

Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time

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ABSTRACT: Chemical elements within calcified structures of organisms such as fish, corals, bivalves, gastropods and foraminiferal shells can provide a record of the environmental characteristics at the time of calcification. To predict accurately the environmental characteristics at the time of calcification, it is important to understand the influence of exposure time on elemental incorporation. We examined the effect of enhanced ambient strontium:calcium (Sr:Ca) ($2\times$ and $4\times$ ambient concentrations) and different periods of exposure (2, 4, 8, 16 and 32 d) on Sr:Ca uptake and incorporation into fish otoliths of the black bream *Acanthopagrus butcheri* (family: Sparidae). Sr:Ca ratios (hereafter referred to as concentrations) in bream otoliths increased with increasing ambient concentrations, with the amount of Sr:Ca incorporated into otoliths being positively affected by the period of exposure. Saturation of Sr:Ca in otoliths occurred after 20 d of exposure. Importantly, by measuring multiple variables of elemental uptake and incorporation, the relative effect of enhanced concentration and exposure time can be separated, which to date has not been possible. This research will allow for greater accuracy when interpreting environmental histories of fish.

KEY WORDS: Trace element · Strontium · Laser ablation ICP-MS · Uptake · Exposure · Transects · Migration

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INTRODUCTION

Elements within calcified structures of fish, corals, molluscs and foraminiferal shells have been used to reconstruct environmental histories of extant and extinct species (e.g. Lea & Spero 1994, Brown & Luoma 1995, Shen et al. 1996, Alibert & McCulloch 1997, Rosenthal et al. 1997, Tzeng et al. 1997, Secor et al. 2001, Ikeda et al. 2003, Zacherl et al. 2003). Environmental histories commonly described include water temperature (Alibert & McCulloch 1997), salinity (Kafemann et al. 2000) and ambient elemental concentrations (Secor et al. 2001), all of which can reconstruct past climatic conditions. For example, past environments inhabited by *Porites* corals have been inferred from Sr concentrations, where temperature has been shown to affect Sr incorporation (Alibert & McCulloch 1997).

Reconstructions of environmental histories commonly use equations that are derived from either manipulative laboratory experiments, using controlled conditions (e.g. Elsdon & Gillanders 2003b), or by measurements during field collection (e.g. Shen et al. 1998). Such equations describe the effects of environmental variables on the elemental concentrations within calcified structures, without accounting for the amount of time organisms are exposed to environmental variables. Many calcified structures, such as fish otoliths (earbones) and spines, are not in direct contact with the surrounding seawater, with physiological (cellular) and physical boundaries existing (concentration gradients in organisms). Elements must pass through these boundaries before being incorporated into the matrix of calcified structures; therefore, it is plausible that changes in environmental variables would not

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result in instantaneous changes in elements within calcified structures. Consequently, the rate at which elements are incorporated into calcified structures would affect the interpretation of environmental histories, both spatially and temporally.

The time required for elements to be incorporated into calcified structures is largely unknown, although there have been several attempts to examine the effect of exposure time to enhanced elemental concentrations on the uptake and incorporation of elements in fish otoliths, spines and vertebrae (Snyder et al. 1992, Ennevor & Beames 1993, Brown & Harris 1995, Pollard et al. 1999). Due to technical constraints, all of these experiments analysed whole calcified structures using solution-based techniques (e.g. whole otolith), and, in doing so, the derived elemental signatures were a mixture over the entire chronology of the structure. As reconstructions of environmental histories require high resolution of elemental concentrations, sample analysis requires the use of fine-scale micromilling or laser instruments (Elsdon & Gillanders 2003a). Nevertheless, experiments using solution-based techniques have shown that an increased period of exposure to environmental variables results in enhanced concentrations of elements being incorporated into calcified structures of fish (e.g. Pollard et al. 1999). Further experiments using more modern sample analysis are needed to assess the effects of exposure time on elemental incorporation; these experiments will provide more accurate interpretations of environmental histories of organisms.

Sr in otoliths is largely derived from the surrounding water (Milton & Chenery 2001), with minimal dietary influence. Numerous experiments have related changes in ambient Sr to changes in calcified structures of fish otoliths (e.g. Schroder et al. 1995, Bath et al. 2000, Elsdon & Gillanders 2003b), fish scales (e.g. Mugiya et al. 1991, Wells et al. 2000) and foraminiferal shells (e.g. Lea & Spero 1992). For Sr, a strong positive relationship exists between ambient and otolith concentrations. Although the relationship between the ambient elements and that of the calcified structure remains near linear for a large number of organisms (such as corals and foraminiferal shells, Lea & Spero 1992, Ferris-Pagès et al. 2002), otoliths display elemental discrimination, due to environmental and biological factors affecting elemental incorporation (such as temperature, Bath et al. 2000, Elsdon & Gillanders 2003b).

Reconstructions of the environmental histories of fish using the elements in calcified structures have been based on fine-scale analysis, using either laser or micromilling techniques (e.g. Kafemann et al. 2000), where peaks in the absolute elemental concentration are related to environmental variables (e.g. Tzeng et al. 2002). Previously, Elsdon & Gillanders (2003a) have

shown that the scale of otolith analysis, fine 30 μm transects versus large 100 μm spot sampling, affects the detected elemental concentrations in laboratory-reared fish. Thus, fine-scale analyses allow for greater and more accurate resolution of environmental histories. The analysis of transects across structures results in a continuous elemental record that can be interpreted using several variables (e.g. height, distance, slope and areas of peaks and troughs; see Fig. 1); each variable relates to a function of elemental uptake and incorporation. Fine-scale transect analysis can be used to assess the effect of exposure time to changes in environmental variables on the elemental incorporation into otoliths. The resulting data should aid the interpretation of environmental histories of fish.

