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$^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio analysis by laser ablation MC-ICP-MS in scales, spines, and fin rays as a non-lethal alternative to otoliths for reconstructing fish life history

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1 **$^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio analysis by laser ablation MC-ICP-MS in scales, spines, and fin**
2 **rays as a non-lethal alternative to otoliths for reconstructing fish life history**

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17 Abstract

18 Strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) in otoliths are a well-established tool to determine origins
19 and movement patterns of fish. However, otolith extraction requires sacrificing fish, and
20 when working with protected or endangered species the use non-lethal samples such as
21 scales, spines, and fin rays, is preferred. Unlike otoliths that are predominantly aragonite,
22 these tissues are comprised of biological apatite. Laser ablation multi-collector inductively
23 coupled plasma mass spectrometry (LA-MC-ICP-MS) analysis of biological apatite can
24 induce significant interference on mass 87, causing inaccurate $^{87}\text{Sr}/^{86}\text{Sr}$ measurements. To
25 quantify this interference, we applied LA-MC-ICP-MS to three marine samples (white
26 seabass, *Atractoscion nobilis*, otolith; green sturgeon, *Acipenser medirostris*, pectoral fin ray;
27 salmon shark, *Lamna ditropis*, tooth) and freshwater walleye (*Sander vitreus*) otoliths,
28 scales, and spines. Instrument conditions that maximize signal intensity resulted in elevated
29 $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in the bioapatite samples, related to a polyatomic interference
30 ($^{40}\text{Ca}^{31}\text{P}^{16}\text{O}$, $^{40}\text{Ar}^{31}\text{P}^{16}\text{O}$). Retuning instrument conditions to reduce oxide levels removed this
31 interference, resulting in accurate $^{87}\text{Sr}/^{86}\text{Sr}$ ratios across all tissue samples. This method
32 provides a novel, non-lethal alternative to otolith analysis to reconstruct fish life histories.

33 Introduction

34 Understanding the origins, movement patterns, and habitat changes of fish is a
35 fundamental question in fish ecology. Measuring strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) in
36 otoliths using *in situ* laser ablation multi-collector inductively coupled plasma mass
37 spectrometry (LA-MC-ICP-MS) techniques is a well-established tool used to retrospectively
38 determine fine scale provenance and migration history (e.g., Barnett-Johnson et al. 2005;
39 Hobbs et al. 2005, 2010; Sturrock et al. 2015; Brennan et al. 2015; Chase et al. 2015). The
40 application of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in such studies are useful because $^{87}\text{Sr}/^{86}\text{Sr}$ isotope
41 ratios of water reflect the composition and age of the underlying geology and thus may vary
42 between tributaries or across a watershed, and are consistent over ecological timescales
43 (Capo et al. 1998, English et al. 2000, Hegg et al. 2013). Otoliths are comprised of daily
44 accretions of calcium carbonate and protein, and incorporate strontium, primarily from the
45 surrounding water, with some contribution from food (Farrell and Campana 1996; Walther
46 and Thorrold 2006; Brown and Severin 2009). Otoliths are ideal for investigating the
47 relationship between water and tissue geochemistry because, once otolith material is
48 deposited it becomes inert and thus is generally not resorbed or altered (Campana and
49 Neilson 1985; Campana and Thorrold 2001). Additionally, there is no significant strontium
50 isotopic fractionation between the water and otolith (Graustein 1989; Blum et al. 2000)
51 Potential effects of the fractionation of the $^{88}\text{Sr}/^{86}\text{Sr}$ isotope ratio (Fietzke and Eisenhauer
52 2006) are corrected for during the MC-ICP-MS analysis by internal normalization. The major
53 drawback to the widespread use of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ isotope analysis is that it requires fish to
54 be sacrificed for otolith extraction. This approach is typically not an option for fish species or
55 populations that are of conservation concern and require non-lethal sampling methods.

56 Alternative fish tissue samples such as scales, spines and fin rays also provide
57 valuable geochemical information that can be utilized to determine movement in fishes

(Wells et al. 2000, 2003; Muhlfeld et al. 2005; Clarke et al. 2007). They are particularly useful either as a non-lethal alternative to otoliths or for fish species that do not exhibit well developed otoliths, such as cartilaginous fishes (Muhlfeld et al. 2005, Clarke et al. 2007, Allen et al. 2009, Wolff et al. 2013, Altenritter et al. 2015). Because these tissues exhibit an internally layered structure that corresponds to time, similar to otoliths, they have long been used for age determinations of fish (Erickson 1983). Several studies have demonstrated that elemental concentrations in fish scales (Sr, Ba, Cd) are incorporated in proportion to concentrations found in surrounding waters where fish reside (Wells et al. 2000, 2003, Clarke et al. 2007, Allen et al. 2009). However, it is currently not well understood if these tissues record the same life history that $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic compositions in otolith do. Additionally, it is not well understood if they are inert and resist chemical exchange and resorption after formation.

In situ LA-MC-ICP-MS has become the method of choice for analyzing $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in otoliths. Sampling spatial resolution capabilities down to 20-100 μm and high precision (<100 ppm) measurements can be used to investigate changes in $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic compositions over the lifetime of a fish at high temporal resolution (Barnett-Johnson et al. 2005). However, before alternative fish tissue samples can reliably be used to make inferences into natal origins and reconstruct life history movement patterns, it is important to first determine if these samples can be analyzed to the same accuracy and precision as otoliths.

In otoliths, strontium is bound within the aragonitic calcium carbonate lattice, where it replaces calcium (Campana 1999, Doubleday et al. 2014). Scales, spines and fin rays do not consist of aragonite, but instead are comprised of biological apatite containing among other elements, phosphorus and rubidium; two elements that, if present, can cause interference when measuring strontium isotope ratios. *In situ* laser ablation studies of biological apatite

83 and similar minerals have demonstrated potential polyatomic interferences, likely stemming
84 from significant molecular-formation; i.e., $^{40}\text{Ca}^{31}\text{P}^{16}\text{O}$, or $^{40}\text{Ar}^{31}\text{P}^{16}\text{O}$, overlapping with ^{87}Sr
85 (Woodhead et al. 2005, Paton et al. 2007, Vroon et al. 2008, Simonetti et al. 2008, Horstwood
86 et al. 2008, Lewis et al. 2014). This interference is particularly problematic, because medium
87 or high mass resolution measurements, which could potentially resolve the interference,
88 commonly do not yield sufficient signal intensity at the spatial resolution needed (10-100
89 μm) for this type of analysis. It is hypothesized that this interference is correlated with the
90 P:Sr composition of the sample material, but also depends on the instrument conditions,
91 specifically with the sampling depth of the inductively coupled plasma. It has been
92 demonstrated that the occurrence of these polyatomic interferences during the analysis of
93 bioapatite teeth can be minimized by reducing the oxide levels (Foster and Vance 2006; de
94 Jong et al. 2007; Lewis et al. 2014).

