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Relative contribution of water and diet to otolith chemistry in freshwater fish

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ABSTRACT: Otolith chemistry is widely used to address key questions relating to fish ecology and fisheries management; however, there is limited research on the relative contributions of water and diet to elements within otoliths. This study explicitly tested the proportional contributions of water and diet in 3 Australian freshwater fish—silver perch, golden perch and Murray cod—in a controlled laboratory experiment. We independently 'spiked' both tank water and diet with enriched stable isotopes of strontium (^{86}Sr) and barium (^{137}Ba), key elements used in otolith chemistry. Hatchery-sourced fingerlings were used in the experiment and were independently exposed to a control, water-spiked or diet-spiked treatment for a period of 31 to 39 d. Otolith material laid down during the experiment was subsequently analysed for relevant isotopes using laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS), and water and diet samples were analysed using solution-based ICP-MS. An isotope mixing model was used to determine the relative contributions of water and diet to Sr and Ba otolith chemistry. For all species, water was the dominant source of elements in the otoliths, contributing between 64 and 71% for Sr and 88 and 92% for Ba. Diet contributed to a far lesser degree, with contributions ranging from 4 to 6% for Sr and 10 to 26% for Ba. The results from this study improve interpretations of otolith chemistry data in freshwater fish, thereby allowing more accurate reconstructions of the environmental and movement histories of wild populations.

KEY WORDS: Otolith chemistry · Freshwater fish · Water · Diet · Relative contributions · Isotopes

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INTRODUCTION

Otolith chemistry is used extensively to address critical issues relating to the population structure, movement patterns and environmental histories of fish (Campana 2005). Although much of the research is focused on marine and estuarine environments, otolith chemistry is being increasingly utilised for a diverse range of applications in freshwater systems. Such applications include the identification of recruitment and natal sources (Crook & Gillanders 2006, Pangle et al. 2010), invasive species' origins (Munro et al. 2005, Wolff et al. 2012) and habitat use (Brazner et al. 2004); the discrimination of wild and hatchery-reared stocks (Bickford & Hannigan 2005, Coghlan et al. 2007) and diadromous and non-diadromous ecotypes (Godbout et al. 2010); and the determination of release-site fidelity (Boehler et al. 2012) and riverine infrastructure impacts (Clarke et al. 2007). Environmental interpretations based on otolith elemental composition rely on the underlying assumption that fish derive elements predominantly from the water, either via the gills in freshwater fish or via the intestine in marine fish (Campana 1999), and that there is a predictable and consistent relationship between the chemistry of the otolith and the chemistry of the water. However, some elements have been shown to be derived from dietary sources (e.g. Kennedy et al. 2000), potentially confounding otolith–water

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chemistry relationships and reducing the accuracy of data interpretation in the field.

Strontium (Sr) and barium (Ba) are perhaps the most informative and commonly used elements in otolith chemistry as they are easily measured in the otolith, heterogeneously distributed throughout the aquatic environment, metabolically inert, and have been found to reflect environmental parameters (Campana et al. 1999, Elsdon et al. 2008, Brown & Severin 2009). Controlled laboratory experiments have shown that Sr and Ba levels in otoliths reflect ambient water concentrations in freshwater species (classified here as either potamodromous or diadromous species raised in freshwater) (Gibson-Reinemer et al. 2009, Collingsworth et al. 2010), which concurs with similar studies on marine (Bath et al. 2000) and estuarine (Elsdon & Gillanders 2003) species. Several studies have also examined the influence of diet on Sr otolith chemistry in freshwater species through diet manipulation (e.g. changing food type or enriching food with specific elements); however, they report conflicting results (Limburg 1995, Milton & Chenery 2001, Marohn et al. 2009), which was also observed in analogous studies on marine and estuarine species (see Hoff & Fuiman 1995, versus Buckel et al. 2004).

Studies that examine the influence of diet and water chemistry on otolith chemistry are vital for building our understanding of otolith–water chemistry relationships and the proportional contributions of both water and diet need to be determined to explicitly test the influence of diet on otolith chemistry. This can be achieved by comparing isotope variations, either naturally occurring or artificially induced, in the diet, water and otolith. We are aware of only 3 studies that have examined the proportional contributions of water and diet to Sr uptake in freshwater species. Of these studies, each concluded that diet had either very little influence (Farrell & Campana 1996, Gibson-Reinemer et al. 2009) or a substantial influence (Kennedy et al. 2000) on Sr otolith chemistry. There are no known studies that have examined the effect of diet on Ba otolith chemistry, either through food manipulation or analysis of natural isotopic variation in freshwater systems. Isotopic studies on marine and estuarine fish species found that Ba (and also Sr) is predominantly sourced from water (Walther & Thorrold 2006, Webb et al. 2012); however, comparisons with freshwater fish are tenuous as the physiological mechanism underlying osmoregulation, and thus elemental uptake, is fundamentally different (Evans 1980).

