Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish

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Abstract: The concentrations of elements and isotopes in fish otoliths may provide a method of reconstructing movements of fish by differentiating between water bodies of different temperatures and salinities. However, before otoliths can be used to reconstruct environmental histories of fish, it is necessary to assess the effects of seawater temperature and salinity on otolith microchemistry. Using controlled laboratory experiments, juvenile black bream, *Acanthopagrus butcheri* (family Sparidae), were reared for 50 days in aquaria of varying temperatures and salinities using three experimental designs: temperature × salinity, temperature only, and salinity only. Temperature and salinity interacted to significantly affect the elemental concentration ratios of Sr:Ca and Ba:Ca and the concentrations of isotopes $\delta^{13}C$ and $\delta^{18}O$ in otoliths. The single-factor experiments showed that temperature significantly affected the concentration ratios of Sr:Ca and Ba:Ca and the concentrations of $\delta^{13}C$ and $\delta^{18}O$ in otoliths, whereas salinity alone did not affect the concentration ratios of any elements but did affect both isotopes. The concentration ratios of Mg:Ca and Mn:Ca varied considerably among fish within the same treatment level and showed little or no effects due to temperature and (or) salinity. The significant interactive effects of temperature and salinity on otolith microchemistry highlight the need for a multifactorial approach to testing hypotheses regarding the environmental histories of fish.

Résumé : Les concentrations d'éléments et d'isotopes dans les otolithes de poissons peuvent fournir une méthode pour retracer les déplacements des poissons en discriminant entre des masses d'eau de températures et de salinités différentes. Cependant, avant que les otolithes ne puissent servir à récapituler l'histoire environnementale des poissons, il est nécessaire de déterminer les effets de la température et de la salinité de l'eau de mer sur la microchimie des otolithes. Dans des expériences de laboratoire contrôlées, nous avons élevé des jeunes « brèmes noires », *Acanthopagrus butcheri* (famille des Sparidae), durant 50 jours dans des aquariums de salinités et de températures différentes selon trois plans d'expérience: température × salinité, température seule et salinité seule. La température et la salinité interagissent pour affecter de façon significative les rapports des concentrations d'éléments Sr:Ca et Ba:Ca,, ainsi que des isotopes $\delta^{13}C$ et $\delta^{18}O$ dans les otolithes. Les expériences qui tiennent compte des facteurs séparément indiquent que la température affecte de façon significative les rapports des concentrations de Sr:Ca, Ba:Ca, $\delta^{13}C$ et $\delta^{18}O$ dans les otolithes, alors que la salinité seule n'affecte pas les rapports des éléments, mais seulement les deux isotopes. Les rapports des concentrations de Mg:Ca varient considérablement chez les poissons soumis à un même traitement et ne sont que peu affectés par la température et/ou par la salinité ou alors ne sont pas affectés du tout. Les effets significatifs de l'interaction de la température et de la salinité dans la microchimie des otolithes mettent en relief la nécessité d'adopter une approche multifactorielle dans l'évaluation des hypothèses sur l'histoire environnementale des poissons.

[Traduit par la Rédaction]

Introduction

One of the most recent developments in fisheries science is the use of elemental and isotopic concentrations of calcified structures as chemical fingerprints or natural tags. These chemical fingerprints can potentially be used to discriminate between fish that have inhabited different water bodies (e.g., discriminate between nursery areas (Gillanders and Kingsford 2000) and different stocks (Edmonds et al. 1999)) and can therefore determine migration patterns of individuals and, more broadly, define fish stocks. The most widely used calcified structure for stock discrimination and movement studies are otoliths, which are suspended in endolymph fluid within the inner ear (Campana 1999). Formation of new otolith material occurs when calcium carbonate ($CaCO_3$) crystallizes out of the endolymph fluid onto the outer surface of the otolith, with this growth corresponding to daily, seasonal, and annual increments (Campana and Neilson 1985). As calcium carbonate crystallizes onto the otolith surface, elements such as carbon (C), oxygen (O), calcium (Ca), strontium (Sr), magnesium (Mg), manganese (Mn), and barium (Ba) along with other elements are incorporated into the

