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Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*

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ABSTRACT: Elements contained within calcified structures of fish, molluscs, bivalves and corals may provide a means to determine the characteristics of the environment occupied by an organism over time. In order to establish these characteristics it is first important to establish a link between one or more environmental variables and the concentration of elements within such a calcified structure. Black bream *Acanthopagrus butcheri* (Family: Sparidae) were reared for 30 d in the laboratory under controlled conditions, during which time the rearing water was spiked with different concentrations of strontium, barium and manganese. The Sr:Ca and Ba:Ca concentration ratios in the otoliths were related to water chemistry, while that of the Mn:Ca ratio was not. Furthermore the Sr:Ca concentration ratio in the rearing water was beyond levels previously examined. Mean partition coefficients (D_{Me}) were calculated for Sr:Ca, Ba:Ca and Mn:Ca ratios, and were 0.131, 0.099 and 0.683 respectively. The relationship between the partition coefficients from each treatment group and the elemental ratio of the rearing waters was non-linear for all elements, suggesting that extrapolation of the data beyond the concentrations used is not justified. These results indicate that it is possible to reconstruct the past environmental characteristics from fish otolith chemistry, based on the concentration of elements in water. Establishing this link allows for the reconstruction of past environments that fish have occupied based on elemental chemistry.

KEY WORDS: Trace metal · Barium · Strontium · Manganese · LA ICP-MS · Uptake

INTRODUCTION

The concentrations of elements in calcified structures of fish (Townsend et al. 1995), molluscs (Anadón et al. 2002), foraminiferans (Lea & Spero 1994) and corals (Shen et al. 1996) can provide a record of the environment at the time of crystallisation. Determination of environmental characteristics from elemental chemistry of calcified structures is possible if the precipitation of elements into calcified structures reflects their concentration in the surrounding water. However, there have been few species of fish for which experiments have directly linked the elemental chemistry of water to that in fish otoliths. Similarly, there have been few experimental investigations that have examined the uptake of elements other than strontium (but see Bath et al. 2000), although the use of elements such as barium and manganese also has potential for determining the environment that fish have inhabited. Thus, if we are to infer that the elements in otoliths of fish are derived from the water, it is important to understand how these element concentrations change in relation to the respective concentrations in water.

Fish otoliths, unlike other calcified structures such as corals, are not in direct contact with the ambient water. Due to the physiological barriers that elements must cross before they are incorporated into otoliths, it is unlikely that elemental deposition directly reflects the elemental concentration in the water (Campana 1999). The degree of partitioning that occurs between the concentration of elements in water and that in calcium carbonate can be described using a partition coeffi-

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cient (Morse & Bender 1990). Elements (defined from here as Me) such as Sr^{2+}, Ba^{2+} and others that can occur in the same state (e.g. Mn^{2+}) are likely substitutes for Ca^{2+} within the calcium carbonate structure of otoliths. Thus, where a simple substitution occurs it is possible to describe the relationship between the Me concentrations and Ca in the water and in the otolith. This relationship can be described based on the elemental ratio of Me to Ca in both the water and otolith, and is expressed as D_{Me}, where D_{Me} is the partition coefficient that relates the concentrations of elements in the water to that in the calcified structures such as an otolith. This simple equation eliminates the need for complex thermodynamic equations (but see Chowdhury & Blust 2001 for an example), which may be impossible to construct given that the elements of Sr, Ba and Mn would have to exist in a solid solution, which is not possible (Lea & Spero 1992).

Partition coefficients cannot describe the processes by which elements are discriminated. Indeed, partition coefficients are likely to be influenced by temperature (Kinsman & Holland 1969, Bath et al. 2000), water pressure (Morse & Bender 1990), and the precipitation rate of the crystal structure itself (Kinsman & Holland 1969, Mucci & Morse 1983). However, they are particularly useful in comparing the discrimination of elements in calcified structures at different water elemental concentrations. Thus, partition coefficients can be given for a particular elemental concentration of water or for an average across all concentrations, allowing for generality of elemental discrimination to be obtained. As partition coefficients are standardised measurements, they are also an ideal unit to use for species and organism comparisons of elemental discrimination, such that a similar coefficient between 2 species indicates similarities in elemental discrimination. To date, the use of partition coefficients in the otolith chemistry literature is limited (but see Bath et al. 2000), which makes comparisons of elemental uptake between different experiments and species difficult.

Several experiments have tested how water chemistry affects concentrations of elements in both fish otoliths and other calcified structures such as foraminiferans and corals. While the type of calcified structures used in fisheries analysis varies, the results remain relatively consistent, with the majority of studies finding a strong positive association between water chemistry and otolith chemistry (Snyder et al. 1992, Ennevor & Beames 1993, Brown & Harris 1995, Schroder et al. 1995, Bath et al. 2000, Milton & Chenery 2001), and in other calcified structures of both fish (e.g. scales, Wells et al. 2000a) and foraminiferans (Lea & Spero 1992, 1994).