95 The purpose of this study was to demonstrate the potential for bias when using *in situ*
96 laser ablation MC-ICP-MS of fish bioapatite (fin ray, spine, and scale) for $^{87}\text{Sr}/^{86}\text{Sr}$ isotope
97 ratio analysis and to provide a simple method to minimize this effect. Accurate reconstruction
98 of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios from fin rays, spines, and scales could provide a potentially less
99 harmful microchemical approach, serving conservation needs of endangered species of fish
100 and fisheries. To achieve this, we first investigated $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio profiles of three
101 ocean-caught samples we use as reference materials at the UC Davis Interdisciplinary Center
102 for Plasma Mass Spectrometry, including a white seabass otolith (*Atractosteomys nobilis*), a
103 salmon shark tooth (*Lamna ditropis*), and the marine part of a green sturgeon (*Acipenser*
104 *medirostris*) pectoral fin ray. The samples were analyzed under instrument tuning conditions
105 optimized for high precision (± 0.0001) $^{87}\text{Sr}/^{86}\text{Sr}$ measurements in calcium carbonate
106 materials including the white seabass otolith, and optimized tuning conditions to minimize
107 oxide levels. We chose marine origin samples as reference materials because the global mean

108 $^{87}\text{Sr}/^{86}\text{Sr}$ isotope composition (0.70918) is well established and has been shown to be spatially
109 homogenous (Hodell et al. 1990; McArthur et al. 2001). The improved tuning conditions of
110 the instrument were then applied to $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio analysis of 10 paired otolith, scales,
111 and spines of walleye (*Sander vitreus*) collected from Boysen Reservoir, Wyoming, to further
112 validate the application of this method to a field setting where natural background $^{87}\text{Sr}/^{86}\text{Sr}$
113 isotope ratios reflect freshwaters.

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Materials and Methods

Marine sample collection and preparation

A green sturgeon (*Acipenser medirostris*) pectoral fin ray was collected by the Yurok Tribe gill-net fishery on the Klamath River between March and July of 2000 on the Yurok Tribe reservation (river km 72) as part of study on the reproductive condition by Van Eenennaam et al. (2006). Only the portion of the pectoral fin ray that represented ocean residency, as previously identified using Sr/Ca ratios by Allen et al., (2009), was used in this experiment. The salmon shark (*Lamna ditropis*) tooth was collected from a salvaged carcass on Salmon Creek beach by Joe Newman, assistant curator at the Bodega Marine Laboratory, UC Davis in December of 2011 and the white seabass (*Atractoscion nobilis*) otolith was collected by the Hubbs SeaWorld Research Institute of Southern California. The samples were mounted in 1/4" diameter brass cylinders with Hillquist AB epoxy. The samples were then ground with a 600 grit diamond wheel and lapped with 3 µm alumina/water slurry on a glass plate. The sturgeon fin ray and shark tooth were polished with Buehler MicroPolish 1.0 µm alumina suspension on Buehler TexMet cloth for 2 minutes and then for 90 seconds using Buehler Micropolish 0.3 µm alumina suspension on Buehler Microcloth. The seabass otolith was polished with Buehler Micropolish 0.05 µm alumina suspension on silk cloth for 3 minutes.

Walleye sample collection and preparation

Walleye (*Sander vitreus*) otolith, spine, and scale samples were collected from Boysen Reservoir, Wyoming by the Wyoming Game and Fish Department as part of annual population monitoring. Tissues were collected by the agency for age and growth analysis. The unused tissues of 10 individual fish were acquired for $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic analysis to compare values across otoliths, scales and spines from the same individual. Sagittal otolith, spines, and scales were placed into individual 1.5 ml vials with ultrapure deionized water

(Milli-Q, 18.2 MOhm/cm³) water and cleaned using an ultrasonic water bath for 15 minutes to remove excess organic tissue. The samples were rinsed again with ultrapure water, placed in new 1.5 ml vials and allowed to dry in a class 100 laminar-flow hood. After drying for 48 h, scales were mounted on petrographic slides using double sided tape ready for laser ablation analysis.

Otoliths were embedded in epoxy and allowed to cure for 48 hours. The core of the otolith was identified and marked using a dissecting microscope and a fine point permanent marker. Two transverse cuts were made on either side of the core using a low speed saw (IsoMet, Buehler) fitted with a 12.7 cm wafering blade to create a thin section that exposed the core and growth rings extending to the edge of the otolith. Sections were then sanded using a MTI Corporation UNIPOL 1210 grinding/polishing machine with 1500 grit sand paper wetted with ultrapure water, dried for 48 hours, and then mounted to petrographic slides for laser ablation analysis (Thorrold and Shuttleworth 2000, Hobbs et al. 2010).

Spines underwent an extra cleaning cycle after drying using a sharp blade to remove skin tissue which adheres tightly to the fish spines. They were then set vertically using a thin layer of modeling clay, centered in a 1 cm diameter tube, and embedded in epoxy. Note, that the inside walls of tube must be first treated with a thin layer of mineral oil so that the epoxy will release when hardening is complete. After 48 hours, two transverse cuts were made within 2-3 mm of the base of the spine using a low speed saw (IsoMet, Buehler) fitted with a 12.7 cm wafering blade to create a thin section. Sections were then sanded using a MTI Corporation UNIPOL 1210 grinding/polishing machine with 1500 grit sand paper wetted with ultrapure water, dried for 24 hours and then mounted to petrographic slides for laser ablation analysis (Thorrold and Shuttleworth 2000, Hobbs et al. 2010).

