

Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*

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ABSTRACT: The use of otolith chemistry to delineate fish populations and trace migration pathways is premised on a significant correlation between the elemental composition of otoliths and physicochemical properties of the ambient environment. However, few experiments have been rigorously designed to address the effects of temperature and salinity on the elemental composition of otoliths. We examined the effects of temperature and salinity on the incorporation of magnesium (Mg), manganese (Mn), and barium (Ba) in the otoliths of larval and early juvenile spot *Leiostomus xanthurus* by rearing fish in the laboratory under controlled environmental conditions. *L. xanthurus* are an estuarine dependent species that traverse varying temperature and salinity regimes throughout their life histories. It is important, therefore, to understand the influence of physicochemical properties of different water masses before attempting to reconstruct important life history transitions based on variations in otolith chemistry. Both $[Mg/Ca]_{\text{otolith}}$ and the Mg partition coefficient, D_{Mg} , were not significantly affected by either temperature or salinity, but were correlated with otolith precipitation and somatic growth rates. Temperature and salinity had significant interaction effects on D_{Mn} , but not on $[Mn/Ca]_{\text{otolith}}$. Finally, D_{Ba} was influenced by salinity but not temperature. These results highlight the complex nature of elemental deposition in otoliths, and suggest that both environmental and physiological effects likely influence elemental ratios in fish otoliths.

KEY WORDS: Otolith chemistry · Temperature · Salinity · Magnesium · Manganese · Barium

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INTRODUCTION

Elemental signatures in the otoliths of teleost fishes are increasingly being used as natural tags of stock structure and to assess usage of juvenile nursery habitats (Edmonds et al. 1989, Campana et al. 1994, Gillanders & Kingsford 1996, Begg et al. 1998, Thorrold et al. 2001). Using the elemental composition of fish otoliths to infer population affinities and past environments is based on the assumption that elemental incorporation in otoliths is proportional to the ambient concentration of a particular element in the water. Water bodies have different elemental compositions based on the geochemistry of the watershed,

anthropogenic, and natural inputs to the system, and the behavior of elemental species as influenced by complexation, biological uptake, and sorption on suspended solids (Bender et al. 1977, Turner et al. 1981, Bruland 1983, Byrne et al. 1988, Morel & Hering 1993). In theory, fish with similar elemental signatures in otoliths originated from the same location, while fish with significantly different signatures are assumed to have come from elsewhere. In practice, however, it is not clear that elemental incorporation in otoliths is solely a function of the physicochemical properties of the ambient water mass. Because fisheries management relies on the accurate classification of fish stocks and habitat use, erroneous interpreta-

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tions of geochemical signatures in otoliths could lead, in turn, to illogical management decisions.

Environmental effects on otolith element incorporation may be confounded by the influence of aragonite precipitation rate on element incorporation in biogenic aragonite. Studies on synthetic aragonite have found no effect of precipitation rate on element incorporation (Kinsman & Holland 1969, Zhong & Mucci 1989), while studies on biogenic aragonite have been contradictory. Some work has suggested that Sr/Ca in corals was correlated with precipitation rates (Weber 1973, deVillers et al. 1994, 1995), although Shen et al. (1996) found no such relationship. In mollusks, Bucharadt & Fritz (1978) found Sr incorporation to be independent of growth rate, but Stecher et al. (1996) speculated that there was a seasonal growth effect on Sr incorporation of 2 species of bivalves. Bath et al. (2000) found no effect of aragonite precipitation rate on Sr and Ba incorporation in fish otoliths. However, no information is available for any of the other elements routinely used in otolith chemistry studies.

Physiological processes also influence otolith composition. Otoliths are isolated from seawater by branchial, intestinal, and the endolymphatic membranes, and fish obviously control ion transport across these membranes for osmoregulatory purposes. Elemental concentrations are, therefore, typically lower in otoliths than in the surrounding environment (Campana 1999). Elemental discrimination does not necessarily invalidate the use of otolith chemistry for reconstructing environmental conditions experienced by individual fish, providing that the degree of regulation of a particular element either remains constant (allowing reconstruction of dissolved levels of an element in the environment) or changes predictably with a variable of interest (typically either temperature or salinity). For instance, otolith Sr has been shown to scale linearly with ambient Sr/Ca ratios in the water, with predictable effects of temperature and salinity on Sr partition coefficients (Bath et al. 2000, Kraus & Secor 2004, Martin et al. 2004). However, similar information is not available for any of the other elements routinely used in analyses of geochemical signatures in otoliths.

Rigorous testing of the factors determining otolith chemistry requires knowledge of the physicochemical properties of the ambient water and the physiological attributes of the fish assigned to different treatments (e.g. Bath et al. 2000). However, few experiments have addressed the influence of temperature and salinity on elemental composition of otoliths with the necessary environmental and biological constraints (Kalish 1989, Hoff & Fuiman 1995, Chesney et al. 1998, Milton & Chenery 2001, Elsdon & Gillanders 2002). The objective of this study was to test the effects of temperature,

salinity, and water elemental composition on the incorporation of Mg, Mn, and Ba in late larval/juvenile spot *Leiostomus xanthurus* otoliths under controlled conditions in the laboratory. Spot spawn in marine water and juveniles inhabit low salinity estuaries, so the species experiences a wide range of temperature, salinity, and water chemistry during its early life history. It may, therefore, be possible to retrospectively identify water mass residency of individual spot if temperature and salinity induce consistent and predictable changes in otolith chemistry.