The lack of information regarding the influence of exposure time on elemental uptake into calcified structures is a severe inhibitor in determining environmental histories of organisms. In the present study, we tested the hypothesis that the effect of exposure to enhanced concentrations of ambient Sr on the concentration of otolith Sr is significantly affected by the exposure time. Specifically, juvenile black bream *Acanthopagrus butcheri* were exposed to enhanced concentrations of Sr, for a range of time periods (from 2 to 32 d), in controlled laboratory conditions. The relative and interactive effects of concentration and exposure time on Sr concentration in otoliths were then documented.

MATERIALS AND METHODS

Experimental design. Juvenile black bream *Acanthopagrus butcheri* of approximately 25 mm total length were obtained from a hatchery and held in replicate 250 l fibreglass holding tanks containing seawater (33‰) of ambient elemental concentration at 20°C. Tanks were supplied with filtration and aeration. The fish were bred from a common brood stock to reduce genetic variability; the influence of genetics on otolith chemistry is unknown. Fish were held in the holding tanks for 10 d before being exposed to experimental conditions. All seawater used during the rearing process was collected weekly from the South Australian Research and Development Institute, Aquatic Sciences Division, and was held in a covered and aerated 2000 l indoor tank. Commercial fish food was used during the experiments. Light was provided from fluorescent tubes on a 12 h day:12 h night cycle, in a constant-temperature room (20°C).

Black bream otoliths were marked with alizarin complexone (36 ppm) to enable old growth to be distinguished from new growth, which was laid down under experimental conditions. After the initial acclimatisa-

tion period of 10 d in 1 of 2 holding tanks, fish were randomly assigned to experimental treatments at densities of 10 to 14 fish per tank. Each tank consisted of a 40 l high-density polyethylene tub supplied with aeration and covered with a clear Plexiglas lid to minimise evaporative loss that may change the water chemistry.

The experiment involved manipulating both the concentration of Sr in the rearing water and the time of exposure to enhanced Sr in an orthogonal 2-way design, to test the relative and interactive effects of these factors on otolith Sr. Treatments consisted of all possible combinations of Sr spiking (2 × and 4 ×) and times of exposure (2, 4, 8, 16 and 32 d). At the start of the experiment, fish were transferred from the holding tanks to experimental tanks and held at an enhanced Sr concentration for the prescribed time period, after which all water was changed back to ambient strontium concentrations (referred to as post-spiking) and held constant until the experiment was terminated (32 d). For each treatment, duplicate tanks were used to assess variability in both the elemental composition of the water and otoliths. A total of 22 tanks were used, 2 of which were control tanks with fish held at ambient Sr concentrations. Sr spiking consisted of the addition of appropriate amounts of standard solution made from $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ mixed with seawater. To maintain water quality, 50% of the rearing water was siphoned off every second day and replaced; any accumulated detritus on the bottom of tanks was removed daily.

During the rearing period, temperature and salinity were monitored on random days ($n = 20$). Replicate water samples were collected on random days ($n = 3$ per tank) to determine the actual concentrations of Sr and Ca in the water. After 32 d the experiment was terminated, at which time all fish were immersed in an ice slurry. The sagittal otoliths were dissected, washed thoroughly, cleaned in Milli-Q water and stored in microcentrifuge tubes. Then, 7 fish from each treatment tank were selected, based on similarities of length, and their otoliths were prepared for elemental analysis.

Elements in otoliths. Otoliths from 7 fish per tank (154 otoliths in total) were embedded in epofix resin that had been spiked with 40 ppm indium to allow for discrimination between the otolith and resin upon analysis. Otoliths were sectioned transversely through the focus (centre section) using a low-speed diamond saw, which was lubricated with Milli-Q water. Sections (approximately 300 μm thick) were polished using 30 and 9 μm lapping film until they were approximately 200 μm thick. Polished sections were fixed to glass slides with epofix resin and allowed to dry, after which time slides were sonicated in Milli-Q water for 5 min and dried in a plastic laminar flow cabinet. Slides were stored in clean, sealable plastic bags until analysed.

The concentration of Sr and Ca in otolith samples was determined using a Merchantek LUV 266 Q-switched Nd: YAG UV laser microprobe, with a pulse rate of 6.00 Hz, energy of 1.0 mJ and spot size of 30 μm . The laser ablation station was connected to a Finnigan MAT ELEMENT HR-ICP-MS (High Resolution–Inductively Coupled Plasma–Mass Spectrometer) with gas flow parameters of coolant 14.00, auxiliary 1.55, sample 1.5 and helium 0.36 l min^{-1} . Ablations occurred inside a sealed chamber with the sample gas being extracted to the HR-ICP-MS via a smoothing manifold in the presence of argon and helium gas. The chamber was purged for 20 s after each ablation, to remove background gas or sample from previous ablations that could contaminate resolved concentrations of elements (Lahaye et al. 1997, Elsdon & Gillanders 2002). Background concentrations of elements within the chamber were measured before each ablation (for 30 s), to correct sample concentrations. During sample ablations the laser scanned at approximately 1.3 $\mu\text{m s}^{-1}$ across the otolith surface, resulting in a continuous transect across the otolith (Sinclair et al. 1998, Fallon et al. 2002). Otoliths were analysed from the centre to the outside edge, with up to 200 μm of otolith material being scanned during each ablation; standard materials were also ablated for a total of 200 μm . The outside edge of the otoliths and end point of the scans were determined when the indium concentration increased, indicating the ablation of resin and not of otolith matrix.