Laser ablation MC-ICP-MS analysis

$^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios were measured at the University of California-Davis Interdisciplinary Center for Plasma Mass Spectrometry. For the *in situ* Sr isotope analysis a Nd:YAG 213 nm laser (New Wave Research UP213) was coupled to a Nu Plasma HR MC-ICP-MS (Nu032). A 55 μm spot laser beam was traversed across the sample surface at a scan rate of 5 $\mu\text{m}/\text{s}$. This track was repeated at three different locations within each sample to assess spatial homogeneity. The operating conditions of the instrument are summarized in Table 1. Different methods have been used to account and correct for the different interferences and mass bias present when analyzing strontium isotopes using LA-MC-ICP-MS (Vroon et al. 2008; Lewis et al. 2014). We normalized the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio for instrumental mass discrimination by monitoring the $^{86}\text{Sr}/^{88}\text{Sr}$ isotope ratio (assuming $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$), and ^{87}Rb was corrected by monitoring the ^{85}Rb signal. The Rb correction is considered robust for samples with low $^{85}\text{Rb}/^{88}\text{Sr}$ ratios (<0.002), which included all samples in this study. For samples with higher Rb concentration alternative interference correction protocols should be used (Müller and Anczkiewicz 2016). Krypton interference originating in the argon supply (^{86}Kr) was subtracted using the on peak zero method before each analysis. Kr contribution was monitored throughout the analyses as increasing amounts of Kr would lead to an increased uncertainty of the individual measurements. Artifact caused by hypothesized polyatomic interference was investigated by measuring strontium oxide and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios through several instrument configurations producing different oxide levels (Table 2). Oxide levels were monitored on mass 104 ($^{88}\text{Sr}+^{16}\text{O}$) using a discrete dynode electron multiplier detector (IC0), because signal intensity was too low for accurate faraday cup collection ($<1\text{mV}$). Accuracy and precision of the tuning was evaluated by monitoring the white seabass otolith and comparing the results to the modern $^{87}\text{Sr}/^{86}\text{Sr}$ isotope value of seawater, 0.70918 (McArthur et al. 2001).

Water sample collection and analysis

Water samples were collected in pre cleaned polypropylene vials in duplicate from five locations within Boysen Reservoir, Wyoming (Fig. 1) using a standard 10 ml syringe and 0.2 μm pore filters (WhatmanTM Puradisk) and transported to a class 100 clean room facility for processing. An aliquot of each water sample was made at a volume totaling approximately 1 μg of Sr. This volume was evaporated to dryness in an acid leached PTFE (Teflon) vial on a hotplate, and strontium was isolated from all other aqueous constituents by selective ion exchange chromatography (Horwitz et al. 1992). Water sample $^{87}\text{Sr}/^{86}\text{Sr}$ isotope analysis was conducted at the University of California-Davis Interdisciplinary Center for Plasma Mass Spectrometry. Sr separates were reconstituted in 2% HNO_3 and introduced in the MC-ICP-MS (Nu Plasma HR) using a desolvating nebulizer introduction system (Nu Instruments DSN-100). Procedural blank levels were measured and contributed $<0.002\%$ of total Sr per sample. Replicate analyses of the National institute of standards and technology (NIST) Sr carbonate standard reference material SRM987 were conducted every six samples to monitor and correct for instrument drift over the course of the day and for analytical artifact among sessions. Standard sample bracketing was used to normalize the SRM987 measurements to an $^{87}\text{Sr}/^{86}\text{Sr}$ isotope value of 0.710249 (e.g., Marzoli et al. 2014). An in-house modern marine coral standard from the South China Sea was processed in parallel with water samples and resulted in $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.70917 ± 0.00003 ($n=22$, 2σ) in good agreement with the modern $^{87}\text{Sr}/^{86}\text{Sr}$ isotope value of seawater (McArthur et al. 2001).

Data analysis

The data from all laser tracks measured for each sample were first checked for spatial homogeneity and then averaged to create a single average value for the sample. The mean $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of the different tissue samples from the same sample at different instrument tuning conditions were statistically compared by paired t-tests using R (R Core

212 Team 2013). For the time resolved analysis a LOESS smooth was applied using the ggplot2
213 package (Wickham 2009) in R.

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Results

Marine samples

The marine samples (white seabass otolith, salmon shark tooth, green sturgeon pectoral fin ray) were measured using different instrument tuning conditions. Observed oxide levels (measured as $^{88}\text{Sr}^{16}\text{O}/^{88}\text{Sr}$) varied significantly and were most dependent on the plasma sampling depth, or distance from the load coil to the sampler cone. As the plasma sampling depth was increased to 2.5 mm the oxide levels decreased three fold and remained stable around 0.0004%, before a small increase to 0.0005% related to the significant loss of ^{88}Sr signal intensity at these tuning conditions (Fig. 2, Table 2). The corresponding $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of the white seabass otolith remained stable at 0.70919 ± 0.00005 ($n=15$, 2σ) for the different instrument conditions used.

In contrast, the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of the salmon shark tooth and green sturgeon pectoral fin ray varied significantly at different plasma sampling depths. At shallower plasma sampling depths coinciding with greater oxide transmission, the obtained $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of up to 0.70953 for the salmon shark tooth, and 0.70954 for the green sturgeon pectoral fin ray, were significantly higher than the average seawater $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio of 0.70918 (McArthur et al. 2001). These values are also significantly higher than the previously obtained $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in this laboratory by drilling and traditional solution MC-ICP-MS analysis of 0.70918 ± 0.00009 ($n=2$, 2σ) for the salmon shark tooth and 0.70917 ± 0.00003 ($n=5$, 2σ) for the green sturgeon pectoral fin ray. Increasing the plasma sampling depth led to a decrease in oxide levels to $\sim 0.0004\%$ ($^{88}\text{Sr}^{16}\text{O}/^{88}\text{Sr}$). At these oxide levels we find a good agreement between the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio of the biapatite samples and seawater with an average offset of the salmon shark tooth of $43 \text{ ppm} \pm 66 \text{ ppm}$ ($n=20$, 2σ) and $55 \text{ ppm} \pm 85 \text{ ppm}$ ($n=11$, 2σ) for the green sturgeon pectoral fin ray (Fig. 2). This is within the accuracy and precision achieved when measuring the white seabass otolith, 39

ppm $\pm$ 65 ppm ($n=12$, 2σ), and within the long term analytical reproducibility of strontium isotopic measurements by *in situ* LA-MC-ICP-MS at the UC Davis Interdisciplinary Center for ICPMS.