MATERIALS AND METHODS

Spot *Leiostomus xanthurus* were hatched on 10 December 1999 at the NOAA, National Ocean Service, Center for Coastal Fisheries and Habitat Research in Beaufort, North Carolina, USA, and reared at 34‰ salinity and 20°C in a common tank in natural seawater for 42 d. At that time they were randomly distributed among 24 acid-washed 20 l high-density polyethylene tanks at a density of 2 fish l⁻¹. Experimental tanks were randomly assigned 4 temperature treatments (17, 20, 23, and 26°C) and 2 salinity treatments (15 and 25‰). For each treatment combination, 3 replicate tanks were used (24 tanks in total). The ambient temperature in the room was maintained at 16°C and electric aquarium heaters were used to regulate temperatures within individual tanks. Artificial seawater (Instant Ocean®) was dissolved in deionized water to regulate salinity. Before initiating the experiment, fish were gradually acclimated to the temperature and salinity treatments for a week to ensure high survival. The light:dark cycle was 12:12 h for the duration of the experiment. Fish were fed an artificial diet (Golden Pearls, 300 to 500 and 500 to 800 µm, Brine Shrimp Direct) twice daily ad libitum. To maintain water quality and salinity, water was changed at 50% volume daily.

Water temperature was measured and recorded daily (Table 1). Every third day, water samples from each tank were collected, filtered through 0.22 µm cellulose nitrate membrane filters, acidified with 40% trace-metal grade HCl to pH 2, and then stored frozen for subsequent analysis.

All surviving fish after terminating the experiment (42 d) were measured (17.3 to 38.0 mm SL) and stored frozen in individual plastic bags. Sagittal otolith pairs were removed from the fish and adhering tissue was removed with acid-washed glass probes in a clean room. Otoliths were cleaned ultrasonically in Milli-Q water for 7 min, triple rinsed with ultrapure H₂O₂ (Ultrex, J. T. Baker), and washed with 3 sequential rinses of Milli-Q water. Otolith pairs were placed on

Table 1. *Leiostomus xanthurus*. Summary of mean water temperature (Temp., °C, ± standard deviation [SD]), salinity treatment (Sal., ‰), dissolved Mg/Ca (mol mol⁻¹, ±SD), Mn/Ca (µmol mol⁻¹, ±SD) and Ba/Ca (µmol mol⁻¹, ±SD) levels, individual element concentrations for Mg (mmol l⁻¹, ±SD), Mn (µmol l⁻¹, ±SD), Ba (µmol l⁻¹, ±SD), and Ca (mmol l⁻¹, ±SD), the number of otoliths analyzed (n), the mean otolith mass (OM, mg, ±SD) and somatic growth rate (GR, mm d⁻¹, ±SD) within each of the 24 individual tanks during the course of the experiment

Tank	Temp.	Sal.	[Mg]	[Mn]	[Ba]	[Ca]	[Mg/Ca]	[Mn/Ca]	[Ba/Ca]	n	OM	GR
9	17.5 (0.7)	15	0.65 (0.08)	37.30 (12.01)	14.13 (1.57)	0.23 (0.03)	4.57 (0.17)	115.86 (27.70)	17.72 (0.81)	7	0.93 (0.23)	0.47 (0.11)
11	18.1 (0.7)	15	0.58 (0.03)	30.05 (15.24)	9.71 (6.82)	0.20 (0.10)	4.79 (0.11)	108.86 (6.76)	14.93 (6.36)	7	0.97 (0.19)	0.46 (0.09)
20	18.5 (0.7)	15	0.59 (0.01)	33.86 (4.63)	12.85 (2.38)	0.19 (0.03)	5.19 (0.81)	133.26 (22.96)	20.07 (3.26)	8	0.61 (0.05)	0.31 (0.04)
24	20.1 (0.9)	15	0.56 (0.02)	29.91 (13.81)	11.73 (5.59)	0.20 (0.10)	4.69 (0.19)	115.87 (18.09)	17.76 (1.75)	8	0.72 (0.18)	0.36 (0.07)
5	20.8 (0.4)	15	0.62 (0.01)	30.33 (7.49)	13.26 (3.12)	0.20 (0.05)	5.03 (0.58)	110.52 (19.08)	19.12 (1.79)	7	0.98 (0.29)	0.45 (0.12)
18	21.2 (1.3)	15	0.71 (0.05)	39.03 (12.98)	15.28 (1.59)	0.25 (0.02)	4.63 (0.13)	111.38 (28.89)	17.63 (1.26)	8	0.89 (0.13)	0.42 (0.07)
10	22.7 (1.8)	15	0.58 (0.08)	33.13 (7.03)	12.37 (2.00)	0.20 (0.03)	4.81 (0.06)	121.25 (14.24)	18.12 (0.39)	8	1.21 (0.19)	0.52 (0.06)
21	23.4 (1.1)	15	0.66 (0.07)	32.38 (4.71)	16.20 (4.43)	0.24 (0.03)	4.65 (0.11)	100.47 (5.48)	20.18 (5.48)	8	1.12 (0.27)	0.48 (0.09)
22	23.8 (2.3)	15	0.62 (0.06)	35.10 (4.02)	13.13 (1.54)	0.22 (0.02)	4.72 (0.09)	120.37 (26.83)	17.70 (0.74)	8	0.79 (0.25)	0.37 (0.08)
4	26.1 (1.3)	15	0.66 (0.09)	30.21 (9.19)	13.88 (1.41)	0.22 (0.02)	4.89 (0.28)	100.13 (33.54)	18.19 (0.18)	8	1.20 (0.30)	0.50 (0.12)
14	26.2 (1.6)	15	0.69 (0.07)	28.40 (2.74)	14.49 (1.29)	0.25 (0.02)	4.61 (0.04)	84.60 (9.43)	17.21 (0.66)	8	0.95 (0.23)	0.42 (0.08)
16	26.8 (1.2)	15	0.61 (0.15)	23.76 (6.78)	12.94 (3.61)	0.21 (0.06)	4.75 (0.33)	84.30 (27.72)	17.64 (0.83)	8	1.21 (0.24)	0.50 (0.07)
1	17.5 (0.6)	25	0.96 (0.22)	45.30 (10.55)	16.91 (5.56)	0.33 (0.08)	4.76 (0.20)	99.86 (12.66)	15.01 (3.78)	8	0.62 (0.20)	0.32 (0.08)
7	17.8 (0.7)	25	1.04 (0.07)	43.24 (8.67)	12.82 (10.96)	0.37 (0.03)	4.71 (0.13)	87.14 (21.77)	10.01 (8.51)	4	0.80 (0.18)	0.31 (0.07)
15	18.7 (0.9)	25	0.96 (0.21)	43.61 (14.00)	16.06 (5.83)	0.33 (0.08)	4.81 (0.25)	95.32 (13.60)	14.56 (4.80)	8	0.54 (0.09)	0.28 (0.05)
2	19.9 (0.4)	25	1.12 (0.25)	38.84 (12.01)	19.77 (9.24)	0.38 (0.11)	4.83 (0.24)	75.62 (18.62)	14.90 (5.03)	8	0.69 (0.19)	0.30 (0.06)
8	20.2 (0.3)	25	1.09 (0.16)	52.91 (31.71)	20.36 (3.74)	0.36 (0.05)	4.67 (0.04)	109.78 (64.82)	16.78 (2.35)	8	0.63 (0.18)	0.26 (0.08)
6	20.3 (0.3)	25	0.99 (0.14)	34.81 (15.74)	15.72 (9.33)	0.35 (0.05)	4.69 (0.12)	71.47 (26.89)	13.18 (7.46)	8	0.70 (0.15)	0.34 (0.06)
23	23.2 (0.3)	25	1.06 (0.09)	50.32 (18.21)	16.16 (7.66)	0.38 (0.04)	4.65 (0.14)	95.98 (27.06)	13.04 (6.60)	8	0.94 (0.34)	0.43 (0.14)
12	23.3 (2.3)	25	1.12 (0.11)	37.92 (10.42)	12.37 (7.91)	0.38 (0.03)	4.82 (0.18)	72.08 (16.43)	9.75 (6.67)	7	1.04 (0.40)	0.43 (0.14)
13	23.5 (2.2)	25	1.16 (0.11)	39.40 (15.36)	22.95 (2.22)	0.41 (0.04)	4.66 (0.21)	68.84 (21.41)	16.35 (0.58)	8	0.89 (0.17)	0.39 (0.06)
19	25.6 (1.3)	25	1.14 (0.07)	28.93 (16.76)	18.33 (8.51)	0.41 (0.03)	4.63 (0.12)	52.60 (30.66)	12.97 (5.75)	8	0.91 (0.29)	0.39 (0.13)
3	25.8 (2.6)	25	0.94 (0.23)	29.31 (9.98)	20.11 (5.40)	0.32 (0.08)	4.83 (0.25)	73.15 (37.13)	18.22 (0.43)	1	0.84	0.33
17	26.3 (0.4)	25	1.05 (0.17)	30.76 (18.97)	17.72 (4.65)	0.37 (0.07)	4.67 (0.17)	64.46 (44.69)	14.31 (4.14)	6	1.06 (0.26)	0.44 (0.10)