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carbonate matrix. Elements in the water pass through three main interfaces (brachial uptake, cellular transport, and crystallization) before they are incorporated into the otolith. The presence of these interfaces, which may concentrate or dilute elements and isotopes, ensures that otoliths, unlike other calcified structures (e.g., bivalve shells (Campana and Thorrold 2001)), do not necessarily directly reflect the elemental or isotopic composition of the surrounding seawater. Thus, the assimilation of elements and isotopes into otoliths is likely to be influenced by both physiological processes (Kalish 1989; Hoff and Fuiman 1993; Campana 1999) and environmental variables such as temperature and salinity (Fowler et al. 1995*a*; Hoff and Fuiman 1995; Thorrold et al. 1997).

Temperature and salinity do not vary independently of one another in seawater and can vary over small spatial scales, both vertically in the water column and horizontally between different water bodies (e.g., Bisagni et al. 2001). Temperature and salinity can also influence elemental and isotopic concentrations in seawater (e.g., Epstein and Mayeda 1953). As fish migrate they may inhabit waters of varying temperature and salinity. Hence, determining the effects of temperature and salinity on the incorporation of elements and isotopes into otoliths enhances our ability to interpret movement and migration patterns (e.g., Milton et al. 2000), stock structure of fish (Edmonds et al. 1999; Campana et al. 2000), and temperature and salinity histories of fish (e.g., Patterson 1999) based on otolith microchemistry.

The influence of temperature and salinity on the concentration of elements in otoliths has largely been determined by field studies where fish collected from environments with different temperature and salinity regimes (e.g., freshwater, estuarine, and marine waters, see Tzeng et al. 1997) are compared. Such studies assume that the otolith area was deposited while the fish inhabited a particular body of water; however, temperature and (or) salinity at the time of otolith deposition is often unknown. There have been several controlled experimental evaluations reporting varied results on the effects of temperature alone (e.g., Tzeng 1996; Chesney et al. 1998; Kawakami et al. 1998) and salinity alone (e.g., Hoff and Fuiman 1995; Secor et al. 1995; Tzeng 1996) on the concentration of elements in otoliths. Most of these experiments have focused on microelements (e.g., Na, Mg, Sr), and there have been relatively few experiments examining trace elements (e.g., Ba and Mn, but see Fowler et al. 1995*a* and 1995*b* and Hoff and Fuiman 1995). There have been fewer experiments successfully rearing fish under orthogonal combinations of temperature and salinity (Secor et al. 1995; Tzeng 1996; Chesney et al. 1998).

Much of the research on the influence of temperature and salinity on carbon and oxygen isotopes has been inferred through correlative studies (e.g., Patterson 1999). Although there are several examples of isotopes being used to discriminate between fish inhabiting different water masses (e.g., Edmonds et al. 1999), the environmental factors that cause these differences in otolith isotopic concentration remain largely unknown. Several experiments have assessed the effect of temperature on otolith oxygen and carbon isotopic concentrations (Kalish 1991*a*; Radtke et al. 1996; Thorrold et al. 1997). All experiments found a positive relationship between oxygen isotopic concentrations and temperature, whereas carbon isotopic results displayed both negative and nonsignificant trends with temperature. The influence of salinity on stable isotope concentrations of otoliths remains untested, and similarly, there have been no orthogonally designed experiments examining the effects of temperature and salinity on isotope concentrations.

The lack of experimental evidence describing the relative and interactive effects of temperature and salinity on otolith elemental and isotopic concentrations represents a significant void in otolith research and is a severe inhibitor to our understanding of the role that otolith microchemistry can play in determining migratory patterns of fish. The objective of this study was to determine if otolith microchemistry of black bream (*Acanthopagrus butcheri*, family Sparidae), a commercially important species that occupies a wide range of temperatures and salinities, is influenced by seawater temperature and salinity. Specifically, controlled laboratory experiments that manipulated temperature and salinity and an orthogonal combination of both factors were used to determine the relative and interactive effects of these parameters on the elemental and isotopic concentrations of bream otoliths.

