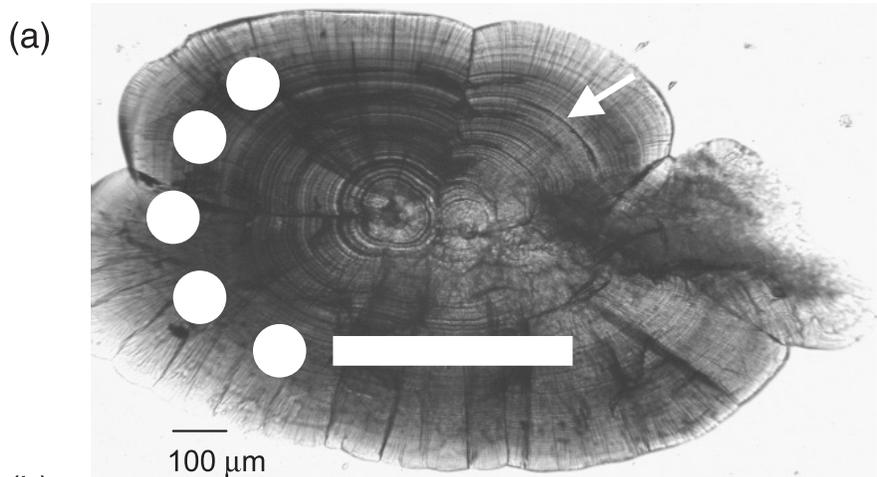
<sup>c</sup>Rb concentration for representative otoliths determined using isotope dilution and TIMS.

Although we evaluated a range of potential interfering elements and molecules (see Ramos et al. 2004), only Kr and Rb were critical for otoliths using LA-MC-ICPMS. We account for the effects of  $^{84}\text{Kr}$  and  $^{86}\text{Kr}$ , present in the Ar gas, by subtracting on-peak baselines (<1 mV  $^{83}\text{Kr}$ ), measured prior to firing the laser, from measured beam intensities. Uncorrected Kr decreases mean  $^{87}\text{Sr}/^{86}\text{Sr}$  by up to 0.00007. The average intensity of  $^{85}\text{Rb}$  in our freshwater otoliths is ~5 mV (Rb/Sr = 0.00008), which increases mean  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios by ~0.002 (40× external precision). To correct for  $^{87}\text{Rb}$ , we use the measured  $^{86}\text{Sr}/^{88}\text{Sr}$  and natural  $^{85}\text{Rb}/^{87}\text{Rb}$  value to calculate a mass bias uncorrected  $^{85}\text{Rb}/^{87}\text{Rb}$  ratio and the measured  $^{85}\text{Rb}$  intensity to calculate and remove  $^{87}\text{Rb}$  contributions to measured  $^{87}\text{Sr}$  intensities. Our technique and equipment do not generate Ca dimers/argides, thus eliminat-

ing a highly influential interferant (Weber et al. 2004; Woodhead et al. 2005).

We measured  $^{87}\text{Sr}/^{86}\text{Sr}$  in the region of the otolith accreted while in the natal tributary, but after yolk absorption and prior to outmigration (~250 μm from primordia). This region was just distal of the dark band diagnostic of the onset of exogenous feeding (Fig. 1; Zhang et al. 1995). Isolating this region is particularly important when characterizing natal river  $^{87}\text{Sr}/^{86}\text{Sr}$  values because the prefeeding value reflects Sr derived from both marine and natal river sources (Bacon et al. 2004).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios within laser tracks did not exhibit trends, suggesting little cross-sampling into areas with different ratios.

Evaluating the accuracy and external precision of measurements in otoliths is challenging, as there are no certified



**Fig. 1.** (a) Image showing laser (bar) and microdrill/thermal ionization mass spectrometry (TIMS) (circles) sampling regions of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) otolith postexogenous feeding check (arrow).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in (b) marine carbonate clam shell reference and otoliths from fish collected from (c) Mokelumne and (d) Feather rivers using laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS) (solid circles) and TIMS (open circles) as a function of Sr concentration (as indicated by  $^{88}\text{Sr}$  laser intensities). Paired LA-MC-ICPMS and microdrilled/TIMS analyses show no significant differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  (two-way analysis of variance,  $F_1 = 0.59$ ,  $p = 0.58$ ). External precision of laser results is conservatively estimated as 2 standard deviations ( $\pm 2\text{SD}$ ) (broken lines) of the population mean (solid line) for marine carbonate ( $\pm 0.00003$ ), Mokelumne River ( $\pm 0.00009$ ), and Feather River ( $\pm 0.00006$ ) otoliths.  $^{88}\text{Sr}$  intensities from LA-MC-ICPMS reflect relative differences in Sr concentration for all samples, as confirmed by isotope dilution using TIMS on a subset of otoliths with concentrations reported in parentheses.

otolith reference materials. A common solution has been to use marine carbonate references, assumed to be in isotopic equilibrium with modern seawater (0.70917), to evaluate accuracy and repeat analyses of these materials to estimate external precision. Our solution uses the low natural variation in  $^{87}\text{Sr}/^{86}\text{Sr}$  in otoliths (among-otolith variation) from fish from the same location, rather than a single non-otolith reference measured over time, to derive a conservative estimate of external precision encompassing both analytical precision and among-otolith variation.