Otoliths were analysed in several sampling sessions along with blank ablations and 2 reference standards, which allowed for comparisons among sampling sessions. Blank ablations that measured the chamber gases in the absence of any sample were analysed before and after the sampling sessions to determine the detection limits of the machine. A reference standard (National Institute of Standards and Technology, NIST 612) was analysed after every 10 otolith ablations to correct for machine drift (Ludden et al. 1995). The concentration of Sr in bream otoliths was corrected, by adjusting data, using a fish otolith standard (a 32 mm pressed powdered disk of finely ground otolith similar to that described by Fallon et al. 1999 for coral). The otolith standard was analysed at the beginning and end of each sample session. The analytical accuracy was determined from the concentrations of the NIST standards, and was averaged across all samples; accuracy was 100% for Ca^{44} and 99% for Sr^{88} . Detection limits determined from blank ablations were 1.40 $\mu\text{g g}^{-1}$ (Ca) and 0.66 $\mu\text{g g}^{-1}$ (Sr), with all otolith values being at least 1 order of magnitude greater.

All data reduction was done off-line in spreadsheets following the general methods described by Sinclair et al. (1998). The concentration of Sr:Ca in each transect

appeared to show noise and required smoothing to determine trends in the data. To reduce the noise along transects, a 9-point running mean was applied to the Sr:Ca ratio. Lower levels (3 and 5 point) of smoothing inadequately reduced noise, and higher levels (11 point) resulted in loss of trends (Sinclair et al. 1998). The Sr:Ca concentrations were then averaged using a 9-point running median to further smooth transects. The results of both the smoothing processes were transects that displayed little noise. Smoothed data were used throughout the rest of the data-reduction process, which included background subtracting, standardising to NIST 612, normalising to calcium (internal standard for ablation yield) and correcting to the otolith standard. Data were standardised to Ca (e.g. Sr:Ca), as strontium is likely to substitute for calcium in the otoliths of fish (Campana 1999).

Interpretation of Sr:Ca concentrations along transects was done by examining several variables of elemental uptake and incorporation (Fig. 1). Height represents the maximum concentration of Sr:Ca incorporated into otoliths for each treatment (Sr:Ca mmol mol^{-1}). Distance represents the otolith distance (μm) from when fish were exposed to enhanced ambient Sr:Ca till when maximum Sr:Ca was incorporated (height). Slope represents the rate of elemental uptake (as a function of height over distance; Sr:Ca, $\text{mmol mol}^{-1} \mu\text{m}^{-1}$). Area represents the total Sr:Ca incorporated into otoliths (as a function of the area under transect curves; Sr:Ca, mmol mol^2). Fig. 1 provides a schematic representation of each variable.

The concentration of Sr:Ca along the transects was partitioned into several sets of data that were representative of $30 \mu\text{m}$ spot analyses. This was done using

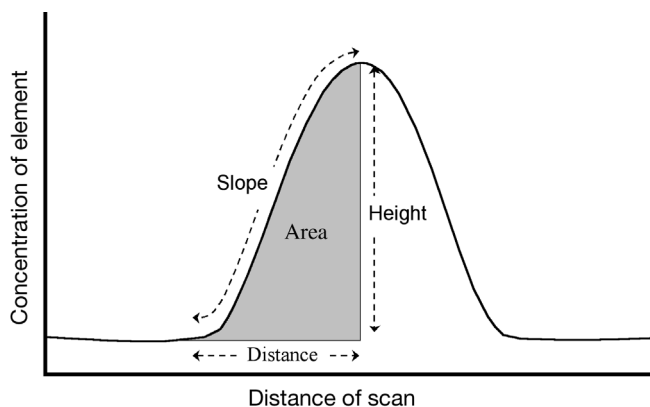


Fig. 1. Measurements (height, distance, slope and area) from transect analysis based on the uptake and incorporation of elements into calcified structures, where height represents the concentration of an element, distance represents the time taken for elemental incorporation and area is the maximal concentration of an element incorporated into a calcified structure per unit of time

blocks of raw elemental counts and standardising them using the methods of Elsdon & Gillanders (2002), whereby data were background subtracted, standardised to NIST 612 and an otolith standard, and presented as Sr:Ca ratios. The reduction of data into $30 \mu\text{m}$ spot increments allowed comparison between the results of the transect analysis and other forms of otolith analysis, such as those obtained using spot or micromill techniques.

Elements in water. Replicate water samples (three 50 ml samples) were collected on randomly selected days from each of the 2 holding tanks, each treatment tank during the spiked period, and 3 random tanks post-spiking. Water samples were collected in polypropylene sample jars, which were acid washed in 10% nitric acid mixed with Milli-Q water to leach metals. Half (25 ml) of each sample was filtered through a $0.45 \mu\text{m}$ filter and acidified with 500 μl of high-grade nitric acid, before being refrigerated (Elsdon & Gillanders 2002). Samples were further diluted (1:10, sample:deionised water) before being analysed by either ICP-MS or ICP-AES.

Water samples were analysed using a Perkin Elmer 3000 DV ICP-AES (Dual View Inductively Coupled Plasma–Atomic Emission Spectrometer) for the analysis of Ca^{44} and a Perkin Elmer Elan 6000 DRC ICP-MS (Dynamic Reaction Cell Inductively Coupled Plasma–Mass Spectrometer) for the analysis of Sr^{88} . Internal standards of indium (In, 5 ppb) and lutetium (Lu, 2 ppb) were used to correct for drift of the ICP-MS and ICP-AES, respectively. Detection limits displayed as micrograms per litre were 5.0 (Ca) and 1.0 (Sr). All sample values were above detection limits. The analytical accuracy of calcium and strontium averaged across all samples was 100% (Ca) and 87% (Sr), with a reproducibility of $<0.0001 \text{ mmol mol}^{-1}$ for both elements. Strontium was standardised to calcium, and was expressed as a ratio (Sr:Ca).