Walleye otolith, spines and scales from Boysen Reservoir

The $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of water samples collected from Boysen Reservoir (0.71094 ± 0.00012 , $n=5$, 2σ) were spatially homogeneous, with the exception of Poison Creek Bay, which was lower than other sites (0.71083 ± 0.00006 , $n=1$, 2σ). A time resolved analysis of one walleye sample (Fish sample ID 11) showed a temporally homogenous profile of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios with no significant changes over the lifetime of the fish (Fig. 3A). The average $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of all walleye otolith (0.71093 ± 0.00018 , $n=10$, 2σ) are in agreement with the water values of Boysen Reservoir. Changes in plasma sampling depth had no significant effect on the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of the walleye otolith 32 ± 53 ppm ($n=10$, 2σ), paired t-test ($t_9=1.19$, $p=0.089$).

Similar to bioapatite marine samples (shark tooth and green sturgeon pectoral fin ray) the average apparent $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of spines (0.71166 ± 0.00019 , $n=10$, 2σ) and scales (0.71151 ± 0.00025 , $n=10$, 2σ) were systematically higher than otoliths from the same individual at instrument conditions of greater oxide transmission and shallower plasma sampling depth (Fig. 4A). This trend is also observed in the time resolved analysis of the single walleye sample (Fish sample ID 11), Figure 3B, C. Spines and scales showed an average relative offset of 1030 ppm and 820 ppm, respectively, compared to their corresponding otolith, far outside of the typical analytical uncertainty of LA-MC-ICP-MS of 100 ppm (Table 3). During instrument conditions tuned for reduced oxide levels, the average difference from the otolith was reduced to $86 \text{ ppm} \pm 99 \text{ ppm}$ ($n=10$, 2σ) for spines and $65 \text{ ppm} \pm 78 \text{ ppm}$ ($n=10$, 2σ) for scales (Fig. 4B). This is an improvement by an order of magnitude and has led to a significant shift in the mean $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios for spines

264 (paired t-test, $t_9=21.63$, $p<0.0005$) and scales (paired t-test, $t_9=13.45$, $p<0.0005$). At
265 instrument conditions that minimize oxide transmission no significant differences were
266 observed for the different tissues from an individual fish.

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Discussion

Over the last two decades, technological advances and increased accessibility to instrumentation such as LA-MC-ICP-MS coupled with the reduced time in obtaining $^{87}\text{Sr}/^{86}\text{Sr}$ values has resulted in a rapid increase in the demand and application of LA-MC-ICP-MS to studies in fisheries ecology and management. Studies involving provenance and reconstruction of life history movement patterns using otolith samples from medium and larger bodied fish have been the most widespread (Kennedy et al. 2002; Barnett-Johnson et al. 2010; Hobbs et al. 2010; Sousa et al. 2016), but applications using small bodied fish are increasing (Chase et al. 2015). As with any rapid expansion in a new methodology, limitations and the need for increased applicability to alternative approaches are quickly realized, and microchemical analysis on alternative tissue samples (i.e. scales, fin rays, dorsal spines), that do not require the sacrifice of the study organism to obtain life history information, are increasingly in demand (Allen et al. 2009; Smith and Whitedge 2010; Wolff et al. 2013; Kerr and Campana 2014). Unfortunately, the demand for $^{87}\text{Sr}/^{86}\text{Sr}$ analysis has grown faster than our ability to test, evaluate and understand its applicability across tissue types differing widely in their structural compositions and chemical characteristics.

This study provides the first direct evidence that *in situ* $^{87}\text{Sr}/^{86}\text{Sr}$ isotope analysis of scales, spines and fin rays, like other bioapatite samples, is affected by significant polyatomic interference on ^{87}Sr . Monitoring, and if present, minimizing this interference is paramount when measuring fish bioapatite tissue samples. If uncorrected, this interference leads to elevated $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in these tissue samples, compared to otolith or water samples, which would render these materials unsuitable as archives for life history reconstruction.

Different measures can be taken to address this interference (Foster and Vance 2006, de Jong et al. 2007, Lewis et al. 2014). Here, we found that a significant reduction in oxide

levels can be achieved by increasing the plasma sampling depth in the MC-ICP-MS. However, sample gas flow pressure and RF power to the plasma may also influence oxide transmission (Horlick et al. 1985; Park and Lee 1991; Uchida and Ito 1994). Absolute instrument tuning conditions vary between different laboratories, instrument setups and analytical sessions. We found that tuning the instrument for maximum signal intensity and stability, and then systematically increasing the torch depth while compensating for symmetric focusing of the mass spectrometer was effective in reducing the oxide levels, resulting in accurate $^{87}\text{Sr}/^{86}\text{Sr}$ results for bioapatite tissue samples. Reducing sample gas flow rates or laser cell gas flow rates can also reduce oxide transmission. However, this can be problematic because it will also effect the laser cell interface with the He and Ar gas flow and ICP, which complicates the tuning.

The effect that this tuning of instrument conditions has on the obtained results is best demonstrated by comparing the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of white seabass otolith, green sturgeon pectoral fin ray and salmon shark tooth (Fig. 2) to the well-established average marine $^{87}\text{Sr}/^{86}\text{Sr}$ ratio (McArthur et al. 2001) obtained using different instrument conditions. These samples were chosen to develop this method because any observed deviation from the average marine $^{87}\text{Sr}/^{86}\text{Sr}$ ratio can be attributed directly to the polyatomic interference. The $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of the white seabass otolith did not vary at different instrument tuning conditions, because the otolith consists of aragonite and does not suffer from this polyatomic interference. In contrast, the salmon shark tooth and green sturgeon fin ray showed significant variation in their $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios, with elevated values and high oxide levels at low plasma sampling depth, and with their expected average marine water $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios and low oxide levels at higher plasma sampling depths. This demonstrates that tuning the instrument to reduce oxide levels is successful in significantly reducing the polyatomic interference, and provides accurate $^{87}\text{Sr}/^{86}\text{Sr}$ results. However, tuning for reduced

oxide levels is accompanied by a significant loss in signal intensity of ~25-40%, meaning that the Sr concentration of the sample may limit this application.

The tissue samples of walleye from Boysen Reservoir provided a case study to further evaluate this method by applying it to freshwater fish samples. The spatial and temporal homogeneity in $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios between the water and otolith values (Fig. 3, Fig. 4) indicates that any variation observed in the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in scales and spines is not related to fish movement but rather due to analytical interference.

The scales and spines showed elevated apparent $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios when the instrument was tuned for maximum signal intensity and low plasma sampling depth (Fig. 4A), but showed similar values to the otolith once the instrument was tuned to reduced oxide levels (Fig. 4B). Based on the low variability of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in Boysen Reservoir the elevated $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios for spines and scales would have led to the misclassification of these fish to different locations outside of Boysen Reservoir. The average offset in $^{87}\text{Sr}/^{86}\text{Sr}$ achieved using tuning conditions that minimize the oxide levels are practically indistinguishable from the otolith and water values and well within the natural variability of the Boysen Reservoir $^{87}\text{Sr}/^{86}\text{Sr}$ isotope values and thus show that these fish are in fact lifelong residents of this watershed.