This study aims to determine the relative contributions of water and diet to Sr and Ba otolith chemistry in 3 potamodromous fish species through the use of enriched stable isotopes. By altering the natural isotopic ratio of diet and water in a controlled laboratory experiment, enriched isotopes were used to independently track the uptake of elements from each source (water and diet) into otoliths, providing a definitive test of proportional contributions.

MATERIALS AND METHODS

Experimental design

Three potamodromous fish species, native to the Murray-Darling Basin, Australia, were utilised in this study: silver perch *Bidyanus bidyanus* (SP), golden perch *Macquaria ambigua* (GP) and Murray cod *Maccullochella peelii* (MC). Juveniles (weighing approx. 0.6 to 2 g) were obtained from the Silverwater Native Fish Hatchery in Grong Grong, New South Wales, in February 2012. Upon arrival at The University of Adelaide, South Australia, each species was held in 200 l holding tanks at ambient temperature (21°C). Aged (dechlorinated) tapwater was used throughout the pre-experimental and experimental periods. SP and MC were fed commercial fish food (Ridley AgriProducts) in the first week after arrival and were gradually weaned onto the experimental diet (composed of gelatine, shrimp, oats and vegetables; see Royes & Chapman 2003), hereafter called 'shrimp diet'. GP were fed a modified version of the diet (composed of just gelatin and fish [*Lates niloticus*]; and called hereafter 'fish diet') throughout the pre-experimental and experimental periods. Twelve days after arrival all fish were marked with calcein, a fluorescent marker, using an osmotic induction method (Crook et al. 2009). Briefly, marking involved immersing fish in a salt solution (3% for MC, 5% for SP and GP) for several minutes (3.5 min for MC and SP, 5 min for GP) and then a freshwater solution containing 1% calcein. This facilitated the differentiation of pre- and post-experimental otolith growth. Fish were then randomly assigned to experimental tanks at a density of 13 fish per tank. Each tank contained 20 l of aged water, along with a small submerged filter and an air stone to aerate and circulate the water.

Experimental treatments involved spiking either the diet or tank water with a combination of enriched stable isotopes of ^{86}Sr and ^{137}Ba. Isotopically enriched $BaCO_3$ and $SrCO_3$ (Oak Ridge National Laboratories)

were used to spike the diet (at concentrations of 0.26 and 0.58 µg g^{-1} for each element, respectively) or water (at concentrations of 0.14 and 0.17 mg l^{-1} for each element, respectively). For the diet, appropriate spiking concentrations (i.e. to achieve a detectable shift from the natural ratio) were estimated from a previous study (Webb et al. 2012). For the water, baseline levels of Sr and Ba in the tank water were measured (Ba = 0.035 mg l^{-1} and Sr = 0.16 mg l^{-1}) with appropriate spiking concentrations subsequently calculated. Prior to spiking, the enriched isotopic compounds were combined, dissolved in a drop of hydrochloric acid and then mixed with ultrapure water. The experimental design consisted of 2 independent experimental treatments (spiked diet or water) plus a control treatment, which were replicated, resulting in a total of 6 tanks per species. Each of the 6 tanks were randomly assigned within one water bath and all water baths were gradually heated to 24°C, providing conditions for optimal fish growth. Fish were exposed to experimental conditions for either 31 d (MC and GP) or 39 d (SP).

Fish were fed daily to satiation and any food or waste remaining after 1 h was siphoned away to avoid potential isotope contamination of the water. It was not possible to accurately measure the amount of food consumed per day due to the use of mechanical filters (which sucked up the finer food particles) and difficulties associated with separating excess food from waste. Water changes (approximately 25 to 50%) were conducted when required and replacement water was spiked with appropriate levels of each enriched stable isotope. At the end of the experiment fish were euthanised through immersion in an ice slurry and immediately frozen until otolith extraction.

Water and diet analyses

Water samples were collected from each tank at the start, middle and end of the experimental period. Each sample was collected using a 25 ml syringe, filtered through a 0.45 µm filter into acid-washed 30 ml plastic vials, acidified with 0.5 ml of ultrapure nitric acid and frozen until analysis. Samples from the water-spiked treatments were diluted 10:1, due to enhanced levels of Sr and Ba isotopes. A second set of samples, diluted 100:1, were also run to measure Ca (due to extremely high levels in the tap water).

Three diet samples from each of the 4 diet types ('shrimp diet' unspiked and spiked; 'fish diet' unspiked and spiked) were prepared for analysis following the method outlined in Woodcock et al. (2012). Briefly, the samples were oven dried, crumbled, dissolved in ultrapure nitric acid, diluted 50:1 and then filtered through a 0.45 µm filter.