Statistical analysis. The univariate analysis of ambient Sr:Ca concentrations in rearing water proceeded in 3 steps. First, to determine if strontium persisted after the spiked period, the concentration of Sr:Ca in the 2 holding tanks was compared to 2 randomly selected tanks post-spiking. Second, to determine if the spiking of strontium significantly increased strontium in the water, the concentration of Sr:Ca was compared in 3 randomly selected tanks of the $2\times$ and $4\times$ treatments, and post-spiking tanks. The third step tested for the effects of strontium spiking and time of exposure on Sr:Ca concentration in rearing water. Analyses in the first 2 tests were done using 2-way ANOVAs with ‘treatment’ as a fixed factor and ‘tank’ as a random nested factor within treatment. The test for the third step was done using a 3-way ANOVA with ‘concentration’ and ‘time’ as fixed factors and ‘tank’ as a random nested factor within treatments.

Univariate ANOVAs were also used to determine if temperature and salinity of the rearing water differed among treatments and if the height, distance, slope and area (see Fig. 1) of otolith Sr:Ca transect curves differed among treatment groups. Three-factor ANOVAs treated both 'concentration' and 'time' as fixed factors and 'tank' as a random factor nested within concentrations. Where significant differences were detected (e.g. $p < 0.05$ or $p < 0.01$ if heterogeneous), means of treatment groups were compared using Student-Newman-Keuls' (SNK) tests, to determine where these differences occurred.

RESULTS

Water strontium concentrations and rearing conditions

Effectiveness of spiking

Post-spiking the Sr:Ca concentration in rearing waters showed no sign of elevation from pre-spiked levels (Fig. 2, Table 1a), with a Sr:Ca difference between pre- and post-spiking concentrations of $<0.015 \text{ mmol mol}^{-1}$. This result indicates that Sr was not enhanced in rearing waters after spiking. The concentration of Sr:Ca in spiked water in both 2 \times and 4 \times treatments was significantly different when compared to post-spiking tanks, indicating that spiking water with $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ resulted in a detectable difference in Sr:Ca during the experimental period (Fig. 2, Table 1b). The spiking of rearing water to 2 \times and 4 \times ambient Sr:Ca resulted in concentrations of 18.17 ± 0.07 and $32.28 \pm 0.28 \text{ mmol mol}^{-1}$, respectively; the ambient Sr:Ca concentration was $10.31 \pm 0.01 \text{ mmol}$

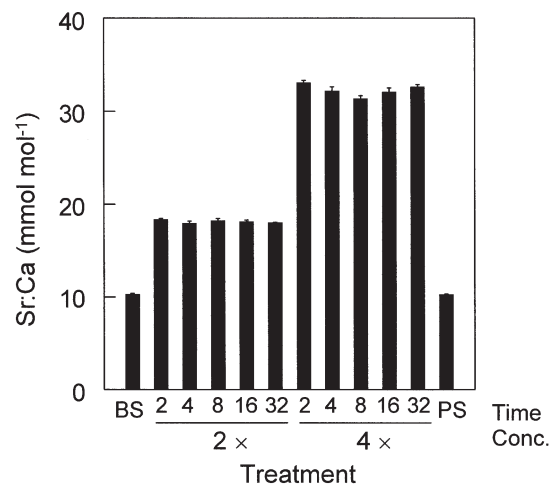


Fig. 2. Concentration of Sr:Ca in the rearing water during the experimental rearing period. BS represents the concentration before elemental spiking, and PS represents that after elemental spiking. Treatments are shown according to concentration (Conc.) and time (in days). Bars represent the mean + SE of the mean of each treatment

mol^{-1} (mean \pm SEM). Thus, final concentrations of spiking with $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ were not quite 2 \times and 4 \times that of ambient Sr:Ca concentrations (see also Fig. 2).

The elemental concentrations among treatments were significantly different for concentration, time and the interaction of concentration \times time. The maximum differences in the Sr:Ca concentrations among any 2 tanks of the 2 \times and 4 \times treatments were 0.412 and 0.984 mmol mol^{-1} , respectively; this suggested that any effect of tank was negligible (see Fig. 2, Table 1c). Importantly, there was no overlap in Sr:Ca concentrations between the 2 \times and 4 \times treatments, with at least 14.7 mmol mol^{-1} separating the means of any 2 treat-

Table 1. Analysis of variance comparing (a) the concentration of Sr:Ca in the rearing water before (2 holding tanks) and after (2 randomly chosen tanks) the spiking period, (b) the concentration of Sr:Ca in rearing water among 2 \times and 4 \times spiking and post-spiking tanks, and (c) the concentration of Sr:Ca in rearing water among treatments of the full design (Conc.: concentration). Cochran's test was used to test homogeneity of variance, with all tests being non-significant

	Source of variation	df	MS	F	p
(a) Before vs after spiking	Treatment	1	0.0008	2.53	0.2530
	Tank (Treatment)	2	0.0003	0.01	0.9892
	Residual	8	0.0310		
(b) Spiked vs post-spiking comparison	Treatment	2	1180.8708	305.72	0.0000
	Tank (Treatment)	6	0.1422	0.42	0.8555
	Residual	18	0.3378		
(c) Treatment comparison	Concentration	1	2988.0824	791.62	0.0000
	Time	4	1.3928	5.96	0.0102
	Tank (Conc. \times Time)	10	0.2336	0.53	0.8593
	Conc. \times Time	4	1.2018	5.14	0.0163
	Residual	40	0.4417		