acid-washed glass slides to dry for 36 h under a class-100 laminar-flow hood. After drying, otoliths were individually weighed to the nearest 10 µg and transferred to individual acid-washed 1.5 ml high-density polyethylene vials.

A maximum of 8 fish were randomly selected from each tank and their otoliths prepared for analysis by inductively coupled plasma mass spectrometry (ICP-MS). If the total number of remaining fish in the tank after the experimental period was less than 8, all remaining fish were used for analyses. Otoliths were dissolved in 70% ultrapure nitric acid (Ultrex II, J. T. Baker) and the solution diluted to achieve a total dissolved solid concentration of 0.1 mg g⁻¹ in 1% HNO₃. Otolith solutions were stored at 4°C until the ICP-MS analysis. Otolith analyses were run on a Thermo Finnigan Element ICP-MS equipped with a self-aspirating (50 µl min⁻¹) PFA nebulizer and a dual-inlet quartz spray chamber. The method measured ²⁵Mg, ⁴⁸Ca, ⁵⁵Mn, and ¹³⁸Ba in low resolution (R = 300) during a 2 min acquisition time.

Quantification of metal/calcium (Me/Ca) ratios followed the procedure outlined by Rosenthal et al. (1999). All samples were standardized to a dissolved solution (0.1 mg g⁻¹) of an otolith reference powder with certified Me/Ca ratios of 89.25 µmol mol⁻¹ for Mg/Ca, 0.257 µmol mol⁻¹ for Mn/Ca, and 2.174 µmol

mol⁻¹ for Ba/Ca (Yoshinaga et al. 2000). The matrix of the standard was therefore matched to the dissolved Ca levels in the samples. Detection limits were calculated as 3σ values of 1% HNO₃ sample blanks (n = 37) that were run throughout the analyses. These limits were 0.2% of the average sample intensity for ²⁵Mg, 0.05% for ⁴⁸Ca, 0.3% for ⁵⁵Mn, and 0.1% for ¹³⁸Ba. An internal laboratory standard was run after each reference sample to estimate precision of the Me/Ca method. The reference material was then treated as an unknown, and Me/Ca values determined as for individual samples above. Measured precision (% relative standard deviation [RSD], n = 37) of the Me/Ca method was 2.7% for Mg/Ca, 2.8% for Mn/Ca and 0.5% for Ba/Ca.

Water samples collected during the experiment were also analyzed using ICP-MS. Every other week, 4 samples were analyzed from each tank. All samples were spiked with an internal elemental standard of Indium (to 4.5 µg g⁻¹). The solutions were then aspirated into a Thermo Finnigan Element 2 ICP-MS, via a self-aspirating nebulizer (50 µl min⁻¹) and Scott's double pass spray chamber. Due to the presence of significant interferences on most of the Ca isotopes, ⁴⁴Ca, ²⁵Mg, ⁵⁵Mn, ¹³⁷Ba, and ¹¹⁵In were measured in medium resolution (nominal R = 4500). The means of 4 water samples from each tank were used in all subsequent

analyses. A seawater reference material (High Purity Standards, seawater CRM [certified reference material]) was used to estimate precision of the water measurements, including Ca, Mg, Mn, and Ba values. Our estimates of precision for element concentrations in the seawater CRM were 1.4 % RSD for Ca, 1.4 % RSD for Mg, 2.7 % RSD for Mn, and 1.7 % RSD for Ba ($n = 8$).