This study has demonstrated that care must be taken when applying *in situ* $^{87}\text{Sr}/^{86}\text{Sr}$ isotope analysis to fish bioapatite tissue samples. Monitoring, and if necessary, tuning to reduce the polyatomic interference, makes it possible to achieve similar analytical accuracy as *in situ* otolith analysis. In contrast to otolith, that are generally considered chemically inert (Campana 1999), it remains to be investigated, if these non-lethal tissues record the same life history information. Processes such as ossification and potential resorption in fin rays and spines (Veinott and Evans 1999; Gillanders 2001), and the loss and regeneration of scales

340 (Campana and Thorrold 2001) point towards potential complexities in the recorded $^{87}\text{Sr}/^{86}\text{Sr}$
341 isotope profiles that need to be considered before this tool can be broadly applied to answer
342 ecological questions.

343 The successful removal of this polyatomic interference, as presented in this study,
344 builds the foundation to validate if these non-lethal tissues record the same life history
345 information as otolith in different fish species. This will provide a powerful new tool for fish
346 ecologists to reconstruct life histories for threatened or endangered fish species where otolith
347 extraction is not a viable option.

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References

- Allen, P.J., Hobbs, J.A., Cech, J.J., Van Eenennaam, J.P., and Doroshov, S.I. 2009. Using Trace Elements in Pectoral Fin Rays to Assess Life History Movements in Sturgeon: Estimating Age at Initial Seawater Entry in Klamath River Green Sturgeon. *Trans. Am. Fish. Soc.* **138**(2): 240–250. doi:10.1577/T08-061.1.
- Altenritter, M.E., Kinnison, M.T., Zydlewski, G.B., Secor, D.H., and Zydlewski, J.D. 2015. Assessing dorsal scute microchemistry for reconstruction of shortnose sturgeon life histories. *Environ. Biol. Fishes* **98**(12): 2321–2335. doi:10.1007/s10641-015-0438-9.
- Barnett-Johnson, R., Ramos, F.C., Grimes, C.B., and MacFarlane, R.B. 2005. Validation of Sr isotopes in otoliths by laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS): opening avenues in fisheries science applications. *Can. J. Fish. Aquat. Sci.* **62**(11): 2425–2430. doi:10.1139/f05-194.
- Barnett-Johnson, R., Teel, D.J., and Casillas, E. 2010. Genetic and otolith isotopic markers identify salmon populations in the Columbia River at broad and fine geographic scales. *Environ. Biol. Fishes* **89**(3-4): 533–546. doi:10.1007/s10641-010-9662-5.
- Blum, J.D., Taliaferro, E.H., Weisse, M.T., and Holmes, R.T. 2000. Changes in Sr/Ca, Ba/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios between trophic levels in two forest ecosystems in the northeastern U.S.A. *Biogeochemistry* **49**(1): 87–101. doi:10.1023/A:1006390707989.
- Brennan, S.R., Zimmerman, C.E., Fernandez, D.P., Cerling, T.E., McPhee, M. V., and Wooller, M.J. 2015. Strontium isotopes delineate fine-scale natal origins and migration histories of Pacific salmon. *Sci. Adv.* **1**(4): e1400124–e1400124. doi:10.1126/sciadv.1400124.
- Brown, R.J., and Severin, K.P. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Can. J. Fish. Aquat. Sci.* **66**(10): 1790–1808. doi:10.1139/F09-112.
- Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser.* **188**: 263–297. doi:10.3354/meps188263.
- Campana, S.E., and Neilson, J.D. 1985. Microstructure of Fish Otoliths. *Can. J. Fish. Aquat. Sci.* **42**(5): 1014–1032. doi:10.1139/f85-127.
- Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.* **58**(1): 30–38. doi:10.1139/f00-177.
- Capo, R.C., Stewart, B.W., and Chadwick, O.A. 1998. Strontium isotopes as tracers of ecosystem processes: theory and methods. *Geoderma* **82**(1-3): 197–225. doi:10.1016/S0016-7061(97)00102-X.
- Chase, N.M., Caldwell, C.A., Carleton, S.A., Gould, W.R., and Hobbs, J.A. 2015. Movement patterns and dispersal potential of Pecos bluntnose shiner (*Notropis simus pecosensis*) revealed using otolith microchemistry. *Can. J. Fish. Aquat. Sci.* **72**(10): 1575–1583. National Research Council of Canada. doi:10.1139/cjfas-2014-0574.
- Clarke, A.D., Telmer, K.H., and Shrimpton, J.M. 2007. Elemental analysis of otoliths, fin rays and scales: a comparison of bony structures to provide population and life-history