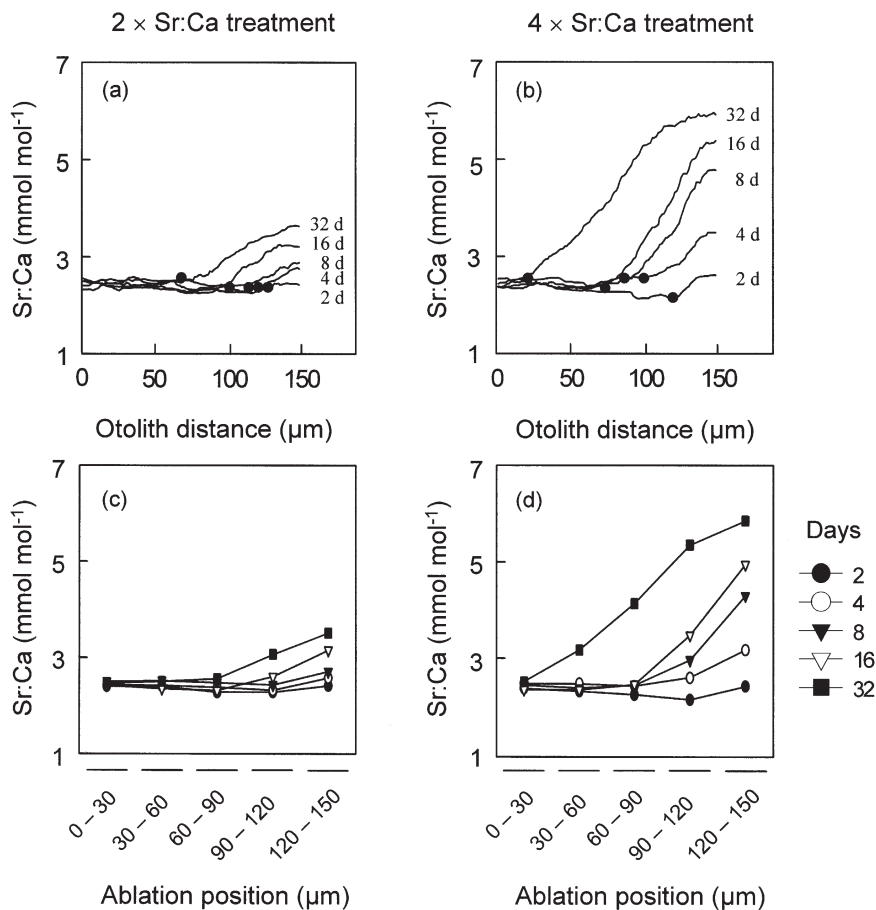


Fig. 3. *Acanthopagrus butcheri*. Strontium concentrations in fish otoliths (Sr:Ca) for 1 representative fish from each treatment analysed using (laser ablation ICP-MS) 30 μm transect analysis (a,b) across the otolith of experimentally reared black bream and corresponding (laser ablation ICP-MS) 30 μm spot analysis (c,d) across the same fish otoliths. Individual transects are plotted such that the starting points of exposures do not match up, but the middle peak for each element does. Black circles in Panels a and b represent the approximate time treatments commenced

ments. Thus, the effects of time and the interaction of concentration \times time on ambient Sr:Ca concentrations were smaller in magnitude compared to the spiking effects.

Temperature and salinity

Temperature and salinity were not significantly different between treatments of either concentration or time (ANOVA: temperature \times conc.: $F_{1,10} = 0.37$, $p = 0.5554$, time: $F_{4,10} = 1.91$, $p = 0.1862$; salinity \times conc.: $F_{1,10} = 0.07$, $p = 0.7923$, time: $F_{4,10} = 0.47$, $p = 0.7569$). The mean temperature and salinity values averaged across all readings were $19.79 \pm 0.01^\circ\text{C}$ and $33.00 \pm 0.09\text{‰}$, respectively (mean \pm SEM). Tank differences in temperature were detected (ANOVA; temperature \times

tank: $F_{10,380} = 6.49$, $p < 0.001$), but, as $< 0.45^\circ\text{C}$ separated the means of duplicate tanks within each treatment, the effect of temperature differences between tanks was small when compared to the effect of enhanced Sr:Ca in water.

Otolith strontium concentrations

Transect Sr:Ca concentrations

Differences between the 2 \times and 4 \times concentration treatments can be seen in the height, distance, slope and area of transect curves (Fig. 3a,b). Otolith transects of fish (*Acanthopagrus butcheri*) reared in 1 \times Sr:Ca concentration (ambient concentration), representing control fish, were stable, and the Sr:Ca concentrations were low (see first 50 μm of otolith distance in Fig. 3a for an example). Representative graphs of 1 fish from each treatment using 30 μm spot analysis (Fig. 3b,c) conform to the same trends as the transect data, thus showing the generality of these results independent of sampling method and broadening their application and relevance.

The amount of otolith material laid down during the rearing process was $4.9 \mu\text{m d}^{-1}$. This growth rate was used to calculate the amount of time taken for Sr:Ca to become saturated in otoliths (based on transect distance calculations). The concentration of Sr:Ca in otoliths increased with time up to approximately 20 d, when the saturation point was reached (otolith distance of 32 d treatments [Fig. 4b]/growth of $4.9 \mu\text{m d}^{-1} \approx 20$ d) (see Fig. 5 for a graphical representation). Fish held at both 2 \times and 4 \times ambient elemental concentrations had almost identical saturation times (2 \times : 18.51 ± 1.46 d, 4 \times : 19.4 ± 0.80 d), as indicated by the non-significant interactive effect between concentration and time for transect distances (Table 2b). Exposure time of >20 d did not result in greater concentrations of Sr:Ca being incorporated into otoliths, as saturation of Sr:Ca in otoliths occurred (see Fig. 5 for an example). Thus, the maximal concentrations displayed in Fig. 4a represent the expected concentrations of Sr:Ca in otoliths at different exposure times.