Water and diet samples were analysed using an Agilent 7500cs inductively coupled plasma-mass spectrometer (ICP-MS) (see Table 1 for operating parameters). To calculate isotope ratios, ^{138}Ba, ^{137}Ba, ^{88}Sr and ^{86}Sr were measured, alongside ^{43}Ca for element:Ca ratios. A natural multi-element stock standard was run for Ca, Ba and Sr at 0, 1, 50, 100 and 500 µg l^{-1}. Two additional standards for each Sr and Ba isotope were also analysed at the following concentrations: ^{138}Ba and ^{137}Ba at 50 and 200 µg l^{-1}; and ^{88}Sr and ^{86}Sr at 100 and 350 µg l^{-1}. Standards and blanks were analysed regularly throughout each session. Agilent Mass Hunter software was used to collect the raw data, which were calibrated against the elemental standards. Both the elemental and isotope standards were used to measure instrument drift and precision. To calculate total Ba:Ca and Sr:Ca ratios (in mmol mol^{-1}) ^{138}Ba and ^{88}Sr were utilised.

Otolith preparation and analyses

Otoliths were dissected from each fish, cleaned in ultrapure water and air-dried. One otolith from each individual was embedded in epoxy resin (Epofix, Struers) spiked with 40 ppm of indium and cut into sections (200–300 µm thick) using a low speed dia-

Table 1. Operating parameters for the Agilent 7500cs inductively coupled plasma mass spectrometer (ICP-MS) and New Wave Nd Yag 213 UV laser with ICP-MS

Solution ICP-MS	
Collision cell	He (5 ml min^{-1})
Cone	Pt
Integration time	0.1 s with 3 replicates for each isotope
Laser	
Wavelength	213 nm
Mode	Q-switch
Frequency	5 Hz
Spot/transect size	30 µm
Transect scan rate	3 µm s^{-1}
Laser power	75%
Carrier	Ar (0.92 l min^{-1})
Laser ICP-MS	
Optional gas	He (58%)
Cone	Pt
Dwell times (ms)	^{138}Ba (300), ^{137}Ba (400), ^{86}Sr and ^{88}Sr (200), ^{43}Ca (100), ^{115}In (50)

mond saw (Isomet, Buehler). Indium (In) was used so that the epoxy resin could be distinguished from the otolith material during analysis. Sections were polished using several grades of lapping film before being fixed onto a glass microscope slide using indium-spiked thermo-plastic glue (CrystalBond™ 509). Sections were viewed under fluorescent light to visualize the calcein mark and ensure that sufficient experimental otolith growth had occurred.

Otolith sections were analysed on a New Wave Nd Yag 213 nm UV laser operated in Q-switch mode connected to an Agilent 7500cs ICP-MS (see Table 1 for operating parameters). For SP and MC, the otolith edge region outside the calcein mark (i.e. experimental growth) was ablated using 30 µm spot analyses (Fig. 1a,b). For GP, relatively little experimental otolith growth occurred (approximately ≤10 µm), thus making spot analyses unfeasible. As an alternative short transects (30 µm wide and approximately 150 µm long) were run perpendicular to the otolith edge, enabling the experimental isotopic signal to be sampled (Fig. 1c,d). The 2 different ablation methods required separate approaches to the isotope data analysis (see below).

To determine enrichment, the following isotopes were measured: ^{138}Ba, ^{137}Ba, ^{88}Sr and ^{86}Sr, alongside ^{43}Ca for element:Ca ratios and ^{115}In to ensure otolith material was ablated at all times. To measure instrument drift and precision a reference standard, NIST612, was analysed approximately every 10 samples, and carbonate standard, MACS-3 (United States Geological Survey), was analysed at the beginning and end of each 7 to 9 h laser session.

All isotope ratio data (^{138}Ba:^{137}Ba and ^{88}Sr:^{86}Sr) were smoothed using a 6-point running mean. For spot analyses (SP and MC) the average value of the smoothed data was used as the isotopic value for each individual (see Fig. 1b for example data). For transect analyses (GP), the minimum value of the smoothed data was used for each individual (see Fig. 1d for example data). As the lighter isotopes were enriched, the minimum values were assumed to represent a shift away from the natural isotopic ratios (i.e. artificially induced isotope ratios will have a lower value than the natural ratio) (see also Munro et al. 2008, Woodcock et al. 2011). To calculate total Ba:Ca and Sr:Ca ratios (in mmol mol^{-1}), ^{138}Ba and ^{88}Sr were utilised.