- 395 information for the Arctic grayling (*Thymallus arcticus*). *Ecol. Freshw. Fish* **16**(3): 354–
396 361. doi:10.1111/j.1600-0633.2007.00232.x.
- 397 Doubleday, Z.A., Harris, H.H., Izzo, C., and Gillanders, B.M. 2014. Strontium Randomly
398 Substituting for Calcium in Fish Otolith Aragonite. *Anal. Chem.* **86**(1): 865–869.
399 doi:10.1021/ac4034278.
- 400 Van Eenennaam, J.P., Linares, J., Doroshov, S.I., Hillemeier, D.C., Willson, T.E., and Nova,
401 A.A. 2006. Reproductive Conditions of the Klamath River Green Sturgeon. *Trans. Am.*
402 *Fish. Soc.* **135**(1): 151–163. doi:10.1577/T05-030.1.
- 403 English, N.B., Quade, J., DeCelles, P.G., and Garziona, C.N. 2000. Geologic control of Sr
404 and major element chemistry in Himalayan Rivers, Nepal. *Geochim. Cosmochim. Acta*
405 **64**(15): 2549–2566. doi:10.1016/S0016-7037(00)00379-3.
- 406 Erickson, C.M. 1983. Age Determination of Manitoban Walleyes Using Otoliths, Dorsal
407 Spines, and Scales. *North Am. J. Fish. Manag.* **3**(2): 176–181.
- 408 Farrell, J., and Campana, S.E. 1996. Regulation of calcium and strontium deposition on the
409 otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comp. Biochem. Physiol. Part A*
410 *Physiol.* **115**(2): 103–109. doi:10.1016/0300-9629(96)00015-1.
- 411 Fietzke, J., and Eisenhauer, A. 2006. Determination of temperature-dependent stable
412 strontium isotope ($^{88}\text{Sr}/^{86}\text{Sr}$) fractionation via bracketing standard MC-ICP-MS.
413 *Geochemistry, Geophys. Geosystems* **7**(8). doi:10.1029/2006GC001243.
- 414 Foster, G.L., and Vance, D. 2006. In situ Nd isotopic analysis of geological materials by laser
415 ablation MC-ICP-MS. *J. Anal. At. Spectrom.* **21**(3): 288. The Royal Society of
416 Chemistry. doi:10.1039/b513945g.
- 417 Gillanders, B.M. 2001. Trace metals in four structures of fish and their use for estimates of
418 stock structure. *Fish. Bull.* **99**(3): 410–419.
- 419 Graustein, W.C. 1989. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios measure the sources and flow of strontium in
420 terrestrial ecosystems. *In* *Stable isotopes in ecological research*. Springer, New York. pp.
421 491–512.
- 422 Hegg, J.C., Kennedy, B.P., and Fremier, A.K. 2013. Predicting strontium isotope variation
423 and fish location with bedrock geology: Understanding the effects of geologic
424 heterogeneity. *Chem. Geol.* **360–361**(2013): 89–98. Elsevier B.V.
425 doi:10.1016/j.chemgeo.2013.10.010.
- 426 Hobbs, J.A., Lewis, L.S., Ikemiyagi, N., Sommer, T., and Baxter, R.D. 2010. The use of
427 otolith strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) to identify nursery habitat for a threatened
428 estuarine fish. *Environ. Biol. Fishes* **89**(3-4): 557–569. doi:10.1007/s10641-010-9672-3.
- 429 Hobbs, J.A., Yin, Q., Burton, J., and Bennett, W.A. 2005. Retrospective determination of
430 natal habitats for an estuarine fish with otolith strontium isotope ratios. *Mar. Freshw.*
431 *Res.* **56**(5): 655. doi:10.1071/MF04136.
- 432 Hodell, D.A., Mead, G.A., and Mueller, P.A. 1990. Variation in the strontium isotopic
433 composition of seawater (8 Ma to present) : Implications for chemical weathering rates
434 and dissolved fluxes to the oceans. *Chem. Geol. Isot. Geosci. Sect.* **80**(4): 291–307.
435 doi:10.1016/0168-9622(90)90011-Z.
- 436 Horlick, G., Tan, S.H., Vaughan, M.A., and Rose, C.A. 1985. The effect of plasma operating

- parameters on analyte signals in inductively coupled plasma-mass spectrometry. *Spectrochim. Acta Part B At. Spectrosc.* **40**(10-12): 1555–1572. doi:10.1016/0584-8547(85)80178-6.
- Horstwood, M.S.A., Evans, J.A., and Montgomery, J. 2008. Determination of Sr isotopes in calcium phosphates using laser ablation inductively coupled plasma mass spectrometry and their application to archaeological tooth enamel. *Geochim. Cosmochim. Acta* **72**(23): 5659–5674. Elsevier Ltd. doi:10.1016/j.gca.2008.08.016.
- Horwitz, E.P., Chiarizia, R., and Dietz, M.L. 1992. A novel Strontium-Selective Extraction Chromatographic Resin*. *Solvent Extr. Ion Exch.* **10**(2): 313–336. doi:10.1080/07366299208918107.
- de Jong, H.N., Foster, G.L., Hawkesworth, C., and Pike, A.W.G. 2007. LA-MC-ICPMS $^{87}\text{Sr}/^{86}\text{Sr}$ on tooth enamel—pitfalls and problems. *Geochim. Cosmochim. Acta* (71): A212.
- Kennedy, B.P., Klaue, A., Blum, J.D., Folt, C.L., and Nislow, K.H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. *Can. J. Fish. Aquat. Sci.* **59**(6): 925–929. doi:10.1139/f02-070.
- Kerr, L.A., and Campana, S.E. 2014. Chemical Composition of Fish Hard Parts as a Natural Marker of Fish Stocks. *In* *Stock Identification Methods*, 2nd edition. Academic Press, San Diego. pp. 205–234. doi:10.1016/B978-0-12-397003-9.00011-4.
- Lewis, J., Coath, C.D., and Pike, A.W.G. 2014. An improved protocol for $^{87}\text{Sr}/^{86}\text{Sr}$ by laser ablation multi-collector inductively coupled plasma mass spectrometry using oxide reduction and a customised plasma interface. *Chem. Geol.* **390**: 173–181. Elsevier B.V. doi:10.1016/j.chemgeo.2014.10.021.
- Marzoli, A., Jourdan, F., Bussy, F., Chiaradia, M., and Costa, F. 2014. Petrogenesis of tholeiitic basalts from the Central Atlantic magmatic province as revealed by mineral major and trace elements and Sr isotopes. *Lithos* **188**: 44–59. Elsevier B.V. doi:10.1016/j.lithos.2013.09.013.
- McArthur, J.M., Howarth, R.J., and Bailey, T.R. 2001. Strontium Isotope Stratigraphy: LOWESS Version 3: Best Fit to the Marine Sr-Isotope Curve for 0–509 Ma and Accompanying Look-up Table for Deriving Numerical Age. *J. Geol.* **109**(2): 155–170. doi:10.1086/319243.
- Muhlfeld, C.C., Marotz, B., Thorrold, S.R., and FitzGerald, J.L. 2005. Geochemical Signatures in Scales Record Stream of Origin in Westslope Cutthroat Trout. *Trans. Am. Fish. Soc.* **134**(4): 945–959. doi:10.1577/T04-029.1.
- Müller, W., and Anczkiewicz, R. 2016. Accuracy of laser-ablation (LA)-MC-ICPMS Sr isotope analysis of (bio)apatite – a problem reassessed. *J. Anal. At. Spectrom.* **31**(1): 259–269. doi:10.1039/C5JA00311C.
- Park, C.J., and Lee, K.W. 1991. Analytical performance evaluation of a 40.68 MHz inductively coupled plasma mass spectrometer. *J. Anal. At. Spectrom.* **6**(6): 431. doi:10.1039/ja9910600431.
- Paton, C., Woodhead, J.D., Hergt, J.M., Phillips, D., and Shee, S. 2007. Strontium Isotope Analysis of Kimberlitic Groundmass Perovskite via LA-MC-ICP-MS. *Geostand. Geoanalytical Res.* (31.4): 321–330. doi:10.1111/j.1751-908X.2007.00131.x.

- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>. R Foundation for Statistical Computing, Vienna, Austria. Available from <http://www.r-project.org>.
- Simonetti, A., Buzon, M.R., and Creaser, R.A. 2008. In-situ elemental and Sr isotope investigation of human tooth enamel by Laser Ablation-(MC)-ICP-MS: Successes and pitfalls. *Archaeometry* **50**(2): 371–385. doi:10.1111/j.1475-4754.2007.00351.x.
- Smith, K.T., and Whitley, G.W. 2010. Fin Ray Chemistry as a Potential Natural Tag for Smallmouth Bass in Northern Illinois Rivers. *J. Freshw. Ecol.* **25**(4): 627–635. doi:10.1080/02705060.2010.9664412.
- Sousa, R.G.C., Humston, R., and Freitas, C.E.C. 2016. Movement patterns of adult peacock bass *Cichla temensis* between tributaries of the middle Negro River basin (Amazonas - Brazil): an otolith geochemical analysis. *Fish. Manag. Ecol.* **23**(1): 76–87. doi:10.1111/fme.12166.
- Sturrock, A.M., Wikert, J.D., Heyne, T., Mesick, C., Hubbard, A.E., Hinkelman, T.M., Weber, P.K., Whitman, G.E., Glessner, J.J., and Johnson, R.C. 2015. Reconstructing the Migratory Behavior and Long-Term Survivorship of Juvenile Chinook Salmon under Contrasting Hydrologic Regimes. *PLoS One*: 1–23. doi:10.5061/dryad.c56rk.
- Thorrold, S.R., and Shuttleworth, S. 2000. In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field inductively coupled plasma mass spectrometry. *Can. J. Fish. Aquat. Sci.* **57**(6): 1232–1242. doi:10.1139/f00-054.
- Uchida, H., and Ito, T. 1994. Comparative study of 27.12 and 40.68 MHz inductively coupled argon plasmas for mass spectrometry on the basis of analytical characteristic distributions. *J. Anal. At. Spectrom.* **9**(9): 1001. doi:10.1039/ja9940901001.
- Veinott, G.I., and Evans, R.D. 1999. An Examination of Elemental Stability in the Fin Ray of the White Sturgeon with Laser Ablation Sampling–Inductively Coupled Plasma–Mass Spectrometry (LAS-ICP-MS). *Trans. Am. Fish. Soc.* **128**(2): 352–361. doi:10.1577/1548-8659(1999)128<0352:AEOESI>2.0.CO;2.
- Vroon, P.Z., van der Wagt, B., Koornneef, J.M., and Davies, G.R. 2008. Problems in obtaining precise and accurate Sr isotope analysis from geological materials using laser ablation MC-ICPMS. *Anal. Bioanal. Chem.* **390**(2): 465–476. doi:10.1007/s00216-007-1742-9.
- Walther, B., and Thorrold, S. 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. *Mar. Ecol. Prog. Ser.* **311**: 125–130. doi:10.3354/meps311125.
- Wells, B.K., Bath, G.E., Thorrold, S.R., and Jones, C.M. 2000. Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry. *Can. J. Fish. Aquat. Sci.* **57**(10): 2122–2129. doi:10.1139/cjfas-57-10-2122.
- Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., and Jones, C.M. 2003. Relationships between Water, Otolith, and Scale Chemistries of Westslope Cutthroat Trout from the Coeur d'Alene River, Idaho: The Potential Application of Hard-Part Chemistry to Describe Movements in Freshwater. *Trans. Am. Fish. Soc.* **132**(3): 409–424. doi:10.1577/1548-8659(2003)132<0409:RBWOAS>2.0.CO;2.
- Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer Sci. Bus. Media.

- 523 Wolff, B.A., Johnson, B.M., and Landress, C.M. 2013. Classification of hatchery and wild
524 fish using natural geochemical signatures in otoliths, fin rays, and scales of an
525 endangered catostomid. *Can. J. Fish. Aquat. Sci.* **70**(12): 1775–1784. doi:10.1139/cjfas-
526 2013-0116.
- 527 Woodhead, J., Swearer, S., Hergt, J., and Maas, R. 2005. In situ Sr-isotope analysis of
528 carbonates by LA-MC-ICP-MS: interference corrections, high spatial resolution and an
529 example from otolith studies. *J. Anal. At. Spectrom.* **20**(1): 22. doi:10.1039/b412730g.
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531 **Tables**

532 **Table 1:** Instrument operating conditions of the Nu Plasma HR (Nu032) and New Wave
533 Research UP213 Nd:YAG 213 nm laser.

Instrument parameters	
Nu Plasma HR (Nu032) MC-ICP-MS	
Forward power	1300 W
Extraction voltage	6000 V
Analyzer pressure	< 5e-8 mbar
Cones	Nickel dry plasma sampler cone + high sensitivity skimmer cone
Torch depth	4-7.5 mm
Detector array	Faraday cups, 10 ¹¹ Ω resistors Ion Counter
Detector configuration	H4 (88), H2(87), Ax (86), L2 (85), IC0 (104), L3 (84), L4 (83)
Gas flows	
Coolant gas	13 l/min
Argon makeup gas	1.05 l/min
Nitrogen	0.005 l/min
Helium gas to cell	0.85 l/min
New Wave Research UP213 laser	
Nd:YAG	213 nm
Supercell	Low volume laminar flow cell
Laser fluence	~ 5.5 J/cm ²
Repetition rate	10 Hz
Spot size	55 µm
Scanning speed	5 µm/s

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Table 2: Strontium isotope ($^{87}\text{Sr}/^{86}\text{Sr}$) data for the white seabass otolith, salmon shark tooth, and green sturgeon pectoral fin ray at different oxide levels.