Interactive effects of concentration and exposure time were detected for the height and the slope of

peaks, while the transect distance and area were non-significant (Fig. 4, Table 2). The interactions detected for both height and slope show a clear increase in concentration of Sr:Ca with increasing time of exposure, with this effect being greater in the 4× concentration treatments than in the 2× treatments (Fig. 4a,c). There was no difference in maximal concentration (height) or rate of uptake (slope) between the 2 d treatments in 2× or 4× concentrations, nor were the 2, 4, or 8 d treatments within the 2× concentration significantly different (SNK test, Fig. 4a,c); however, differences in height and slope were detected for all other levels of concentration and time. For distance and area, both concentration and time of exposure were highly significant (Fig. 4b,d, Table 2b,d), with all levels of time being different from one another within each level of concentration (SNK tests, Fig. 4b,d).

Area versus slope

To distinguish between treatments of concentration and time, a plot of area versus slope was done (Fig. 6). Although fish exposed to elevated Sr:Ca for short periods of time remain indistinguishable, those exposed for longer periods (i.e. 8, 16 and 32 d) group out according to both area (total Sr:Ca in otoliths) and slope (rate of uptake). Equations of these lines are:

$$2\times \text{ treatments: } y = 8426.6x + 0.899 \quad r^2 = 0.6613$$

$$4\times \text{ treatments: } y = 4485.9x + 16.507 \quad r^2 = 0.9859$$

where y is area and x is slope. The slopes of all lines were significantly different from 0 slope.

DISCUSSION

Uptake and incorporation of strontium

A key finding of the current experiment was a clear increase in otolith Sr:Ca (height) in *Acanthopagrus butcheri* with increasing water Sr:Ca from 2× to 4× ambient concentrations. Such a relationship between ambient and otolith Sr:Ca has been found in several manipulative experiments (e.g. Bath et al. 2000, Eldson & Gillanders 2003b). These studies consistently show

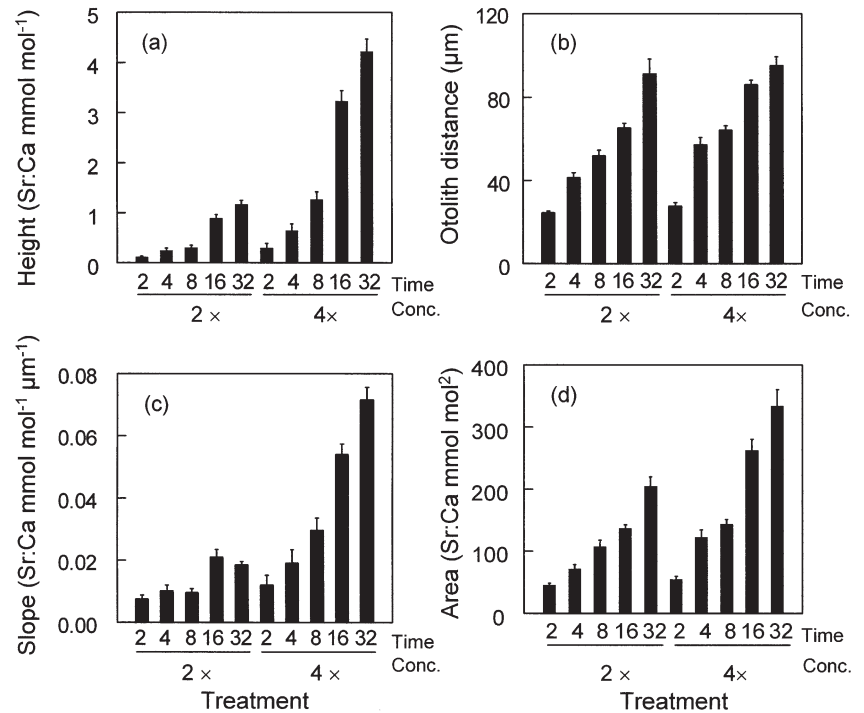


Fig. 4. *Acanthopagrus butcheri*. Strontium concentrations (Sr:Ca) in otoliths of black bream reared under treatments of enhanced elemental concentration (Conc.) for different times of exposure: (a) the maximum Sr:Ca concentration incorporated as a function of transect height, (b) the distance (time) to maximum Sr:Ca concentration incorporation, (c) slope (rate) of uptake and (d) the area or total Sr:Ca concentration incorporated into otoliths. In all graphs '0' represents control fish that were reared at 1× (ambient) Sr:Ca concentration. Bars represent the mean + SE of the mean of each treatment

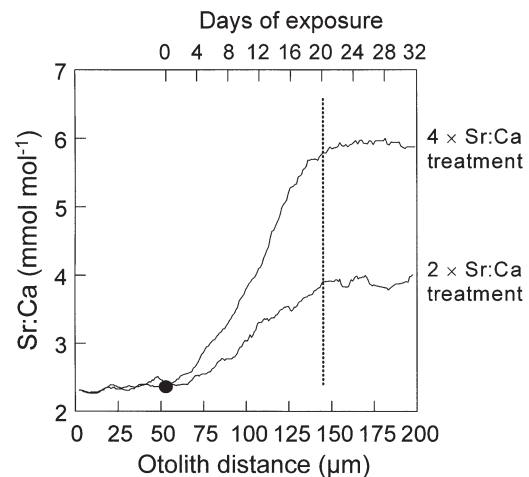


Fig. 5. *Acanthopagrus butcheri*. Sr:Ca concentrations in fish otoliths for 1 representative fish each from the 2× and 4× enhanced elemental treatments, over 32 d. Individual transects are plotted such that the starting points of the treatments match up (●). Dotted line represents the saturation of Sr:Ca in otoliths, after which very little change in otolith Sr:Ca concentration occurred