Many experiments determining the relationship between water chemistry and otolith chemistry have been for mass-marking purposes, where Sr has been used (e.g. Schroder et al. 1995, Townsend et al. 1995). Given that mass-marking protocols often require large and distinguishable signatures to be imprinted on the fish otoliths, this has led to high concentrations of Sr in the water (e.g. 1.0 g l^{-1}, Pollard et al. 1999). Concentrations this high may not mean a lot biologically, given that oceans and estuaries typically have lower Sr concentrations (e.g. approximately 7.8 µg l^{-1}, Bruland 1983), and thus fish are unlikely to experience such high concentrations in the wild. However, using lower concentrations (e.g. Milton & Chenery 2001), which are not largely enhanced from ambient values, have still detected positive relationships. Thus, the Sr concentration in water surrounding a fish has been determined as a major contributor to the Sr concentration detected in otoliths.

The effect that enhanced concentrations of other elements (e.g. Ba and Mn) have on the chemistry of calcified structures has been examined in less detail. The concentration of Ba in otoliths, scales and foraminiferans displays a near linear relationship with elemental concentration in the water (Mugiya et al. 1991, Lea & Spero 1992, 1994, Bath et al. 2000, Wells et al. 2000b). The effect that spiking water with Mn has on its concentration in otoliths is not well documented in the literature. However, the positive relationship that occurs for other elements (such as Sr and Ba) has not been reported for Mn (see Mugiya et al. 1991). Positive relationships between copper, mercury and lead in water and otoliths of fish have also been determined using experiments (Geffen et al. 1998, Milton & Chenery 2001).

The objective of this study was to determine whether the otolith chemistry of black bream *Acanthopagrus butcheri* is influenced by the concentration of elements in water. Specifically, controlled laboratory experiments that manipulated the concentration of Sr, Ba and Mn in water were used to determine the elemental response of otoliths. The spiked Sr concentrations in water covered a larger range than previously examined, whilst 'natural' sea water was used to eliminate the artificially high concentration of Ba at ambient levels in artificial seawater (Bath et al. 2002). Partition coefficients have also been calculated for each of these elements, for which these are the first coefficients determined for Mn. The determination of partition coefficients allows for the generalisation and comparison of results from this experiment to that of others using different species.

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 tanks, equipped with filtration and aeration. The fish were bred from a common brood stock to reduce the influence of genetic variability on otolith chemistry. Fish were held in these conditions 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 (SARDI) Aquatic Sciences division, and was held in a covered and aerated 2000 l indoor tank. Fish were fed a diet of commercial fish food (Skretting formula) throughout the duration of the experiment. Light was provided from fluorescent tubes on a 12:12 h light:dark cycle, in a room of constant temperature (20°C).

The otoliths were marked with alizarin complexone (36 ppm) at the hatchery to enable old growth to be distinguished from new growth, the latter of which had been laid down under experimental conditions. After the initial acclimatisation period, 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, with adequate aeration and a clear plexiglass lid to minimise evaporation that may influence the elemental composition of the water.

Experimental treatments consisted of manipulating the concentrations of elements (Sr, Ba and Mn) in rearing water using 5 treatment levels (ambient concentrations): 1, 2, 4, 8, and 16× ambient. The concentrations of all spiked elements were designed to span levels experienced by wild black bream, with some levels (e.g. 8 and 16×) being as high as natural levels in estuaries and run-off drains, which are expected to exceed oceanic levels. For each treatment, duplicate tanks were used to assess variability in both the elemental composition of the water and otoliths. The concentration of elements was manipulated by the addition of appropriate amounts of standard solutions made from $SrCl_2 \cdot 6H_2O$, $BaCl_2 \cdot 2H_2O$ and $MnCl_2 \cdot 4H_2O$ mixed with seawater, such that all elements were spiked at the same level in each treatment. The concentrations of these elements in ambient water were determined from prior water analysis (Elsdon & Gillanders 2002). Experimental conditions were established by placing fish in water containing the enhanced elemental concentrations. To maintain water quality, 50% of water was siphoned off every second day and replaced with freshly spiked water. Any accumulated detritus on the bottom of tanks was siphoned away daily. Fish were reared in treatment tanks for 32 d. During this period, temperature and salinity were monitored on random days (n = 21 samples), with 3 replicate water samples being collected from each tank to determine the actual concentrations of elements in the water. After fish were exposed to the treatments for 32 d, all fish were immersed in an ice slurry and their standard lengths and weights recorded. The sagittal otoliths were dissected, washed thoroughly and cleaned in Milli-Q water and allowed to dry before being stored in microcentrifuge tubes. Seven fish from each treatment tank were then selected, based on similarities of length and weight, and their otoliths were prepared for elemental analysis.

Elements in otoliths. One otolith from each of the 7 fish per tank was embedded in epofix resin, sectioned transversely through the focus (centre section) using a low-speed diamond saw lubricated with deionised water. Approximately 300 µm thick sections were then polished down to 200 µm using 9 µm lapping film. These sections were cleaned in Milli-Q water and dried on glass, after which they were sonicated for 5 min and dried in a plastic laminar flow cabinet, before being mounted on microscope slides. Slides were stored in clean sealable plastic bags until analysis.

Outside edges of the sectioned otoliths were sampled to ensure that the material analysed had accumulated during the time that fish were exposed to experimental conditions, which was determined by the position of the alizarin ring on the otoliths. Analysis was done using a Merchantek LUV 266 Q-switched Nd: YAG UV laser microprobe, with a pulse rate of 6.00 Hz and an ablation spot size of 100 µm. The laser ablation station was connected to a Finnigan MAT ELEMENT HR-ICP-MS (High Resolution-Inductively Coupled Plasma-Mass Spectrometer), with operating conditions identical to those in Elsdon & Gillanders (2002). Ablation of the otolith material took place in a sealed chamber, with the sample gas being extracted from the chamber to the HR-ICP-MS via a smoothing manifold in an argon and helium gas stream.