### Solution analysis

Accuracy of LA-MC-ICPMS analyses is evaluated using samples obtained by microdrilling five holes ( $\sim 100\ \mu\text{m}$  diameter) replicating laser tracks along the same otolith growth zones (Fig. 1). Microdrilled samples were collected as slurries (with water), dried, and weighed. A high-purity  $^{84}\text{Sr}$  spike was added that allowed for the determination of both Sr concentrations and unspiked  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios using isotope dilution. Samples were dissolved in distilled 6 mol  $\text{HCl}\cdot\text{L}^{-1}$ . Sr was separated with cation exchange resin and 2.5 mol  $\text{HCl}\cdot\text{L}^{-1}$ , with total process blanks  $\leq 45\ \text{pg}$ . Rb was also collected for select samples to determine Rb/Sr ratios (Table 1). Purified Sr solutions were dried, loaded onto rhenium filaments (with  $\text{Ta}_2\text{O}_5$ , phosphoric acid), and analyzed using a VG Sector 54 (TIMS) in dynamic mode with  $^{87}\text{Sr}/^{86}\text{Sr}$  normalized to 0.1194.

### Statistical analysis

Accuracy of LA-MC-ICPMS is defined as differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  between LA-MC-ICPMS and TIMS and is evaluated using a two-way analysis of variance (ANOVA) with river blocked as a random variable and instrument type as fixed. Variance distributions between LA-MC-ICPMS and TIMS are tested using a two-sample variance test. External precision is estimated as 2SD for each river group. Differences in Sr concentrations among rivers and the marine carbonate reference are quantified for a subset of otoliths by isotope dilution on TIMS and analyzed using an ANOVA with fixed factors.

## Results and discussion

Our analytical methodology produces accurate  $^{87}\text{Sr}/^{86}\text{Sr}$  values using LA-MC-ICPMS. Paired LA-MC-ICPMS and TIMS analyses show no significant differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  for instrument type (ANOVA,  $F_{[1,1]} = 0.59$ ,  $p = 0.58$ ; Table 1). In addition, the nonsignificant interaction between river (random) and instrument type indicates the accuracy of the technique can be generalized across rivers (Table 1). LA-MC-ICPMS  $^{87}\text{Sr}/^{86}\text{Sr}$  means for Feather and Mokelumne

rivers are  $0.70615 \pm 0.00006$  (2SD) and  $0.70690 \pm 0.00009$  (2SD), respectively, while purified TIMS averaged  $0.70615 \pm 0.00007$  (2SD) and  $0.70686 \pm 0.00005$  (2SD), respectively. Similarly, there are no significant differences in variances between the two instrument types (variance test,  $F_{[12,12]} = 1.11$ ,  $p = 0.85$ ).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from LA-MC-ICPMS and TIMS for Feather River are identical and overlap water values ( $0.70620 \pm 0.0002$  (2SD),  $t$  test,  $t_{10} = 0.98$ ,  $p = 0.35$ ; Ingram and Weber 1999). Together, nonsignificant differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  means or variances between instruments and agreement between LA-MC-ICPMS and water values demonstrate that the analysis produces accurate results and thus is of utility for otolith studies.

External precisions of LA-MC-ICPMS are  $\pm 0.00006$  and  $\pm 0.00009$  for otoliths from Feather and Mokelumne rivers, respectively, and  $\pm 0.00003$  for the marine carbonate reference (Fig. 1). Strontium concentrations differ from Feather River (311 ppm) and Mokelumne River (842 ppm) otoliths and from marine carbonate reference (1200 ppm; ANOVA,  $F_{[2,8]} = 11.4$ ,  $p = 0.005$ ), indicating that external precision does not vary significantly in freshwater otoliths that range in Sr concentration from 300 to 800 ppm. Individual otoliths analyzed by TIMS and LA-MC-ICPMS average within-run precisions (2SE) of  $\pm 0.00002$  and  $\pm 0.00004$ , respectively.

Measurements of Sr isotope ratios in marine carbonate references using other analytical instruments have resulted in significantly poorer precisions. Analyses of  $^{87}\text{Sr}/^{86}\text{Sr}$  on single collector LA-ICPMS result in lower precisions (0.001) due to limitations of nonsimultaneous collection of multiple isotopes and potential uncorrected isobaric interferences (Thorrold and Shuttleworth 2000). In contrast, secondary ion mass spectrometry (SIMS) analyses can achieve impressive spatial resolution ( $\sim 2\ \mu\text{m}$ ), but low precisions (0.001), largely due to lower analyte intensities and the presence of additional interferents (e.g., calcium dimers; Weber et al. 2004).

### Conclusions

We successfully obtain in situ Sr isotope ratios in otoliths with high accuracy, within-run precisions ( $\pm 0.00004$ ), external precisions (0.00003–0.00009), and spatial resolution (50  $\mu\text{m}$ ) using LA-MC-ICPMS. Our technique achieves external precision 10-fold better than previous in situ techniques on similar samples, approaching TIMS values. For many ecological systems, natural variability is greater than measurement precisions. This suggests that Sr isotope TIMS analyses may be more precise than necessary and the increased sampling efficiency of LA-MC-ICPMS (300 samples in 10 days) is an important benefit, making many applications to fisheries science now feasible (Barnett-Johnson 2004). Use of multi-elemental concentrations in freshwater fish ecology is in-

creasing, yet because of higher within-river variations and temporal variability in elemental concentrations observed in aquatic systems, Sr isotopes may prove to be more robust markers, depending on respective underlying geology (Bacon et al. 2004). Now accurate and precise  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios can be used independently or in combination with multi-elemental concentrations to characterize spatial structure in stocks, movement of fish among freshwater sources, or timing of movement between freshwater and marine environments for a variety of fishes.

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