Table 2. *Acanthopagrus butcheri*. Analysis of variance comparing the (a) maximum Sr:Ca concentration (height), (b) time period of uptake (distance), (c) rate of uptake (slope) and (d) total concentration of Sr:Ca in otoliths of fish (area), for each treatment (Conc.: concentration). Cochran's test was used to test homogeneity of variance; tests that were significant (height, distance, slope and area) were $\ln(x + 1)$ -transformed after which homogeneity was achieved in all cases

Source of variation		df	MS	F	p
(a) Height	Concentration	1	7.9736	273.42	0.0000
	Time	4	4.7577	163.14	0.0000
	Tank (Conc. \times Time)	10	0.0292	0.98	0.4679
	Conc. \times Time	4	0.6502	22.30	0.0001
	Residual	100	0.0298		
(b) Distance	Concentration	1	0.7964	15.21	0.0030
	Time	4	5.6585	108.06	0.0000
	Tank (Conc. \times Time)	10	0.0524	1.11	0.3587
	Conc. \times Time	4	0.1568	2.99	0.0726
	Residual	100	0.0470		
(c) Slope	Concentration	1	0.0185	175.90	0.0000
	Time	4	0.0060	56.96	0.0000
	Tank (Conc. \times Time)	10	0.0001	0.94	0.4999
	Conc. \times Time	4	0.0026	24.51	0.0000
	Residual	100	0.0001		
(d) Area	Concentration	1	5.4693	77.06	0.0000
	Time	4	9.8572	138.88	0.0000
	Tank (Conc. \times Time)	10	0.0710	0.96	0.4794
	Conc. \times Time	4	0.2108	2.97	0.0740
	Residual	100	0.0736		

that an increase in ambient concentration results in a significant increase in Sr concentration within calcified structures. For fish, this result is consistent between freshwater (see Mugiya et al. 1991) and saltwater species (see Bath et al. 2000). Comparisons of data from the literature with the results of the current experiment are difficult, given that different sampling techniques can affect the detected concentrations of elements when organisms, such as fish, have been reared for short periods of time (Elsdon & Gillanders 2003a). Consistency in the direction of effects between the results of the current experiment and those found in the literature is particularly important for interpreting concentrations of elements in otoliths, given that the current experiment is among the first to examine fish otoliths from experimental treatments by transect analysis using laser ablation ICP-MS.

Manipulating exposure times adds an additional degree of complexity to interpreting the effect of ambient Sr:Ca on otolith Sr:Ca. Interactions between concentration and time in the current experiment, for height (maximum concentration) and area (total concentration), were such that Sr:Ca was incorporated into otoliths more rapidly and at greater concentrations when fish were exposed to 4 \times ambient Sr:Ca, compared to at 2 \times ambient Sr:Ca. Previous studies assessing the influence of exposure time to enhanced concentrations of elements in fish spines and otoliths

(Snyder et al. 1992, Ennevor & Beames 1993, Brown & Harris 1995, Pollard et al. 1999), analysed structures using solution-based techniques. In these studies, where whole spines and otoliths were dissolved in acid, the resulting concentration is a signal incorporating experimental and non-experimental periods. Despite the use of solution analysis, Pollard et al. (1999) did detect a significant interactive effect of concentration and exposure time on Sr concentration in snapper *Pagrus auratus* spines; this interaction was identical to the interaction we found in the current experiment (*P. auratus* is a closely related species to black bream *A. butcheri*). Consistency between experiments in both direction and the interaction of effects indicates that exposure time is an important factor worthy of investigation.

The relationship between otolith Sr:Ca and exposure time indicates that elemental uptake into otoliths is not immediate. The lag effect of elemental

uptake into otoliths has implications for the interpretations of past experiments in which fish were reared for short periods of time. Experiments in which fish were

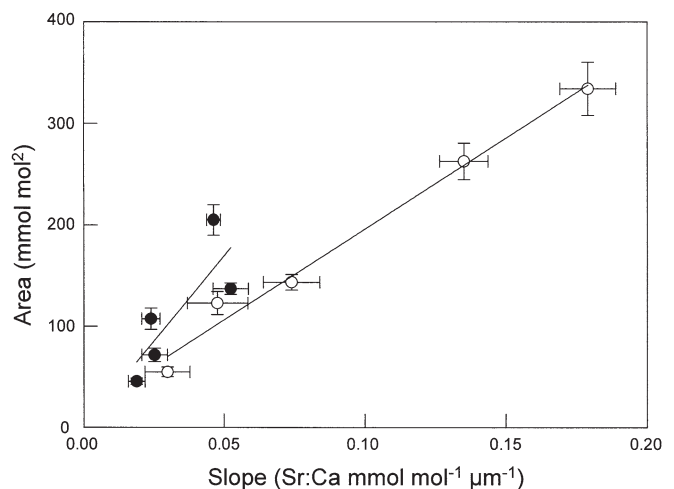


Fig. 6. *Acanthopagrus butcheri*. Plot of area (total Sr:Ca concentration) versus slope (rate of Sr:Ca uptake) in otoliths of black bream reared under enhanced concentrations of Sr:Ca (2 \times and 4 \times) for increasing time periods (2, 4, 8, 16 and 32 d). Black circles represent treatment tanks of 2 \times ambient Sr:Ca, and clear circles represent treatment tanks of 4 \times ambient Sr:Ca. Bars represent the mean \pm SE of the mean of each treatment. The value of '0' represents control fish that were reared at 1 \times (ambient) Sr:Ca concentration