Otoliths were analysed in several sampling sessions, and the chamber was purged after each ablation to remove any background gases that may have contained contaminants. Blank ablations, which consisted of measuring the chamber gases in the absence of any samples, were analysed before and after the sampling session in order 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 that may be attributed to changes in room temperature, plasma or electronics. The concentrations of Sr and Ba in bream otoliths were corrected using a fish otolith standard (pressed powdered disk of finely ground otolith) that was analysed at the beginning and end of each sample session. The analytical accuracy was determined from the concentrations of the NIST standards and averaged across all samples; accuracy was 100% for the elements of Ca^{44}, Ba^{138}, Mn^{55}, and 99% for Sr^{88}.

Detection limits were determined from blank ablations and are displayed as µg g^{-1}, Ca (1.40), Ba (0.03), Mn (0.65) and Sr (0.66), with all otolith values being at least 1 order of magnitude greater. Reproducibility of the results was 0.3892 mmol mol^{-1} (Sr:Ca), 0.0001 µmol mol^{-1} (Ba:Ca), and 0.0003 µmol mol^{-1} (Mn:Ca). The concentration of each element (Sr, Ba, Mn) was standardised to calcium by using ratios (e.g. Sr:Ca); the data are represented as concentration ratios and not absolute concentrations of individual elements. This was done as elements are likely to be substituted for calcium in the otoliths of fish (Campana 1999).

Elements in water. During the rearing period, 3 × 50 ml water samples were collected from each treatment tank. Water samples were collected in 70 ml polypropylene sample jars, which were acid washed to leach metals. Half (25 ml) of each sample was filtered through a 0.45 µm filter and acidified with 500 µl of high-grade nitric acid, before being refrigerated. Samples were further diluted (1:10, sample:deionised water) before being analysed by either ICP-MS, or ICP-AES (Atomic Emission Spectrometer).

Water samples were analysed using a Perkin Elmer 3000 DV (Dual View) ICP-AES for the analysis of Ca^{44}, and a Perkin Elmer Elan 6000 DRC (Dynamic Reaction Cell) ICP-MS for the analysis of Sr^{88}, Ba^{138}, and Mn^{55}. Internal standards of indium (In, 5 ppb) and lutetium (Lu, 2 ppb) were used to correct for drift of both the ICP-MS and ICP-AES, respectively. Detection limits were displayed as µg l^{-1}, Ca (5.0), Sr (1.0), Ba (1.0), Mn (1.0), with all sample values being above detection limits.

The analytical accuracy of elements averaged across all sampling was 100% (Ca), 87% (Sr), 104% (Ba) and 100% (Mn), with a reproducibility of <0.0001 mmol mol^{-1} (Ca, Sr), <0.0001 µmol mol^{-1} (Ba) and 0.0004 µmol mol^{-1} (Mn). The concentration of each element (Sr, Ba, Mn) was standardised to calcium by ratioing (e.g. Sr:Ca).

Statistical analysis. Univariate ANOVA was used to determine whether the rearing conditions (temperature and salinity), fish growth (standard length and weight) and concentration of elements in both water and otoliths differed within treatment groups. Two-

Table 1. *Acanthopagrus butcheri.* Summary of the rearing conditions within treatment tanks for each of 1, 2, 4, 8, and 16× ambient elemental concentrations (tr: treatment level). Data are displayed as means ± SE. Units: Sr:Ca (mmol mol^{-1}), Ba:Ca and Mn:Ca (µmol mol^{-1}), Ca (µmol ml^{-1}), Sr (µmol $ml^{-1} \times 10^{-2}$), Ba and Mn (µmol $ml^{-1} \times 10^{-5}$)

Tr	Temperature °C (n = 16)	Salinity ‰ (n = 16)	Sr:Ca (n = 3)	Ba:Ca (n = 3)	Mn:Ca (n = 3)	Sr (n = 3)	Ba (n = 3)	Mn (n = 3)	Ca (n = 3)
1×	20.15 ± 0.61	32.94 ± 0.48	10.27 ± 0.04	5.22 ± 0.04	3.78 ± 0.96	9.74 ± 0.00	4.95 ± 0.04	3.58 ± 0.91	9.48 ± 0.00
1×	19.67 ± 0.04	33.24 ± 0.41	10.43 ± 0.05	5.42 ± 0.05	3.93 ± 1.06	9.63 ± 0.00	5.00 ± 0.05	3.64 ± 1.00	9.23 ± 0.14
2×	19.97 ± 0.06	33.03 ± 0.46	18.06 ± 0.00	12.54 ± 0.51	8.26 ± 0.92	17.12 ± 0.00	11.89 ± 0.49	7.83 ± 0.86	9.48 ± 0.00
2×	19.66 ± 0.05	32.75 ± 0.43	18.06 ± 0.00	12.54 ± 0.05	8.13 ± 0.65	17.11 ± 0.00	11.89 ± 0.48	7.71 ± 0.62	9.48 ± 0.00
4×	20.33 ± 0.06	32.79 ± 0.41	32.50 ± 0.00	24.06 ± 1.43	16.57 ± 2.08	30.81 ± 0.00	22.81 ± 1.35	15.71 ± 1.97	9.48 ± 0.00
4×	20.36 ± 0.05	33.26 ± 0.43	32.78 ± 0.47	23.52 ± 0.89	17.34 ± 2.49	30.82 ± 0.01	22.09 ± 0.64	16.26 ± 2.21	9.34 ± 0.08
8×	20.37 ± 0.09	33.14 ± 0.42	65.36 ± 0.86	30.98 ± 2.12	40.44 ± 3.72	60.87 ± 0.00	28.88 ± 2.16	37.62 ± 3.21	9.31 ± 0.07
8×	20.31 ± 0.10	32.65 ± 0.49	61.69 ± 2.11	30.17 ± 2.83	39.39 ± 4.15	58.97 ± 0.02	28.88 ± 2.86	37.62 ± 3.69	9.56 ± 0.08
16×	20.16 ± 0.05	33.26 ± 0.36	123.67 ± 5.03	30.73 ± 2.40	80.12 ± 8.54	115.27 ± 0.06	28.64 ± 2.32	74.63 ± 7.93	9.31 ± 0.08
16×	20.77 ± 0.13	32.76 ± 0.51	125.87 ± 3.51	31.65 ± 0.34	85.92 ± 6.26	118.92 ± 0.03	29.90 ± 0.38	81.18 ± 6.01	9.45 ± 0.03