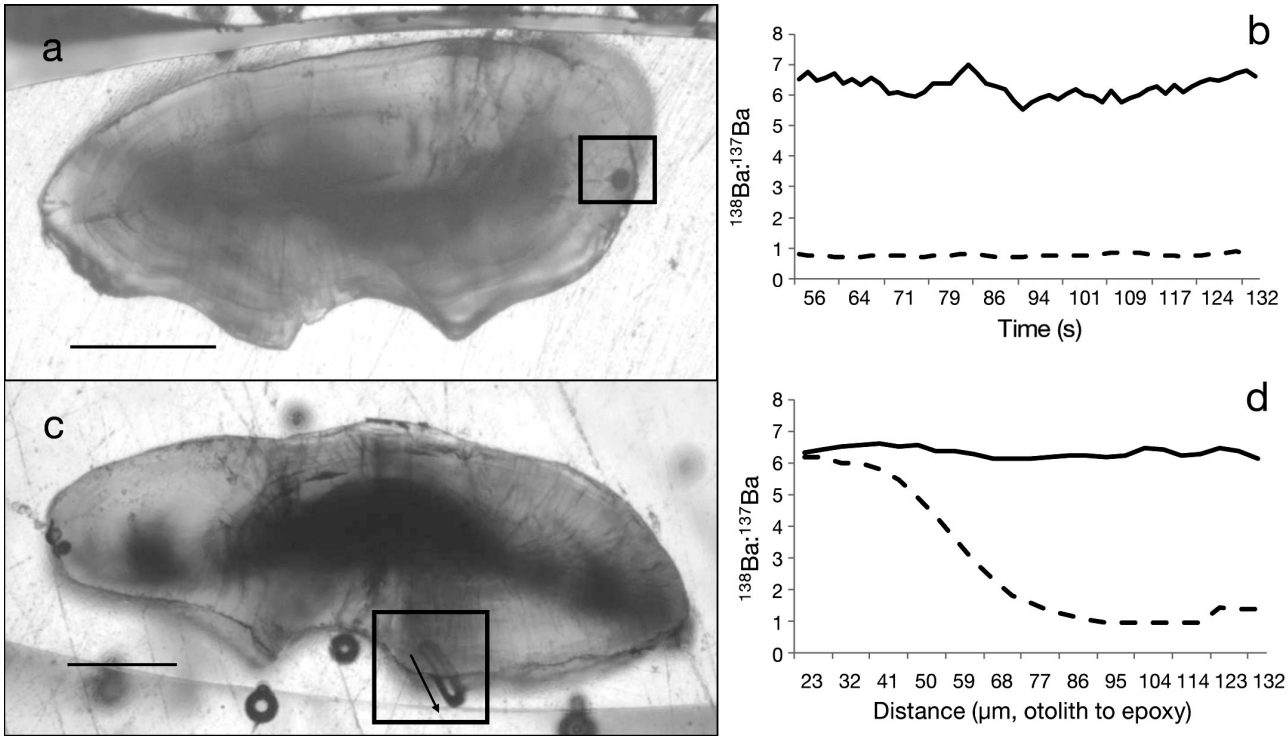

Fig. 1. Comparison of the 2 ablation methods utilised with relevant data outputs. (a) Sectioned silver perch otolith with 30 µm spot ablation crater (indicated by square). (b) Smoothed ^{138}Ba:^{137}Ba data from 2 spot analyses (solid line: individual exposed to control treatment; dashed line: individual exposed to water-spiked treatment). (c) Sectioned golden perch otolith with 150 × 30 µm ablation transect (indicated by square) running from otolith to epoxy. (d) Smoothed ^{138}Ba:^{137}Ba data from 2 transect analyses (solid and dashed lines as above). Scale bars (a,c): 200 µm

Statistical analyses

Differences in ^{138}Ba:^{137}Ba and ^{88}Sr:^{86}Sr otolith ratios among treatments and replicates (n = 6) were tested for each species using 1-way ANOVA and Tukey's HSD test. Data were examined for normality and homogeneity of variances using graphical methods and Levene's test. To meet model assumptions, all data were transformed using power transformations (y^{-3} and $y^{-0.7}$ for Sr and Ba, respectively). A range of common transformations were initially tested, but they did not improve homogeneity of variances. No significant differences were observed between replicate tanks for all species, thus transformed data from each replicate were pooled and re-analysed using the same tests. Otolith data from one of the spiked diet treatment tanks, which indicated very slight levels of isotope contamination in the water, were included in the analysis as they were not significantly different from the uncontaminated replicate. All statistical tests were performed in SPSS (version 19).

The percent contribution of tank water and diet to otolith Sr and Ba was determined for the 2 independent experiments (water-spiked vs. diet-spiked) using a simple isotope mixing model detailed in Kennedy et al. (2000):

$$\%\text{Element}_{(\text{water})} = \left[1-\left(\frac{\text{Isotope ratio}_{(\text{water})}-\text{Isotope ratio}_{(\text{otolith})}}{\text{Isotope ratio}_{(\text{water})}-\text{Isotope ratio}_{(\text{diet})}}\right)\right]\times 100$$

$$\%\text{Element}_{(\text{diet})} = \left[1-\left(\frac{\text{Isotope ratio}_{(\text{otolith})}-\text{Isotope ratio}_{(\text{diet})}}{\text{Isotope ratio}_{(\text{water})}-\text{Isotope ratio}_{(\text{diet})}}\right)\right]\times 100$$

Water and diet ratios used in the above model were averaged across the multiple samples taken from each tank/diet type.