Partition coefficients (D_{Me}) were calculated by dividing the metal/calcium (Me/Ca) ratio measured in an otolith by the mean Me/Ca ratio measured in the treatment tank water (Morse & Bender 1990). Otolith Me/Ca values from individual fish were averaged within tanks, and then the 3 tank averages were used as replicates for each of the 8 treatments.

Otolith mass was used as a proxy for otolith precipitation rate. Because all fish were the same age, otolith precipitation rate was greater in individuals with larger otoliths. Analysis of covariance (ANCOVA) was used to test the influence of otolith precipitation rate on each D_{Me} , using otolith mass as a covariate and temperature and salinity as independent categorical variables. Using Pearson correlations, the influence of otolith precipitation rate was also tested by correlating otolith mass with D_{Me} within each of the 23 tanks of sufficient sample sizes. This provided a test of rate effects on D_{Me} because all fish within the tanks experienced identical environmental conditions (Bath et al. 2000, Martin et al. 2004).

Change in standard length (SL) over the course of the experiment was used as a proxy for somatic growth rate. Individual growth rates were calculated as the difference between the mean SL of pooled fish at the beginning of the experiment and the SL of individual fish at the end of the experiment divided by the number of experiment days. The means of fish growth rates were calculated for individual tanks. ANCOVAs were used to test the influence of somatic growth rate on each D_{Me} , using growth rate as a covariate and temperature and salinity as independent categorical variables. Finally, using Pearson correlations, the influence of somatic growth rate on otolith precipitation rate was tested by correlating growth rate with D_{Me} within each of the 23 tanks with sufficient sample sizes.

Two-way analysis of variance (ANOVA) was used to test for significant differences in $[Me/Ca]_{water}$, $[Me/Ca]_{otolith}$, and D_{Me} among temperature and salinity treatments. Salinity and temperature were treated as independent categorical variables, and $[Me/Ca]_{water}$, $[Me/Ca]_{otolith}$, and D_{Me} as dependent variables in the analyses. The assumptions of ANOVA were met: the data were normally distributed and variances were homogeneous among factor levels. To control for experiment-wise error, the critical p-value ($\alpha = 0.05$) was adjusted ($\alpha = 0.017$) to account for the 3 individual ANOVAs performed.

RESULTS

Water chemistry

Elemental concentrations in the tank water were significantly different between salinity treatments, but not among temperatures. As expected, Ca, Mg, Mn, and Ba concentrations in the water were higher at 25‰ than at 15‰ ($[Ca]_{water}$: $lt|_{0.05,(2),22} = 14.62$, $p = 0.000$; $[Mg]_{water}$: $lt|_{0.05,(2),22} = 16.57$, $p = 0.000$; $[Mn]_{water}$: $lt|_{0.05,(2),22} = 2.99$, $p = 0.003$; $[Ba]_{water}$: $lt|_{0.05,(2),22} = 4.03$, $p = 0.000$). $[Mg/Ca]_{water}$ was not significantly different between salinity treatments ($F = 0.565$, $p = 0.463$, $n = 24$) or temperature treatments ($F = 0.339$, $p = 0.797$, $n = 24$). However, $[Mn/Ca]_{water}$ values were significantly higher at 15‰ than at 25‰ ($F = 32.325$, $p = 0.000$, $n = 24$), and differed significantly among temperature treatments ($F = 6.648$, $p = 0.004$, $n = 24$). Similarly, $[Ba/Ca]_{water}$ values were also significantly higher at 15‰ than at 25‰, ($F = 19.282$, $p = 0.000$, $n = 24$), but not among temperature treatments ($F = 0.366$, $p = 0.779$, $n = 24$). Lower absolute Mn, Ba, and proportionately lower Ca concentrations in 15‰ salinity tanks led to higher Mn/Ca and Ba/Ca ratios in the low salinity treatment (Table 1, Fig. 1) presumably because Mn/Ca and Ba/Ca ratios were higher in the deionized water source than in the 25‰ salinity artificial seawater.

Otolith [Me/Ca] and Me/Ca partition coefficients

Leiostomus xanthurus were initially reared in a single large tank before being randomly assigned and transferred to smaller tanks for the duration of the experiment. We assumed that conditions during the initial rearing period had little effect on the resultant otolith chemistry of the experimental fish because otoliths from these fish averaged less than 50 μg compared to a mean value of 890 μg for otoliths from fish at the end of the experiment.

The Mg/Ca values in the *Leiostomus xanthurus* otoliths ($n = 173$) ranged from 0.676 to 4.74 mmol mol^{-1} , with an overall mean of 1.80 mmol mol^{-1} . Magnesium partition coefficients (D_{Mg}) were considerably less than 1 (among treatment mean = 0.00038, range 0.00014 to 0.001), suggesting that Mg ions were actively excluded from the otolith either during movement across membranes or at the site of aragonite precipitation in the endolymph. We found no significant effects of temperature or salinity on Mg/Ca ratios, and a non-significant interaction (Table 2, Fig. 2). D_{Mg} showed a similar pattern to the Mg/Ca ratios, with no significant effects of temperature or salinity, and an insignificant interaction (Table 3, Fig. 3).