Table 2. *Acanthopagrus butcheri.* ANOVA comparing the elemental ratio among water and fish otoliths from each concentration treatment. Cochran's *C*-test was used to test homogeneity of variance, with tests that were significant (Water: Sr:Ca, Mn:Ca; Otoliths: Sr:Ca, Ba:Ca) being Ln(x + 1) transformed. Conc. = concentration

Source of variation	df	Sr:Ca MS	Sr:Ca *F*	Sr:Ca p	Ba:Ca MS	Ba:Ca *F*	Ba:Ca p	Mn:Ca MS	Mn:Ca *F*	Mn:Ca p
Water										
Concentration	4	5.4642	4621.90	0.0000	780.2746	1416.09	0.0000	7.9386	3131.04	0.0000
Tank (Conc.)	5	0.0012	1.00	0.4454	0.5510	0.08	0.9939	0.0025	0.05	0.9984
Residual	20	0.0012			6.5138			0.0536		
Otoliths										
Concentration	4	3.8347	380.69	0.0000	1.4742	55.68	0.0002	4.2930	6.49	0.0324
Tank (Conc.)	5	0.0101	0.50	0.7713	0.0265	1.38	0.2459	0.6614	0.23	0.9488
Residual	60	0.0199			0.0192			2.8977		

factor ANOVAs treated 'Concentration' as a fixed factor, and 'Tank' as a random factor nested within concentrations. If variances were heterogeneous (Cochran's *C*-test, $p < 0.05$) data were Ln(x + 1) transformed, after which homogeneity of variance was obtained. Where significant differences were detected (e.g. $p < 0.05$), means of treatment groups were compared using Student-Newman-Keuls (SNK) tests to determine where these differences occurred.

A partition coefficient was calculated for each tank as a mean ± 95% confidence interval (CI). An average partition coefficient of the linear regression line was then calculated based on the mean coefficient from each tank. Although a zero intercept was not determined between the concentrations of elements (both Sr and Ba) in the otoliths of fish and the surrounding water, this relationship was assumed in order to assess changes in partition coefficients within treatment groups. Average partition coefficients for each treatment were calculated from the coefficients of each tank in order to plot them against the elemental concentration in the water. Data are displayed as means ± SE.

RESULTS

Rearing conditions

The concentration ratios of elements in the rearing water differed significantly between treatments, and no differences in concentration ratios were detected between tanks of each treatment (Tables 1 & 2, Fig. 1). The concentration ratios for both Sr:Ca and Mn:Ca in the water were different between each treatment level of 1, 2, 4, 8 and 16× elemental spiking (Fig. 1a,e, SNK tests), with the elemental concentration ratios of water increasing according to the treatment spiking regime, although the increase was not linear for Sr:Ca. Similarly, the concentration ratio of Ba:Ca increased with treatment spiking regime (again non-linear), however the treatments of 8 and 16× elemental spiking were not significantly different (Fig. 1c, SNK tests).

Temperature and salinity regimes of the rearing water did not differ between treatment groups (ANOVA: temperature: $F_{4,5} = 2.52$, $p = 0.1694$; salinity: $F_{4,5} = 0.2940$, $p = 0.9479$, Table 1). Although there were significant differences in temperature detected between some tanks (ANOVA: tank: $F_{5,200} = 12.52$, $p = 0.0000$, Table 1), less than 0.7°C separated the means of all duplicate treatment tanks, suggesting that temperature was likely to have little effect. There was no difference in fish length and weight between treatment groups (ANOVA: length: $F_{4,5} = 1.91$, $p = 0.2478$; weight: $F_{4,5} = 3.45$, $p = 0.1034$).