RESULTS

Water and diet chemistry

For all species, elemental water concentrations remained relatively consistent throughout the experiment, having a group mean (±SE) of 0.94 ± 0.01 mmol mol^{-1} for Ba:Ca and 6.54 ± 0.04 mmol mol^{-1} for Sr:Ca (Table 2). Slight variations in elemental concentrations were presumably due to changes in the chemistry of the tap water over the course of the experiment. The isotope ratios of the water-spiked treatments were significantly altered for all spiked tanks and remained consistent throughout the experiment, having a group mean of 0.58 ± 0.01 for ^{138}Ba:^{137}Ba and 1.48 ± 0.02 for ^{88}Sr:^{86}Sr, compared with the natural ratios of 6.38 and 8.38, respectively (Table 2). Overall, the water samples from the control tanks were slightly higher in Sr and lower in Ba than the natural ratios; however, this appears to be an analytical artefact rather than a representation of real ratios as a similar pattern was observed in the natural stock standards (control tanks: mean ^{88}Sr:^{86}Sr = 8.69 ± 0.02, mean ^{138}Ba:^{137}Ba = 6.24 ± 0.02; standards: mean ^{88}Sr:^{86}Sr = 8.79 ± 0.05; mean ^{138}Ba:^{137}Ba = 6.27 ± 0.01). Water isotope ratios from the spiked diet tanks were similar to those of the control tanks, suggesting that isotopes did not leach out of the food to contaminate the water. However, an exception was one of the spiked diet tanks for MC, which showed both lower isotope ratios and greater variation among samples over the experimental period. It is likely that the tank was contaminated during the experiment, possibly as a result of human error. Isotope ratios in the spiked diet were significantly altered in both diet types, with elemental con-

Table 2. Mean (±SE) elemental (mmol mol^{-1}) and isotopic composition of tank water for each treatment and replicate

Tank	Sr:Ca	Ba:Ca	^{88}Sr:^{86}Sr	^{138}Ba:^{137}Ba
Murray cod				
Control 1	5.85 ± 0.09	0.76 ± 0.02	8.70 ± 0.04	6.22 ± 0.02
Control 2	5.90 ± 0.20	0.77 ± 0.02	8.76 ± 0.06	6.28 ± 0.03
Spiked diet 1	6.10 ± 0.28	0.81 ± 0.03	8.61 ± 0.02	6.18 ± 0.05
Spiked diet 2	5.96 ± 0.24	0.76 ± 0.02	8.42 ± 0.12	5.73 ± 0.23
Spiked water 1	6.33 ± 0.15	1.08 ± 0.06	1.46 ± 0.05	0.57 ± 0.01
Spiked water 2	5.80 ± 0.20	1.02 ± 0.03	1.49 ± 0.08	0.60 ± 0.01
Silver perch				
Control 1	7.18 ± 0.22	0.87 ± 0.04	8.66 ± 0.06	6.20 ± 0.02
Control 2	6.95 ± 0.24	0.89 ± 0.04	8.69 ± 0.04	6.31 ± 0.01
Spiked diet 1	6.64 ± 0.29	0.88 ± 0.06	8.64 ± 0.05	6.16 ± 0.04
Spiked diet 2	6.50 ± 0.16	0.84 ± 0.01	8.62 ± 0.06	6.13 ± 0.03
Spiked water 1	6.78 ± 0.17	1.19 ± 0.05	1.42 ± 0.03	0.56 ± 0.01
Spiked water 2	6.12 ± 0.12	1.05 ± 0.02	1.47 ± 0.05	0.57 ± 0.02
Golden perch				
Control 1	6.61 ± 0.10	0.89 ± 0.03	8.63 ± 0.07	6.15 ± 0.04
Control 2	7.16 ± 0.22	0.96 ± 0.03	8.72 ± 0.06	6.28 ± 0.02
Spiked diet 1	6.91 ± 0.30	0.93 ± 0.03	8.58 ± 0.07	6.19 ± 0.05
Spiked diet 2	7.47 ± 0.50	0.99 ± 0.08	8.60 ± 0.05	6.15 ± 0.02
Spiked water 1	6.71 ± 0.25	1.16 ± 0.03	1.53 ± 0.05	0.58 ± 0.01
Spiked water 2	6.73 ± 0.50	1.16 ± 0.09	1.54 ± 0.06	0.58 ± 0.01

Table 3. Mean (±SE) elemental (mmol mol^{-1}) and isotopic composition of each diet type. 1: prawn diet; 2: fish diet

Diet type	Sr:Ca	Ba:Ca	$^{88}Sr:^{86}Sr$	$^{138}Ba:^{137}Ba$
1 – unspiked	6.58 ± 0.34	0.50 ± 0.12	8.69 ± 0.02	6.21 ± 0.01
1 – spiked	6.86 ± 0.11	0.44 ± 0.04	3.46 ± 0.12	1.74 ± 0.12
2 – unspiked	6.69 ± 0.28	0.26 ± 0.04	8.36 ± 0.12	6.14 ± 0.14
2 – spiked	6.35 ± 0.22	0.53 ± 0.16	1.50 ± 0.12	0.60 ± 0.01

centrations remaining relatively consistent between spiked and unspiked diets (Table 3).