reared for <20 d (see Elsdon & Gillanders 2003a for a review) would provide underestimates of otolith Sr concentrations (Secor et al. 1995). Likewise, analyses that incorporate otolith material laid down in the first 20 d of exposure will underestimate otolith Sr concentration (Elsdon & Gillanders 2003b); this includes solution-based analyses (Bath et al. 2000, Martin et al. 2004). An example of underestimation of Sr is given by Elsdon & Gillanders (2003a), who showed that otolith elemental concentrations from fish reared for 32 d under constant conditions can be underestimated if analysed using large portions of otolith material (100 μm spot analysis on outside edge) versus smaller portions of otolith (30 μm spot analysis). The differences in elemental concentrations between 100 and 30 μm spot analyses are clear although both techniques sampled otolith material that was laid down under constant experimental conditions. Therefore, short rearing periods and different sampling techniques can lead to lower chemical concentrations being detected in otoliths. As a result, derived partition coefficients do not represent steady-state conditions. The effect of exposure time on elemental uptake into otoliths may explain differences in the partition coefficient between sampling techniques and between experiments (e.g. Bath et al. 2000, Elsdon & Gillanders 2003b, Martin et al. 2004). We believe that more work is needed in this area; until such time generalisation of the results of experiments will remain difficult.

When fish in the current experiment were exposed to enhanced elemental concentrations for 32 d, a distinct plateau of Sr:Ca concentration was observed to occur after approximately 20 d of exposure, suggesting that Sr:Ca became saturated in otoliths. This result is novel for fish otoliths. Although other fields of research, such as coral calcification, have suggested that saturation of Sr:Ca in skeletal material does not occur (see Ferris-Pagès et al. 2002), we have clearly shown that elemental saturation is reached in otoliths. Given the difference between coral and otolith elemental concentrations (Campana & Thorrold 2001), the discrepancies in partition coefficients (Martin et al. 2004) and the results we present here on elemental uptake, we believe that comparisons of effects among different calcified structures are not justified. Clearly, different models of calcification and elemental incorporation occur between fish and invertebrates; therefore, the applications and results from one field may not transcend to the other. Importantly, we have identified a lag effect of elemental uptake using transect analyses across fish otoliths, and have identified a minimum period of exposure time required for otoliths to fully incorporate Sr:Ca from the ambient environment. These data should allow for the residency time of fish in different environments to be calculated.

Applications to environmental reconstructions

Reconstructing environmental histories of fish is reliant on predictable effects of environmental variables on the elements in calcified structures. Individual measures of elemental uptake and incorporation into otoliths, when considered independently (from measures such as distance, slope, or area), do not allow for accurate classification of environments inhabited at specific points in time. In the current study, when variables such as the area underneath peaks (total Sr:Ca concentration) and slope (rate of elemental uptake) were combined, greater discrimination was found between fish exposed for different periods of time to varying ambient Sr:Ca concentrations. Such discrimination would allow differentiation between fish that occupied different habitats for different periods of time. Thus, the results of the current experiment also cast doubt on the usefulness of linear equations that use the effects of only 1 environmental variable (see Elsdon & Gillanders 2003b for an example) to reconstruct the environmental histories of fish. Questioning the usefulness of linear equations is especially pertinent for fish that can move through environments quickly, without enough time being spent in any single environment to fully incorporate elements.

Questions regarding the influence of growth rates on reconstructions are equally important, if reconstructions bridge several years of fish growth. Where differences in otolith growth occur with fish age (e.g. Jones 2000), interpretations of otolith chemistry would be altered by the amount of otolith precipitated between the annuli. Although attempts could be made to scale results to fish growth, where ontogeny may affect otolith chemistry, this would require additional testing to that done in the current study. Studies that have examined Sr:Ca concentrations across otoliths, where growth rates change (e.g. Limburg 1995), require cautious interpretation with regard to environmental histories. Nevertheless, reconstructions of environmental histories for short periods of constant growth (e.g. Katayama et al. 2000) are less susceptible to errors in interpretation.

Interpreting reconstructions and limitations

The type and scale of environmental histories derived from otolith chemistry largely depend on the biology of the organism (Elsdon & Gillanders 2003a). Resident fish, which are either territorial or very confined in movement, can be analysed based solely on the changes in environmental variables surrounding the organism. Interpretation of the environmental histories of resident fish, therefore, requires knowledge of

the fish's age, its growth rate and the stability of the surrounding environmental variables (e.g. Hatje et al. 2001) to be factored into reconstructions. Reconstructions of the environmental histories of migratory fish are quite complex and require knowledge of several additional factors in order to interpret whether the organism has moved or whether the environment occupied has changed. These factors include: (1) the rate of fish movement, (2) the rate of change of environmental variables and (3) knowledge of how growth rates may change with age and water temperature or with metabolism (see Elsdon & Gillanders 2003a for a review). Once these factors have been defined, information from the current experiment, such as the area and the slope of peaks along transects, can be used to deduce the amount of time fish have spent in different environments.

The complexity of interpreting environmental histories from otolith chemistry increases when multiple environmental variables influence or interact to affect otolith chemistry (e.g. Elsdon & Gillanders 2002). The results we present are useful in interpreting the environmental histories of fish based solely on ambient chemistry and the effect of exposure time. However, when temperature and salinity also affect otolith chemistry (Hoff & Fuiman 1995, Secor et al. 1995, Bath et al. 2000, Yamashita et al. 2000, Elsdon & Gillanders 2002, Martin et al. 2004), the delayed effect of these on elemental incorporation remain unknown. Therefore, our results are best suited for environmental reconstructions in habitats where temperature and salinity are stable, such as in estuaries with similar temperatures to the surrounding coastal environment. In situations where this occurs, we suggest that combining multiple variables of Sr uptake and incorporation into otoliths, such as the area and slope of transects, would allow more accurate discrimination of environmental histories.

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