Effect of spiking on otoliths

The concentration ratios of elements in fish otoliths were significantly different between treatment groups, with no differences detected between tanks of each treatment (Table 2, Fig. 1). The concentration ratio of Sr:Ca in otoliths increased significantly with treatment levels from 1 to 16× elemental spiking (Fig. 1b, SNK tests). Ba:Ca increased with increasing treatment level from 1 to 16× elemental spiking; however, there were no differences in elemental concentration detected for the 2 highest treatments levels (8 and 16× ambient; Fig. 1d, SNK tests). Significant differences were

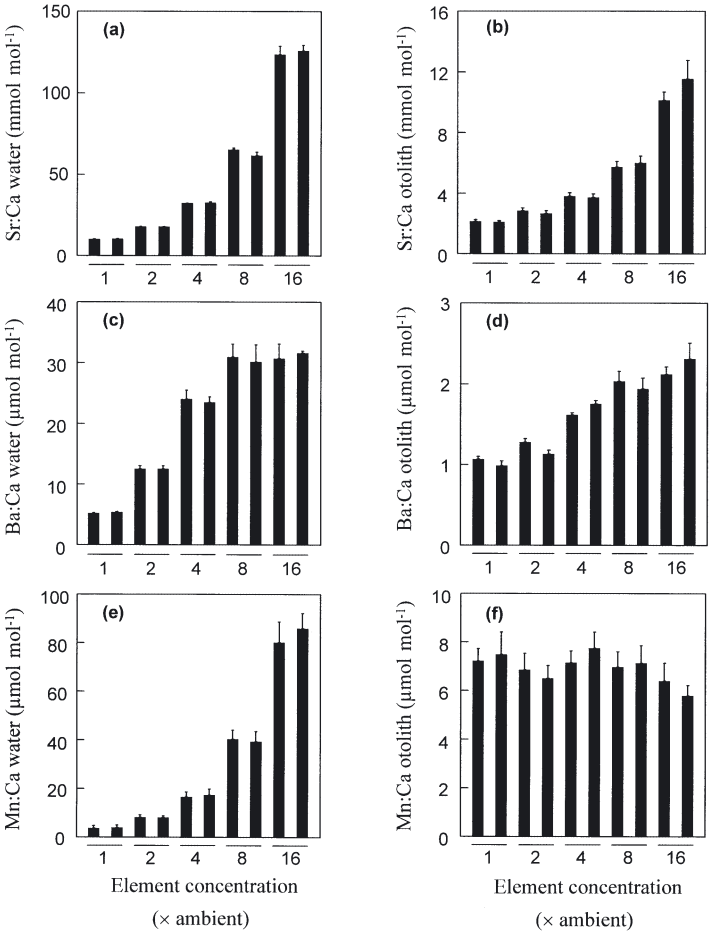

Fig. 1. *Acanthopagrus butcheri.* Mean elemental concentration (±SE) of (a) Sr:Ca, (c) Ba:Ca, and (e) Mn:Ca in the rearing water of each treatment tank, and the corresponding concentration in otoliths (b) Sr:Ca, (d) Ba:Ca and (f) Mn:Ca of black bream within each treatment level. Replicate tanks for each treatment are shown

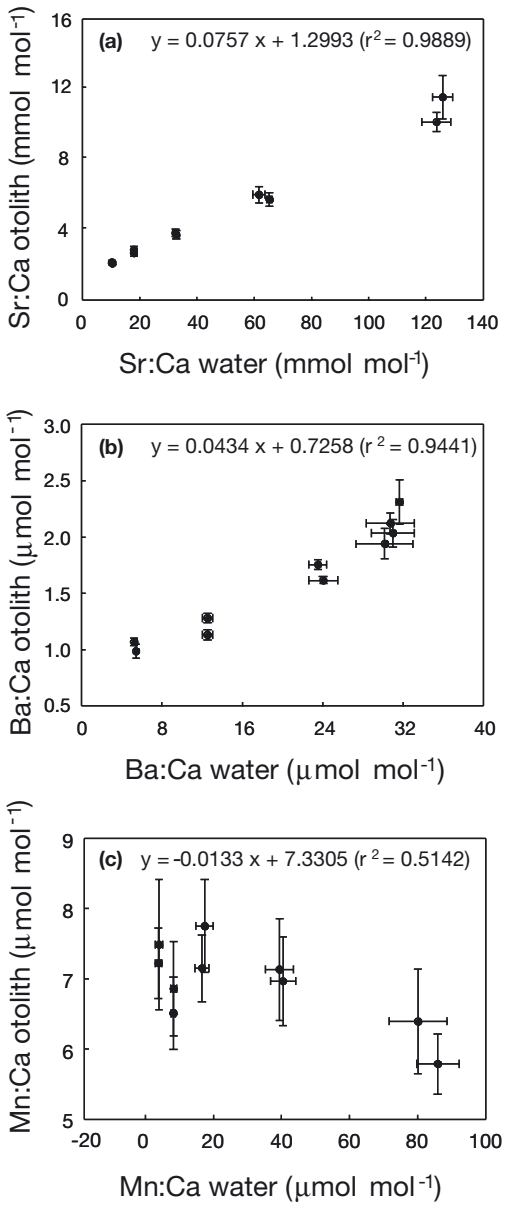

Fig. 2. *Acanthopagrus butcheri*. Plots of elemental concentration in otoliths versus the actual elemental concentration in the water for each element: (a) Sr:Ca, (b) Ba:Ca, and (c) Mn:Ca. Data represent mean concentrations (±SE) for each tank within each treatment level. The relationship between the water and otolith elemental concentration are displayed with linear regression equations and regression coefficients (r^2)

detected for the concentration ratio of Mn:Ca among treatment groups. SNK tests yielded ambiguous results, with the 1× treatment level being higher than the 8 and 16× treatment levels, but not different to 2 and 4× treatment level, yet there were no differences detected among treatments of 2, 4, 8 and 16× (Fig. 1f). However, there is clearly a slightly negative trend in otolith Mn:Ca with that in water, with the detection of this relationship likely to be influenced by the large variation in the Mn:Ca concentration in otoliths of fish and the surrounding water.