Otolith chemistry

Otolith Sr and Ba ratios were significantly different among treatments for all species (Table 4, Fig. 2). Post hoc tests indicated that all treatments were significantly different from each other for all species ($p < 0.05$), except between the diet-spiked and control treatments for GP. Sr and Ba isotopic ratios from the water-spiked treatments were dramatically different from the control treatments for all species, with mean isotope ratios ranging from 3.56 to 4.09 for $^{138}Ba:^{137}Ba$ and 1.03 to 1.25 for $^{88}Sr:^{86}Sr$ (Fig. 2). In contrast, ratios from the diet-spiked treatments showed much smaller (but largely significant) shifts from the control treatments. Sr isotope ratios from the control treatment were slightly higher than the natural ratio for both MC and SP (where the average values of the smoothed data were taken); however, again, this appears to be an analytical artefact. Sr isotope ratios in the control treatment were consistent among individuals for both species and very similar to the internal carbonate standard measured at the beginning and end of each laser session (mean SP = 8.72 ± 0.02; mean MC = 8.82 ± 0.04; mean carbonate standard = 8.84 ± 0.04). Sr and Ba isotope ratios for GP were lower than MC and SP, most likely as a result of taking the minimum value of the smoothed isotope ratio data.

Table 4. One-way ANOVA comparing differences in $^{138}Ba:^{137}Ba$ and $^{88}Sr:^{86}Sr$ in otoliths among treatments for each species

Source	df	MS	*F*	p
Murray cod				
$^{88}Sr:^{86}Sr$				
Treatment	2	1634374	406	<0.001
Error	43	4022		
$^{138}Ba:^{137}Ba$				
Treatment	2	29.6	775	<0.001
Error	43	0.038		
Silver perch				
$^{88}Sr:^{86}Sr$				
Treatment	2	1456274	752	<0.001
Error	44	1934		
$^{138}Ba:^{137}Ba$				
Treatment	2	28	903	<0.001
Error	44	0.31		
Golden perch				
$^{88}Sr:^{86}Sr$				
Treatment	2	1404684	553	<0.001
Error	50	2539		
$^{138}Ba:^{137}Ba$				
Treatment	2	28	664	<0.001
Error	50	0.042		

Percent contributions

Percent contributions indicate that water was the predominant source of Sr and Ba in otoliths for all species, with mean percentages ranging from 64 to 71% for Sr and 88 to 92% for Ba (Fig. 3). Food contributed to otolith chemistry to a much lesser degree, with percent contributions ranging from 4 to 6% for Sr and 10 to 26% for Ba. Overall, there was remarkable consistency in results among species, except for the contribution of diet in SP, which was double that of MC and GP. For all species, the combined percent contribution of water and diet to otolith chemistry was noticeably different between the 2 elements (mean combined contribution: Sr = 71%, Ba = 105%).

DISCUSSION

Here we show that water is the predominant source of Sr and Ba in the otoliths of 3 potamodromous fish species, which represent 3 different genera and 2 families. Although limited, results from similar isotope studies examining relative contributions of water and/or diet in a range of aquatic environments indicate that water is the predominant source of Sr and Ba in the otolith, with contributions ranging from 62 to 88% for Sr and 59 to 92% for Ba (Table 5). Isotope studies examining proportional contributions of C (Solomon et al. 2006), Ca (Farrell & Campana 1996) and Mg (Woodcock et al. 2012) also found that water was the primary contributor for these 3 elements in freshwater teleosts. An exception to this pattern, however, was observed in the study by Kennedy et