Torch depth [mm]	^{88}Sr [V]	White seabass otolith			Salmon shark tooth		Green sturgeon fin ray	
		Oxides ($^{88}\text{Sr}+^{16}\text{O}$: ^{88}Sr)	$^{87}\text{Sr}/^{86}\text{Sr}$	$\pm\text{se}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\pm\text{se}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\pm\text{se}$
4.45	3.64	0.0014%	0.70918	0.00003				
4.65	4.19	0.0011%	0.70919	0.00002	0.70951	0.00003	0.70954	0.00002
4.85	5.10	0.0010%	0.70923	0.00003	0.70953	0.00002		
5.05	3.90	0.0007%	0.70923	0.00003	0.70948	0.00004	0.70944	0.00002
5.25	4.05	0.0007%	0.70917	0.00002	0.70948	0.00003	0.70938	0.00002
5.45	2.23	0.0005%	0.70922	0.00002	0.70931	0.00002	0.70934	0.00003
5.65	2.37	0.0005%	0.70915	0.00004			0.70931	0.00003
5.85	2.57	0.0004%	0.70918	0.00004	0.70921	0.00002		
6.05	2.75	0.0004%	0.70920	0.00003	0.70925	0.00003	0.70920	0.00003
6.25	3.08	0.0004%	0.70921	0.00002	0.70924	0.00003		
6.45	2.48	0.0004%	0.70918	0.00004	0.70922	0.00003	0.70923	0.00002
6.65	2.69	0.0004%	0.70918	0.00003	0.70917	0.00003	0.70918	0.00002
6.85	2.06	0.0004%	0.70920	0.00003			0.70921	0.00002
7.05	1.66	0.0004%	0.70917	0.00002	0.70919	0.00004	0.70916	0.00002
7.25	1.44	0.0005%	0.70918	0.00002	0.70919	0.00003		

540 **Table 3:** Summary of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope data for the walleye samples, tuned for maximum
541 signal intensity and tuned for reduced oxide levels. ΔSpine and ΔScale are defined as the ppm
542 difference between the tissue and the corresponding otolith from the same fish.

Instrument tuned for maximum signal intensity and stability						
Fish ID	Otolith	$\pm\text{se}$	Spine	$\pm\text{se}$	Scale	$\pm\text{se}$
3	0.71081	0.00005	0.71167	0.00003	0.71157	0.00004
4	0.71096	0.00003	0.71179	0.00004	0.71175	0.00012
5	0.71087	0.00004	0.71179	0.00004	0.71159	0.00004
6	0.71103	0.00004	0.71151	0.00003	0.71136	0.00004
7	0.71090	0.00006	0.71157	0.00002	0.71131	0.00002
8	0.71083	0.00002	0.71170	0.00002	0.71151	0.00004
9	0.71105	0.00003	0.71167	0.00002	0.71154	0.00002
10	0.71104	0.00004	0.71158	0.00003	0.71157	0.00004
11	0.71090	0.00002	0.71171	0.00003	0.71149	0.00005
12	0.71087	0.00002	0.71158	0.00002	0.71141	0.00002
Instrument tuned for reduced oxide levels						
Fish ID	Otolith	$\pm\text{se}$	Spine	$\pm\text{se}$	Scale	$\pm\text{se}$
3	0.71081	0.00005	0.71078	0.00005	0.71090	0.00007
4	0.71097	0.00006	0.71094	0.00005	0.71106	0.00006
5	0.71083	0.00006	0.71097	0.00007	0.71082	0.00008
6	0.71099	0.00005	0.71094	0.00005	0.71097	0.00007
7	0.71092	0.00004	0.71093	0.00009	0.71096	0.00009
8	0.71081	0.00005	0.71095	0.00007	0.71090	0.00008
9	0.71102	0.00005	0.71098	0.00009	0.71106	0.00009
10	0.71100	0.00005	0.71094	0.00004	0.71096	0.00006
11	0.71089	0.00005	0.71094	0.00008	0.71093	0.00008
12	0.71088	0.00004	0.71094	0.00004	0.71089	0.00005
Effect of the tuning for reduced oxide levels						
Fish ID	ΔSpine uncorrected[ppm]	ΔScale uncorrected[ppm]	ΔSpine corrected [ppm]	$\pm 2\sigma$	ΔScale corrected [ppm]	$\pm 2\sigma$
3	996	757	46	84	127	4
4	1140	833	34	143	126	36
5	873	683	205	138	11	98
6	1222	949	72	44	27	61
7	945	581	14	133	51	61
8	1116	1050	204	119	131	56
9	680	465	55	46	61	104
10	1210	1062	78	99	54	62
11	1299	1014	70	73	49	130
12	757	745	85	114	16	166
Average	1024	814	86	99	65	78

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Fig. 1: The Boysen Reservoir Dam in central Wyoming was the source of walleye otoliths, scales, and spines. Water samples were collected from 5 sites within the reservoir. Samples were chosen from this location, because of homogeneous spatial and temporal strontium isotope ratios in water and otolith tissues.
279x361mm (300 x 300 DPI)

Fig. 2: Plot of the oxide levels under different instrument conditions and corresponding $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios from the white seabass otolith, salmon shark tooth and green sturgeon pectoral fin ray. Blue box represents the average $^{87}\text{Sr}/^{86}\text{Sr}$ seawater value of 0.70918 ± 0.00005 (McArthur et al. 2001). The white seabass otolith, which should not be affected by the polyatomic interference, does not exhibit any variation in its $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios at different instrument tuning conditions. In contrast, the salmon shark tooth and the green sturgeon pectoral fin ray vary significantly with highly elevated $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios at shallow plasma sampling depth. At oxide levels $\sim 0.0004\%$ measurements of both the salmon shark tooth and green sturgeon pectoral fin ray result in $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in agreement with the average $^{87}\text{Sr}/^{86}\text{Sr}$ isotope for seawater, an indication that the tuning was successful in removing the polyatomic interference.

78x51mm (300 x 300 DPI)

Fig. 3: Time resolved analysis of the three different tissue types from one walleye sample (Fish sample ID 11) under different tuning conditions. The path of the analysis is shown as a white line in the microscope images and crossed the whole sample through the core. The red line in the graphs represents data obtained during instrument conditions tuned for maximum signal intensity and the black line data obtained during instrument conditions that minimize the oxide levels. In the otolith (A) no difference is observed between different instrument conditions, while for spine (B) and scale (C) significant differences are observed. Grey outlines represent the 95% confidence interval in the LOESS smooth.

279x380mm (300 x 300 DPI)

Fig. 4: Plot of all walleye tissue samples analyzed for $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios under standard instrument analytical conditions tuned for maximum intensity (A) reveal that scales and spines do not reflect the values obtained from otolith and water samples. Using methods to reduce oxide levels (B) all tissue types produce results within the expected $^{87}\text{Sr}/^{86}\text{Sr}$ isotope range of Boysen Reservoir.
106x91mm (300 x 300 DPI)