There were strong relationships between the concentration of elements in the water and that in the otolith for Sr:Ca and Ba:Ca, but not Mn:Ca. The concentrations of Sr:Ca and Ba:Ca in the otolith increased with that in the water (Fig. 2a,b). The mean calculated partition coefficient of D_{Sr} was 0.131 ± 0.011 (95% CI), whereas that of D_{Ba} from this line was 0.099 ± 0.043 (95% CI). The relationship between the concentration ratio of Mn:Ca in the otolith and that in the water showed a slight negative trend. This was mostly due to the low concentration ratio of Mn:Ca in otoliths at a high concentration ratio of Mn:Ca in the water (Fig. 2c). A mean partition coefficient of D_{Mn} was determined to be 0.683 ± 0.501 (95% CI).

A non-linear relationship was found between the average partition coefficient of each treatment level and the elemental concentration ratio in water for all of the elements (Fig. 3) The partition coefficients for the elemental ratios of D_{Sr}, D_{Ba} and D_{Mn} decreased with the increasing elemental ratio in water. The partition coefficient for D_{Sr} only stabilised at Sr:Ca water concentrations above approximately 65 mmol mol^{-1}. However, lower concentrations of Sr:Ca in the water significantly increased the partition coefficient of the element (Fig. 3a). Partition coefficients only became stable for D_{Ba} at water concentration ratios above approximately 24 µmol mol^{-1}, again with lower concentration ratios of Ba:Ca yielding higher partition coefficient values (Fig. 3b). Similarly, the partition coefficient for D_{Mn} increased at low concentration ratios of Mn:Ca in the water, and only stabilised when Mn:Ca in the water was greater than approximately 40 µmol mol^{-1} (Fig. 3c).

DISCUSSION

Rearing conditions

The concentration ratios of Sr:Ca and Mn:Ca in the water were significantly different between each treatment group, and conformed with the expected linear increase in elemental concentration with an increase in concentration from 1 to 16× treatment levels. However, there was some discrepancy with Ba:Ca, where the concentration ratio increased linearly from 1 to 8× as expected, but there were no differences detected between the 8 and 16× treatment levels. This is most probably due to the saturation of Ba in seawater, whereby seawater is saturated at concentrations above approximately 35 µg l^{-1} at 25°C and 1 ATM of pressure (Church & Wolgemuth 1972). The concentrations

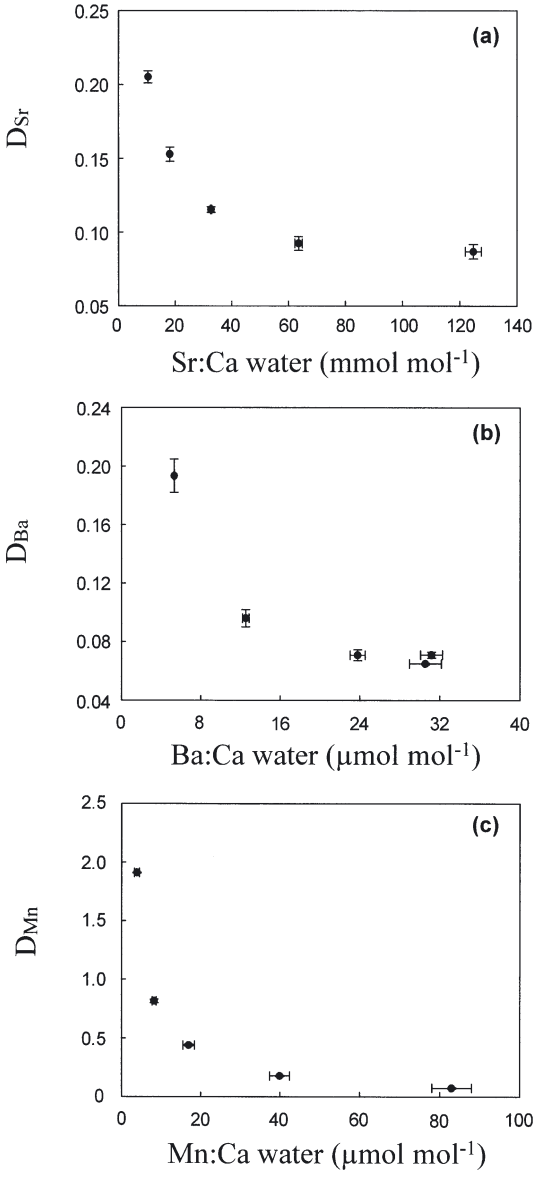

Fig. 3. *Acanthopagrus butcheri*. Plots of partition coefficients ($D_{element}$) versus the actual elemental concentration in the water for each element: (a) Sr:Ca, (b) Ba:Ca, and (c) Mn:Ca. Data represent mean concentrations (±SE) for each treatment

detected in the 8× treatment level may therefore be saturated, and the Ba chloride added to the 16× treatment level tanks had relatively little effect on the already-saturated solution. Nevertheless, the concentrations of elements in the treatment groups generally increased from 1 to 16× treatment levels.