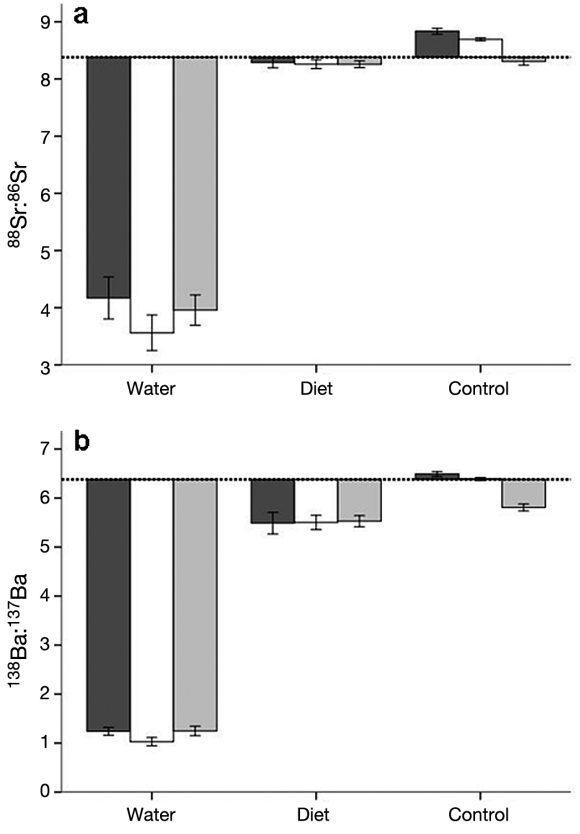

Fig. 2. Mean (±SE) otolith ratios for (a) $^{88}Sr:^{86}Sr$ and (b) $^{138}Ba:^{137}Ba$ for each species reared in water-spiked, diet-spiked and control treatments. Black dotted line indicates natural isotope ratio. Dark grey: Murray cod; white: silver perch; light grey: golden perch

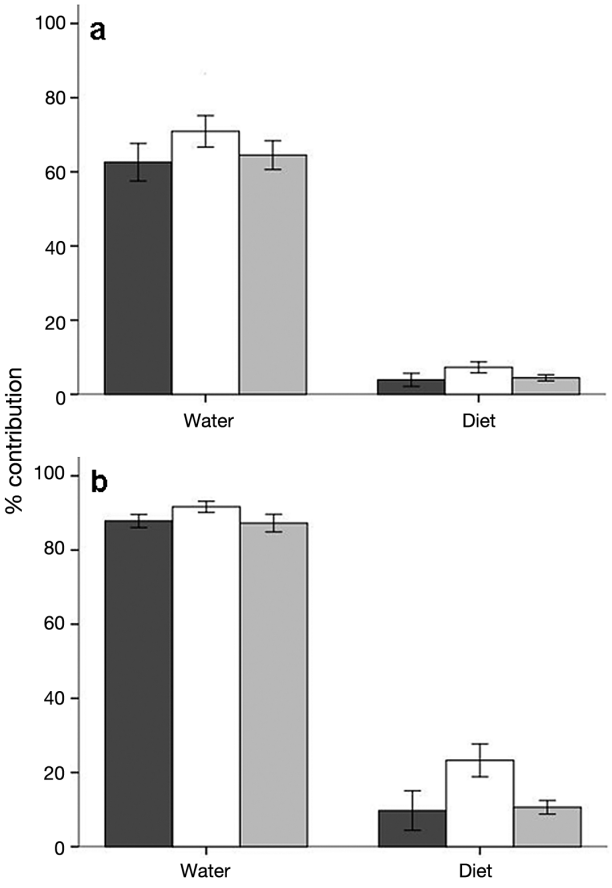

Fig. 3. Mean (±SE) percent contributions of water and diet for (a) Sr and (b) Ba in the otoliths of each species. Dark grey: Murray cod; white: silver perch; light grey: golden perch

Table 5. Summary of literature examining proportional contributions of water and/or diet to otolith chemistry based on isotopic variations. FW: freshwater; E: estuarine; M: marine. *ranges associated with different temperature and salinity treatments

Species	Family	Salinity	Isotope type	Water % Sr	Water % Ba	Diet % Sr	Diet % Ba	Source
Silver perch *Bidyanus bidyanus*	Terapontidae	FW	Enriched	71	92	6	15	Present study
Murray cod *Maccullochella peelii*	Percichthyidae	FW	Enriched	64	89	8	18	Present study
Golden perch *Macquaria ambigua*	Percichthyidae	FW	Enriched	64	87	2	5	Present study
Nile tilapia *Oreochromis niloticus*	Cichlidae	FW	Radio	88	–	12	–	Farrell & Campana (1996)
Rainbow trout *Oncorhynchus mykiss*	Salmonidae	FW	Natural	66	–	34	–	Gibson-Reinemer et al. (2009)
Atlantic salmon *Salmo salar*	Salmonidae	FW	Natural	30	–	70	–	Kennedy et al. (2000)
Black bream *Acanthopagrus butcheri*	Sparidae	E	Enriched	62–84	59–84	16–38	16–41	Webb et al. (2012)*
Mummichog *Fundulus heteroclitus*	Fundulidae	M	Enriched	83	98	–	–	Walther & Thorrold (2006)

al. (2000), which found that diet contributed 70% of the Sr in otoliths in a freshwater-raised diadromous species. Unlike other studies on Sr and Ba, however, percent contributions in the present study did not equal 100%. This is because we examined both water and diet and tested the proportional contributions of each independently (i.e. different individuals were exposed to the enriched diet and water treatments). The studies detailed in Table 5 either examined water alone, and assumed the remaining percentage was derived from diet, or combined water and diet treatments in the same tank (i.e. individuals were exposed to both treatments simultaneously). In the present study, variations from 100% were likely due, in part, to analytical error and individual variability. Additionally, the observed difference in percent contributions between Sr and Ba may relate to relative differences in the incorporation rates of the 2 elements in the otolith from both water and diet. Little is known about the relative incorporation rates of Sr and Ba (but see Elsdon & Gillanders 2005, Woodcock et al. 2013); nonetheless, exposing individuals to experimental conditions for longer time periods maybe an important factor to consider in future studies.