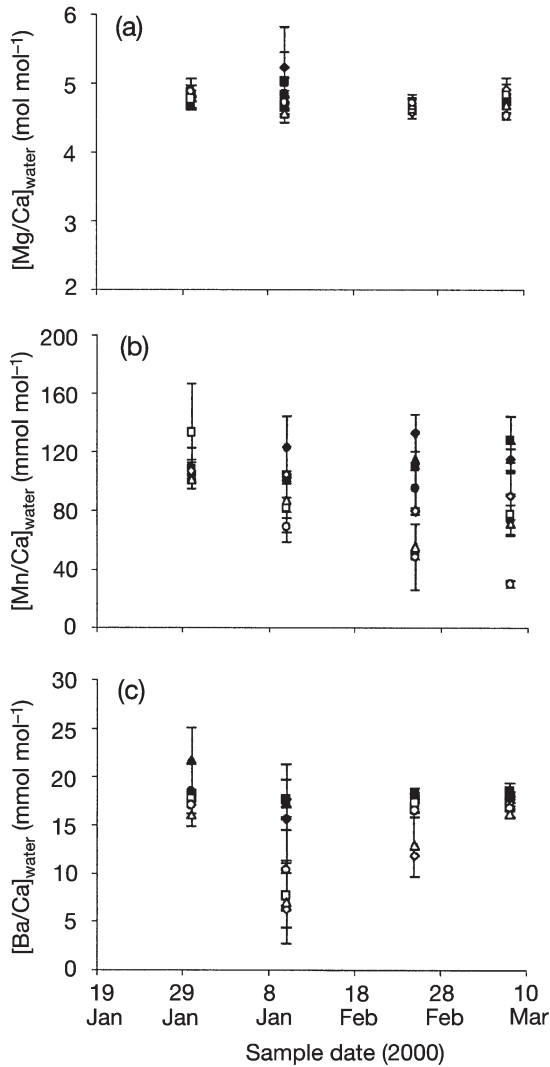


Fig. 1. Calculated metal/calcium ratios in the tank water (a) Mg/Ca, (b) Mn/Ca, and (c) Ba/Ca concentrations \pm SE by sample date for the duration of the experiment. Solid symbols represent the 15‰ salinity treatment tanks and the open symbols represent the 25‰ salinity treatment tanks. Each of the 4 temperature treatments is represented by a different symbol: 17°C (\blacklozenge), 20°C (\blacksquare), 23°C (\blacktriangle), and 26°C (\blacklozenge)

Manganese values ($[Mn/Ca]_{otolith}$) in the *Leiostomus xanthurus* otoliths varied by more than an order of magnitude (6.32 to 101.01 $\mu\text{mol mol}^{-1}$) with an overall mean of 18.055 $\mu\text{mol mol}^{-1}$. Estimates of the Mn/Ca partition coefficient (D_{Mn}) varied over a similar range (0.055 to 0.92), with an overall mean of 0.196. We found no significant effects of temperature, salinity, or their interaction on $[Mn/Ca]_{otolith}$ (Table 2, Fig. 2). However, among-tank variation in water chemistry clearly influenced our results because D_{Mn} were significantly affected by both temperature and salinity,

Table 2. *Leiostomus xanthurus*. Results of 2-way ANOVA testing the effect of temperature (T), salinity (S) and the temperature–salinity interaction (T \times S) on \log_e -transformed Mg/Ca, Mn/Ca and Ba/Ca ratios in the otoliths of juvenile *L. xanthurus*. p is not significant for all entries

	Factor	df	MS	F
$[Mg/Ca]_{otolith}$	T	3	483322.15	3.660
	S	1	361225.57	2.736
	T \times S	3	47976.76	0.363
$[Mn/Ca]_{otolith}$	T	3	33.497	1.766
	S	1	5.484	0.289
	T \times S	3	33.343	1.758
$[Ba/Ca]_{otolith}$	T	3	2.567	0.717
	S	1	12.285	3.431
	T \times S	3	2.273	0.635

and the interaction term (Table 3). The significant interaction was generated by the observation that D_{Mn} increased with temperature in the 15‰ salinity treatment but not in the 25‰ treatment.

The Ba/Ca ratios in *Leiostomus xanthurus* otoliths ranged from 2.02 to 15.8 $\mu\text{mol mol}^{-1}$, with an overall mean of 6.01 $\mu\text{mol mol}^{-1}$. Barium partition coefficients (D_{Ba}) were closer to equilibrium with ambient water than either D_{Mn} or D_{Mg} , with an overall mean of 0.37 and a range of 0.11 to 1.23. Temperature, salinity, and the temperature–salinity interaction had no significant effect on $[Ba/Ca]_{otolith}$ (Table 2, Fig. 2). As with Mn, however, we attributed at least some of the variability in $[Ba/Ca]_{otolith}$ to among-tank variability in $[Ba/Ca]_{water}$ within treatments. We found a significant effect of salinity on D_{Ba} , with non-significant temperature effect, and non-significant interaction between the 2 factors (Table 3). D_{Ba} were higher at 15‰ than at 25‰ (Fig. 3).

Table 3. *Leiostomus xanthurus*. Results of 2-way ANOVA testing the effect of temperature (T), salinity (S) and the temperature–salinity interaction (T \times S) on \log_e -transformed Mg, Mn, and Ba partition coefficients (D_{Mg} , D_{Mn} and D_{Ba}) in the otoliths of juvenile *L. xanthurus* (ns = non-significant, *significant at $\alpha = 0.017$)

	Factor	df	MS	F	p
D_{Mg}	T	3	0.019	3.584	ns
	S	1	0.018	3.452	ns
	T \times S	3	0.002	0.450	ns
D_{Mn}	T	3	0.020	13.617	*
	S	1	0.020	13.316	*
	T \times S	3	0.008	5.187	*
D_{Ba}	T	3	0.021	0.640	ns
	S	1	0.267	8.158	*
	T \times S	3	0.021	0.648	ns