Environmental variables (e.g. temperature and salinity) showed little difference between treatments. Temperature varied less than 1.5°C and salinity varied less than 1‰ across all of the treatment groups. Similarly, there was no difference in fish growth (length and weight) between treatments. Hence, the manipulations of concentrations are unlikely to be confounded by temperature and/or salinity, or fish growth, which have previously been shown to influence otolith chemistry of black bream (Elsdon & Gillanders 2002).

Effect of strontium

The concentration ratios of Sr:Ca in the otoliths of black bream followed the same trend as that of the water elemental concentration; this trend has also been detected in other studies examining the uptake of Sr into fish otoliths (e.g. Brown & Harris 1995, Pollard et al. 1999). However, the range of Sr concentrations used in this experiment was significantly higher that those used in previous studies (with the exception of mass-marking experiments), with a range spanning 10 to 125 mmol mol^{-1}, compared to the lower ranges examined by Bath et al. (2000) of 12 to 23 mmol mol^{-1}, and Milton & Chenery (2001) of between 0.1 and 0.9 mmol mol^{-1}. Thus, a linear relationship between the concentration of elements in water and that in otoliths extends over a wide range of water concentrations, and there is no evidence that the otolith becomes saturated.

Our results also follow trends seen in experiments that aimed to mark otoliths with Sr in a bid to create an elementally enhanced region on the otolith. Many of these studies used concentrations of Sr higher than those used by Bath et al. (2000) and Milton & Chenery (2001), with average concentrations being up to 40× ambient elemental concentrations (equivalent to 1.0 g l^{-1} of Sr) (e.g. Pollard et al. 1999). The present study is the first experimental study to examine the otolith chemistry of fish reared across a large array of Sr concentrations, and as such provides information beyond what is currently known about Sr in fish otoliths.

The trend of increasing Sr:Ca concentration ratios in the otoliths of black bream is explained with a linear equation, and the data showed a close fit. The non-zero intercept of this equation indicates sources of Sr other than water. However, the influence of Sr from other sources (e.g. possible dietary contributions) is thought to be minor compared to sources from the surrounding water. An average partition coefficient was calculated from this relationship (D_{Sr} = 0.131 ± 0.011), showing that significant discrimination occurred between the concentration of elements in the water and those in the otoliths. The partition coefficient found in this experiment is slightly lower than that of Bath et al. (2000) of 0.182 at 20°C and 0.205 at 25°C. However, all of these coefficients are considerably low and show that elemental discrimination has occurred. Whilst the exact cause for discrimination is not known,

it is likely that the otolith precipitation rate (Mucci & Morse 1983) and the many boundaries that elements have to pass through before being incorporated are likely to be influencing factors (Campana 1999). The inference of discrimination can be made when the average partition coefficient of black bream is compared to corals, which have a partition coefficient of approximately 1, as they are in direct contact with the surrounding water and experience little discrimination due to cellular boundaries (Shen et al. 1996).

The relationship between the partition coefficient and the actual concentration ratios of Sr:Ca for each treatment did not result in a linear relationship, which would be expected if the degree of elemental discrimination was constant at each Sr:Ca concentration (Siemann & Schramm 2002). A non-linear relationship also indicates that the relationship between the elemental concentration of otoliths and water cannot be extrapolated beyond these data points. However, the non-linear relationship between the partition coefficient and water Sr:Ca concentration may in part be due to the non-zero intercept that has been assumed to occur between Sr in otoliths and Sr in water. As similar plots have not previously been presented for Sr in otoliths, comparisons of the generality of these results are difficult. However, it can be concluded that the degree of discrimination that occurs between water and calcified structures is likely to be concentration dependent, highlighting the need for experimental manipulations that use broad ranges of elemental concentrations in water.

Effect of barium

The concentration of Ba:Ca in the otoliths of fish showed an increase with increasing water concentration, as has been found previously (Mugiya et al. 1991, Bath et al. 2000). The concentration of Ba:Ca in the treatment levels 1 to 16× ranged from 5 to 30 µmol mol^{-1}, this range having been previously used by Bath et al. (2000). Although the range of concentrations used by Bath et al. (2000) was higher (up to 231 µmol mol^{-1}) than in our study, this may be a reflection of the artificial saltwater mix used by Bath et al. (2000). Whilst the saturation level of the water used by Bath et al. (2000) may have been higher than oceanic waters, the results remain consistent between the 2 experiments.

The relationship of Ba in the water and that in black bream otoliths is also consistent with that of other calcified structures such as foraminiferans (Lea & Spero 1994). Indeed, the slope of the linear equation determined from black bream (0.0434) is similar to that detected for other species of fish by both Bath et al. (2000) and Milton & Chenery (2001) (0.039 and 0.03 respectively). A mean partition coefficient of 0.099 was calculated for Ba in the otolith versus the water, and again, like Sr, this shows that considerable discrimination occurred. Whilst the partition coefficient of Ba in bream (D_{Ba} = 0.099) is slightly higher than that calculated by Bath et al. (2000) (D_{Ba} = 0.06), they both still remain very similar to other calcified structures such as foraminiferans (D_{Ba} = 0.16) (Lea & Spero 1992).