Even if water is the predominant source of elements to the otolith, the results from the present study and others suggest that diet has some varying level of influence on Sr and Ba otolith chemistry (2 to 41%, excluding Kennedy et al. 2000; Table 5). Furthermore, studies that have examined the effect of diet on Sr and Ba otolith chemistry, either through artificial enrichment of food or changing natural food types, suggest that diet has either no effect (Hoff & Fuiman 1995, Milton & Chenery 2001, Marohn et al. 2009), or a significant and/or detectable effect on otolith chemistry (Limburg 1995, Gallahar & Kingsford 1996, Buckel et al. 2004, Engstedt et al. 2012). In regards to the latter group, however, it is difficult to assess to what level diet influences otolith chemistry relative to water, particularly if diet effect has only been tested. A variety of factors, such as taxonomic differences, temperature, salinity, life history stage, food availability, feeding and growth rates, captivity and elemental concentration in the water, may at varying degrees confound the proportional contributions of water and diet to otolith chemistry; however, these factors have been largely untested. Although not relevant to freshwater systems, one study examining an estuarine species found that although temperature alone did not influence the percent contribution of water to otolith chemistry, high temperatures were shown to influence the incorporation of Sr at difference salinities (Webb et al. 2012). Results from Webb et al. (2012) also suggested that Sr and Ba were still predominantly derived from water regardless of elemental concentration in the water, which has also been observed in a similar study on Mg (Woodcock et al. 2012). In our study many of the above factors were constant among species and treatments and results were generally consistent; nonetheless, based on daily observations, feeding activity, overall condition and level of stress associated with captivity did appear to vary among species and may explain, in addition to taxonomy, why diet had a greater contribution to otolith chemistry in SP (the 'hardiest' of the 3 species).

In light of the current evidence presented, which implies that water is the predominant source of elements in the otolith, a key question researchers may ask is to what level is diet seen as having a significant or noteworthy influence on otolith chemistry? This may depend on the question being asked and the magnitude of relative variations in the physical and chemical properties of the water and diet being examined. For instance, Buckel et al. (2004) states that although statistically significant differences were found between otolith Sr levels in shrimp- and fish-fed individuals, the differences were relatively small compared with results from studies examining fish collected across large salinity gradients. The authors concluded, therefore, that diet effects may influence the successful detection of smaller salinity gradients, but should not result in the misclassification of individuals from low and high salinity environments. In regard to measuring movement of fish across heterogeneous environments (e.g. marine to freshwater, upper to lower reaches of a river, and among tributaries with distinct bedrock geochemistry), a relatively minor contribution from diet, as observed in the present study, may have a significant effect on how otolith chemistry data, and thus movement patterns, are interpreted. When investigating movement across relatively homogeneous water bodies (e.g. a single tributary or the open ocean), however, the proportional contribution of diet, even if small, should be considered, as well as the level of spatial and temporal diet variation among individuals. Another application of otolith chemistry, which may be impacted by small dietary effects, is the reconstruction of environmental parameters such as temperature, dissolved oxygen and salinity, where the aim is more focused on tracking a changing environment (and its potential impact on the fish) rather than delineating fish movement (e.g. Limburg et al. 2011). A study examining the effect of diet on Sr and

Ba levels in cuttlefish statoliths (analogues to otoliths) suggested that even the small effects they observed (up to 10%) could affect the accuracy of Ba as a temperature proxy by 2°C (Zumholz et al. 2006). In contrast, the source of the chemical variation in otoliths may be relatively irrelevant in some applications, such as in the discrimination of wild and hatchery-reared stocks (e.g. Coghlan et al. 2007), whereby a distinct signature between the two is all that is required.

Our study has increased our understanding of the relative contributions of water and diet to otolith composition and reinforced our confidence in Sr and Ba as informative and useful elements for understanding the environmental histories and movement patterns of fish living in freshwater environments. Of the fish studied, evidence suggests that water is the predominant source of Sr and Ba in the otolith, which aligns, for the most part, with similar isotope-based studies. However, depending on the question being answered, relatively minor dietary contributions could affect how otolith chemistry data, and thus movement and environmental histories, are interpreted. Furthermore, to address discrepancies among studies and the substantial level of variation in how diet may affect otolith chemistry, further research is required that not only allows direct comparisons between the effects of water and diet, but also investigates potential factors that may influence such variability.

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