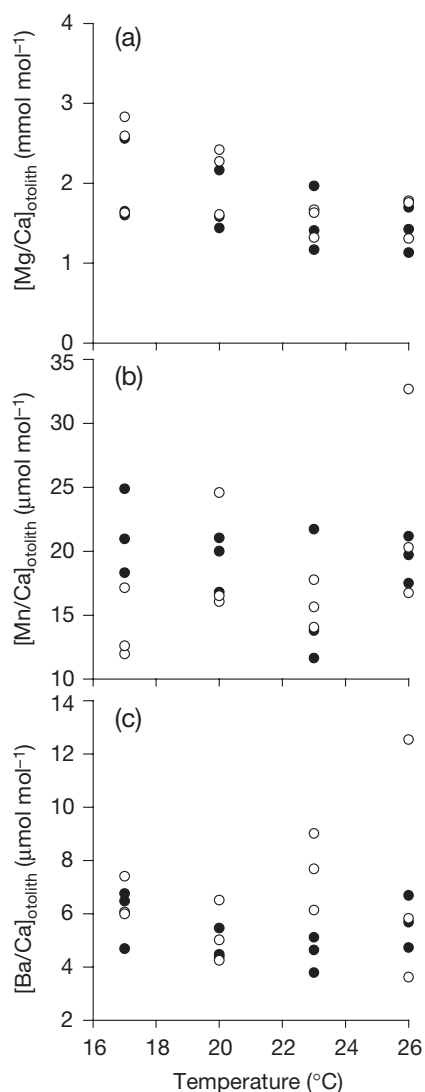


Fig. 2. *Leiestomus xanthurus*. (a) Mg/Ca, (b) Mn/Ca, and (c) Ba/Ca ratios in otoliths of laboratory-reared *L. xanthurus* as a function of tank temperatures at 2 salinity levels, 15‰ (●) and 25‰ (○)

Biom mineralization and growth rate effects

We used otolith mass as a proxy for otolith precipitation rates to examine potential effects of calcification rate on otolith chemistry using ANCOVA (Table 4). Otolith mass had a significant effect on D_{Mg} ($F = 56.65$, $p = 0.000$, $n = 24$), no significant effect on D_{Mn} ($F = 0.06$, $p = 0.81$, $n = 24$), and no significant effect on D_{Ba} ($F = 0.57$, $p = 0.46$, $n = 24$). However, it is possible that rate effects were aliased to some degree by significant variability in D_{Me} as a function of temperature and salinity. We therefore ran Pearson correlations between otolith mass and D_{Me} for each of 23 tanks, because individual fish within a tank had experienced identical condi-

tions. A total of 13 out of 23 correlations between otolith mass and D_{Mg} were negative, and 4 out of 23 correlations were significant after applying the Bonferroni correction for multiple tests. A total of 15 out of 23 correlations were negative for D_{Mn} , but none of the 23 correlations were significant after the appropriate Bonferroni correction. Similarly, although a total of 10 out of 23 correlations between D_{Ba} and otolith mass were negative, only 3 of the 23 within-tank correlations were statistically significant.

Somatic growth rates of *Leiestomus xanthurus* varied significantly with temperature ($F = 4.52$, $p = 0.019$,

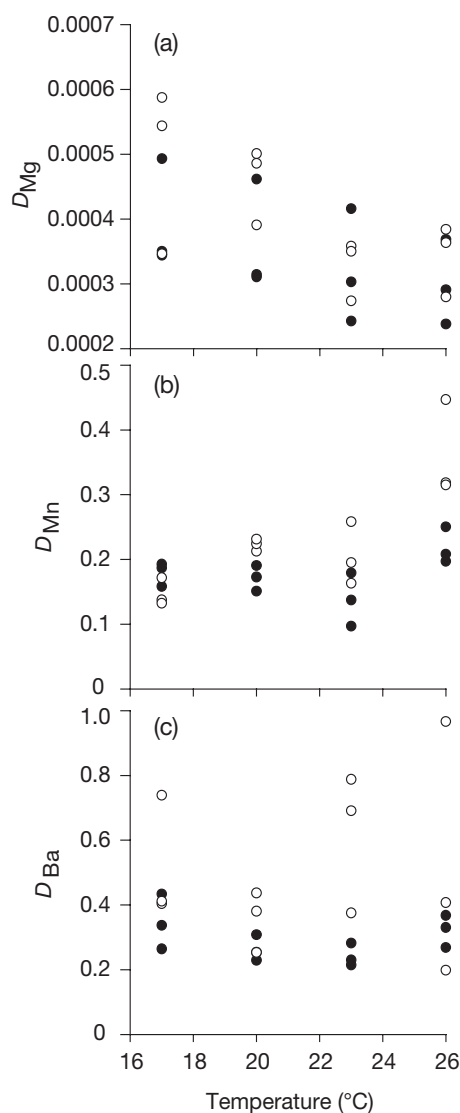


Fig. 3. *Leiestomus xanthurus*. (a) Mg/Ca partition coefficients (D_{Mg}), (b) Mn/Ca partition coefficients (D_{Mn}), and (c) Ba/Ca partition coefficients (D_{Ba}) for otoliths of laboratory-reared *L. xanthurus* as a function of tank temperatures at 2 salinity levels, 15‰ (●) and 25‰ (○)