The relationship between the partition coefficient of each treatment group and the actual concentration ratio of Ba:Ca in the water shows a non-linear relationship, which, similar to Sr, may be influenced by the non-zero intercept between otoliths and water Ba concentrations. This relationship is almost identical to that determined by Bath et al. (2000), and shows that the extrapolation of results beyond the data points is not appropriate. Indeed, the variation of otolith Ba:Ca may also indicate that changes in other environmental variables may influence the relationship between the elemental concentration of calcified structures and the water, with temperature already known to cause such changes (Bath et al. 2000).

Effects of manganese

Unlike the near-linear relationships that occur with Sr and Ba in otoliths and water, Mn showed a slight decrease in otolith elemental concentrations with increasing water concentration and large variation among replicate fish in each tank. As there are few published results of Mn effects on otolith chemistry, generalisation about how the results for black bream compare are difficult. However, it appears that the contribution of Mn in the water to otolith concentration is low (Mugiya et al. 1991). Penreath (1973) determined that the concentration of Mn in the water surrounding fish (*Pleuronectes platessa* L.) only accounted for 0.1% of the Mn detected in their body tissue.

Interestingly, plotting the concentration ratio of Mn:Ca in the otoliths versus that in the water showed that at low water concentrations, the obtained partition coefficient is close to 1 and approached 2 at ambient levels. Thus, at low and ambient concentrations there seems to be a positive discrimination (incorporation) of Mn. However, the incorporation of Mn in otoliths (if not the entire fish) is regulated at concentrations above ambient. Given that Mn is both toxic to organisms in high concentrations (Rainbow 1997), and used in many metabolic processes (e.g. cellular catalyst, Lehman & Joyce 1993), regulation of Mn to constant levels may be required to prevent death. Evidence that toxic elements like Mn are regulated is not uncommon in the invertebrate literature. For example, decapod crus-

taceans are known to excrete zinc in order to balance uptake with that in the body tissue (White & Rainbow 1984). The large variation of Mn in bream otoliths found in the present study may indeed represent the regulation of Mn by individual fish, especially in treatments where variation in ambient Mn is considerably lower. Thus, differences that are detected in the otoliths of fish (Gillanders & Kingsford 1996, 2000, Sanchez-Jerez et al. 2002) may not represent changes in water chemistry. Indeed, such changes may represent dietary uptake or trophic links. Dietary uptake of Mn into the liver, muscle, and bones of fish *Pleuronectes platessa* L. has previously been shown to be more significant that uptake from water (Pentreath 1976).

Application for determining environmental histories of fish

The present study has demonstrated that black bream, reared in enhanced concentrations of elements Sr and Ba, show a clear and significant increase in the concentration of these elements in their otoliths. Partition coefficients, D_{Me}, indicate that there is a degree of discrimination between the water and otolith chemistry, with plots of these coefficients versus water indicating that predictions of the relationship occuring at concentrations beyond those used in the experiment are not justified. The same linear relationship between water and otolith elemental concentration did not occur for Mn, which we believe represents the regulation of this element by fish. Given that Mn is both toxic and used in metabolic processes, regulation to a constant concentration seems plausible. However, some evidence has shown that the most likely source of Mn in otoliths is from trophic or direct dietary uptake, which fish may not be able to regulate (see Pentreath 1976 for the influence of diet on Mn in fish tissue and bone).

The simultaneous manipulation of elements in the present study does not allow for a clear distinction of the competition of elements for binding sites in the calcium carbonate matrix of otoliths. However, both Sr and Ba showed increasing concentrations consistent with patterns of other studies (Bath et al. 2000). The incorporation of Mn in otoliths is slightly in reverse to that of Sr and Ba, which may represent competition for binding sites by Sr and Ba. However, the incorporation of Mn in otoliths from water sources has not been tested extensively; thus, there is a need for further investigation of Mn incorporation in fish. Investigations should include experiments that spike elements into rearing water of fish in different combinations of concentrations to determine the degree of competition for binding sites within the otolith matrix.

The results of this study indicate the usefulness of laboratory experiments in determining how individual environmental variables influence otolith chemistry. Knowing that Sr and Ba are influenced by the concentration of elements in water, and that Mn seems to be less influenced by ambient concentrations, may lead to these elements being used to answer different ecological questions. For instance, Sr and Ba are most suited to the reconstruction of fish movement along concentration gradients, where predictable equations such as those presented in this study can be used to determine the type of water bodies that fish inhabited at different life-history periods. However, if Mn is indeed resistant by diet (which seems possible given available evidence, Pentreath 1976), then Mn may be useful in determining areas of inhabitancy where food sources contain different concentrations of Mn (Sanchez-Jerez et al. 2002).

It should be noted that the influence of water chemistry on otolith chemistry in this study does not take into account other factors that may influence the concentrations detected. Where factors such as temperature and salinity are known to influence the uptake of elements (Elsdon & Gillanders 2002), it is likely that interactions will occur between these environmental variables and the concentration of elements in water. This may be particularly important if otolith chemistry is used to determine past migrations of fish based on ambient elemental levels. If migratory fish move through dilution or temperature gradients, as is common in estuaries, then interactions affecting the trace-metal uptake rate may confound the derived movements of these fish. Thus, it would be ideal to test how a combination of environmental variables influences otolith chemistry, if otoliths are to become even more useful tools in fisheries science than they presently are.

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