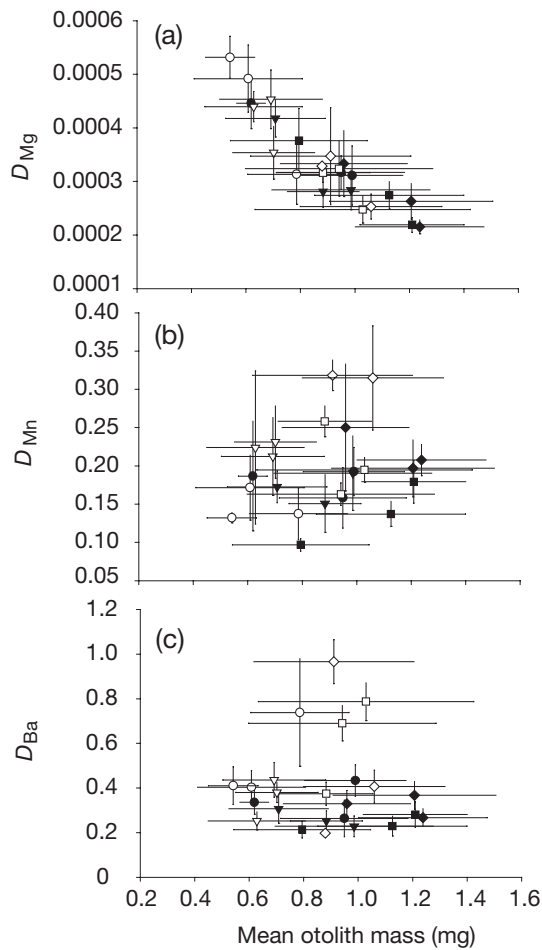


Fig. 4. *Leiestomus xanthurus*. (a) Mg/Ca partition coefficients (D_{Mg}), (b) Mn/Ca partition coefficients (D_{Mn}), and (c) Ba/Ca partition coefficients (D_{Ba}) for otoliths of laboratory-reared *L. xanthurus* as a function of otolith mass within each tank. Each of the 4 temperature treatments is represented by a different symbol: 17°C (●), 20°C (▼), 23°C (■), and 26°C (◆) at 2 salinity levels, 15‰ (solid) and 25‰ (open)

$n = 24$), and salinity ($F = 11.43$, $p = 0.004$, $n = 24$). Growth rates also influenced D_{Mg} ($F = 18.14$, $p = 0.001$, $n = 24$), but had no detectable effect on either D_{Mn} ($F = 0.08$, $p = 0.79$, $n = 24$) or D_{Ba} ($F = 0.02$, $p = 0.90$, $n = 24$) (Fig. 4). Within tank correlations between growth rate and D_{Mg} found that 13 out of 23 correlations were negative, and 5 out of 23 correlations were significant after Bonferroni adjustment. Correlations between growth rate and D_{Mn} demonstrated that 13 out of 23 correlations were negative, although none of the 23 correlations were statistically significant. Finally, 12 out of 23 correlations between D_{Ba} and growth rate within each of the tanks were negative, and 5 of the 23 correlations were significant after Bonferroni adjustment.

DISCUSSION

Numerous environmental and biological effects are likely to influence the elemental composition of fish otoliths. Many of these effects are also likely to covary. For instance, fish growth rate, otolith precipitation rate, and temperature are all likely to be positively correlated in the field collections. We reared fish in the laboratory under controlled environmental conditions to examine temperature and salinity effects on the incorporation of Mg, Mn, and Ba in fish otoliths while minimizing other influences on otolith chemistry. All fish used in the experiment were from the same brood stock and were the same age, therefore limiting possible genetic or ontogenetic effects on otolith elemental incorporation. Diets among treatments were identical, thereby eliminating the effect of food on otolith elemental incorporation. The elemental composition of the water was monitored throughout the experiment, allowing us to account for significant differences in water chemistry among treatments. Therefore, we were able to test the effects of temperature and salinity on the elemental composition of otoliths without the confounding effects of genetics, ontogeny, diet, or water compositional differences.

Water chemistry

Mg/Ca ratios were relatively constant among all treatment tanks throughout the experiment, with no significant difference among temperature and salinity treatments. There was greater variation in Mn/Ca and Ba/Ca among treatments, despite our attempts to maintain constant elemental ratios in all the tanks. Both Mn/Ca and Ba/Ca were significantly higher at 15‰ than at 25‰. The water for the lower salinity treatment was a dilution of the higher salinity water, and therefore both Mn/Ca and Ba/Ca were higher in the deionized water used to generate the 15‰ treatment than in the artificial seawater at 25‰. A similar result was also noted for Sr/Ca ratios in the same experiment (Martin et al. 2004). Nonetheless, by collecting water samples at regular intervals throughout the experiment we were able to quantify the water chemistry experienced by fish in each of the tanks and therefore account for any variability in water elemental composition within tanks throughout the experiment by calculating partition coefficients (Morse & Bender 1990).

Mg partition coefficients

Several studies have suggested that Mg/Ca ratios in the aragonitic skeletons of corals (Mitsuguchi et al.

1996, 2003) and mollusk shells (Crick & Ottensmeyer 1983, Vander Putten et al. 2000) may vary with temperature. Magnesium thermometry is more established in calcitic skeletons of foraminifera (Lea et al. 1999, Elderfield & Ganssen 2000, Rosenthal & Lohmann 2002) where, as with coral aragonite, D_{Mg} is positively correlated with temperature. Although D_{Mg} consistently declined with increasing temperature, the effect of temperature was not significant ($p = 0.037$; $\alpha =$ Bonferroni corrected 0.017). Because D_{Mg} in otoliths are considerably lower than those of aragonitic coral skeletons, we suspect that there is considerably more biological control over Mg/Ca ratios in fish otoliths than in the skeletons of hermatypic corals. It will, therefore, likely be difficult to reconstruct temperature profiles based on Mg/Ca ratios in otoliths.

Mn partition coefficients

We found evidence of complex interactions between otolith D_{Mn} , temperature, and salinity. Specifically, salinity appeared to influence D_{Mn} only at the highest temperature in our experiment. At least some of the variability in $[Mn/Ca]_{otolith}$ may have been due to our inability to maintain constant $[Mn/Ca]_{water}$ in the individual tanks. Dissolved Mn is rapidly oxidized in estuarine waters, aided by microbial catalysis which plays a dominant role in the scavenging of Mn onto particles in the aquatic environment (Sunda & Huntsman 1987, von Langen et al. 1997, Klinkhammer & McManus 2001). We suspect that microbial-aided oxidation was also occurring in the experimental tanks. Bacterial growth was observed in the tanks, even though water was changed daily (50%) and solid particles were removed by siphoning. It was therefore possible that bacteria served as catalysts forming manganese oxides, which precipitated out of solution, contributing to unstable Mn/Ca concentrations in the tanks over time.

It has proved difficult to ascertain any correlation between $[Mn/Ca]_{otolith}$ and $[Mn/Ca]_{water}$ even under laboratory conditions. Elsdon & Gillanders (2002) found no effect of either temperature or salinity on Mn/Ca ratios in the otoliths of juvenile black bream *Acanthopagrus butcheri*. More tellingly, in a later study the researchers were unable to detect any correlation between $[Mn/Ca]_{otolith}$ and $[Mn/Ca]_{water}$ in an experiment in which individual tanks were spiked with Mn up to 16 times above ambient concentrations (Elsdon & Gillanders 2003). The results were somewhat surprising, as several authors have found that Mn was a useful element when attempting to discriminate among fish from different geographic locations based on otolith geochemistry (Thorrold et al. 1998a,b, Rooker et al. 2001, 2003). We suggest, however, that it is pre-

ture to ignore the effect of water chemistry on Mn/Ca ratios in otoliths. D_{Mn} certainly explained more variation among treatments in our experiment than did $[Mn/Ca]_{otolith}$. Nonetheless, more experimentation is clearly required before we obtain a mechanistic explanation of Mn/Ca variations in fish otoliths.

Ba partition coefficients

All studies to date have found that Ba/Ca ratios in otoliths accurately reflect dissolved Ba levels in the environment (Bath et al. 2000, Milton & Chenery 2001, Elsdon & Gillanders 2003). Effects of temperature and salinity on D_{Ba} have, however, been more variable when tested. We found that salinity but not temperature affected D_{Ba} in larval spot otoliths. Bath et al. (2000) also found that temperature had no effect on D_{Ba} in the otoliths of *Leiostomus xanthurus*, although they did not test for salinity effects in their experiment. Elsdon & Gillanders (2002) reported significant interactions between temperature and salinity on Ba/Ca ratios in the otoliths of *Acanthopagrus butcheri*. Unfortunately Elsdon & Gillanders (2002) did not report D_{Ba} ; therefore it was difficult to draw any definitive conclusions from their study. Experiments on inorganic aragonite found that D_{Ba} is negatively correlated with temperature between 10 and 50°C, with the temperature effect being an order of magnitude larger than that of D_{Sr} (Dietzel et al. 2003). A recent study found that temperature was also negatively correlated with D_{Ba} in the aragonitic protoconchs of larval gastropod mollusks (Zacherl et al. 2003). Therefore, although Ba/Ca ratios in calcified structures may have potential for recovering temperature histories, the work by Zacherl et al. (2003) remains the only convincing example of temperature effects on Ba/Ca ratios in biogenic aragonite.

We were able to detect significant effects of salinity on D_{Ba} , with higher values at 15‰ than at 25‰. Similar to Sr (Martin et al. 2004), absolute Ba levels in the tank waters appeared to influence otolith Ba/Ca ratios beyond that predicted by $[Ba/Ca]_{water}$. We argued, based on the findings of Chowdhury & Blust (2001, 2002), that positive, non-linear inhibition of Ca ions by Sr ions at uptake sites on branchial or intestinal membranes would result in the pattern of $[Sr]_{water}$ and $[Sr/Ca]_{otolith}$ that we observed. Although Chowdhury & Blust (2001, 2002) did not examine Ba in their experiments, it is certainly possible that Ba ions may also inhibit Ca ions in a similar manner to Sr. Whatever the mechanism, Ba/Ca levels in larval spot otoliths reflect both $[Ba/Ca]_{water}$ and $[Ba]_{water}$ but not temperature, and therefore $[Ba/Ca]_{otolith}$ is likely to accurately reflect dissolved Ba levels in aquatic systems.

Biom mineralization and fish growth rate effects

There is little consensus on the effect of precipitation rate effects on elemental uptake in biogenic aragonite. A couple of studies have suggested that magnesium incorporation in coral skeletons is independent of skeletal extension rates (Watanabe et al. 2001, Mitsu-gushi et al. 2003). We found that D_{Mg} was negatively correlated with both otolith precipitation rate and somatic growth rate. Precipitation rate effects are presumably kinetic in nature and occur at the site of aragonite deposition in the endolymph. Metabolic effects are likely to occur during transport of ions across membranes or within the blood plasma. However, because precipitation and growth rates covaried significantly it was impossible to determine the influence of either effect in isolation. Indeed, somatic growth, otolith precipitation rates, and temperature are often highly correlated in the field, and so it will be challenging to design an experiment to examine these effects independently.

We found no evidence that D_{Mn} or D_{Ba} in otoliths were affected by either otolith precipitation or metabolic rates. The results were similar to those obtained for D_{Sr} in the same experiment (Martin et al. 2004). The partition coefficients of all 3 elements are significantly lower than 1, indicating that there clearly is a degree of physiological control over Mn, Sr, and Ba ratios in fish otoliths. Nonetheless, physicochemical properties of the ambient water appear to be primarily responsible for natural variations in concentrations of these elements.

CONCLUSION

We were able to test the effects of temperature and salinity on the elemental composition of *Leiostomus xanthurus* otoliths without the confounding effects of genetics, ontogeny, diet, or water compositional differences. Physicochemical properties of the ambient water appear to be primarily responsible for natural variations in concentrations of Mn, Sr, and Ba ratios in spot otoliths, while Mg incorporation in spot otoliths is confounded by the negative correlation with otolith precipitation and somatic growth rates. Our results draw attention to the complexity of element incorporation in otoliths, and propose that both environmental and physiological factors influence elemental ratios in fish otoliths. Thus, we emphasize the importance of acknowledging and measuring these variables to validate geochemical signatures in fish otoliths.

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