Materials and methods

Experimental design

Juvenile black bream (*A. butcheri*) ~20 mm in total length were obtained from a local hatchery (Clean Seas Aquaculture, Arno Bay, South Australia) and held in a 300-L fibreglass tank equipped with adequate air and water filtration. The fish were bred from a common brood stock, thus reducing genetic variability. Fish were allowed to acclimatize for 2 days under similar conditions to those in which they were reared (22°C and 30‰), after which they were subjected to experimental treatments. All seawater used during the rearing process was collected weekly from the South Australian Research and Development Institute (SARDI) Aquatic Sciences facility and was held in a 2000-L indoor aerated tank, which was covered to reduce evaporation. Fish were fed a mixed diet of commercial bream food, flake food, live brine shrimp (*Artemia* sp.), and black worms (*Tubifex* sp.) throughout the duration of the experiment. Light was provided from fluorescent tubes on a 12-h day – 12-h night cycle in a constant-temperature room.

Otoliths were marked at the end of the acclimatization period by immersing fish in a tetracycline hydrochloride bath (250 $mg \cdot L^{-1}$) for 16 h (e.g., Kalish 1989). This enabled the differentiation of old otolith growth (at the hatchery) from new otolith growth (under experimental conditions). Immediately after the tetracycline bath, fish were randomly assigned to experimental treatments at densities of 11–13 fish per tank. Each experimental tank consisted of a 40-L high density polyethylene tub (sterilized using bleach) with adequate aeration and a clear plexiglass cover to minimize evaporation (and hence salinity changes).

Three experiments were set up: (1) temperature × salinity manipulations arranged in an orthogonal two-way design to test the relative and interactive effect of these two factors, (2) temperature manipulations in a one-way design to test the effect of a broad range of temperatures (12, 16, 20, 24, 28°C) at a constant salinity (30‰), and (3) salinity manipulations in a one-way design to test the effect of a broad range of salinities (5, 11, 17, 23, 30‰) at a constant temper-

ature (16°C). Treatments in the orthogonal two-way experiment of temperature × salinity consisted of all possible combinations of three temperatures (16, 20, and 24°C) and three salinities (5, 17, and 30‰). For all experiments, each treatment level had duplicate tanks.

The experimental conditions in each tank containing fish were established by adjusting the temperature and salinity of the water at a rate of 2°C per day and 3.5‰ per day until the desired temperatures and (or) salinities were obtained. Temperature conditions were maintained using chiller units (12°C tanks) and aquarium heaters (20°C and higher temperature tanks). The desired salinity for each treatment was established by mixing seawater with deionized water to the desired concentration. One-quarter of the water in each tank was changed every 2nd day, at which time any accumulated detritus was siphoned away. During the rearing period, temperature and salinity were monitored daily.

After 50 days of exposure to the experimental conditions, all fish were immersed in an ice slurry. For each fish, the standard length was measured and the sagittal otoliths were dissected, washed and cleaned in deionized (Milli-Q, Millipore) water, and allowed to dry before being stored in microcentrifuge tubes. One otolith from each pair was weighed using an electrobalance (accuracy to 0.00001 g). The otoliths of seven fish from each tank (the maximum number available at the end of the experiment for a balanced design) were then chosen for analysis of elemental and isotopic concentrations based on the similarities of otolith weights because otolith weight is likely to influence otolith chemistry. Previous work has found that the analysis of seven fish is adequate given the low variability of otolith chemistry among replicates (Dove et al. 1996).

General sample preparation

All sample cleaning and diluting was done using deionized water, as was the cleaning of all materials. Plasticware and glassware were soaked in 10% HNO_3 for at least 24 h and rinsed several times in deionized water before use. The final preparation of samples was done in a plastic laminar flow cabinet to reduce possible airborne sources of contamination.

Elements in otoliths

An otolith from each fish was embedded in Epofix resin (Struers), sectioned transversely through the focus (centre section) to a thickness of approximately 300 μm using a low-speed diamond saw, and polished using 9-μm aluminium oxide lapping film to approximately 200-μm thickness. Polished sections were cleaned in deionized water and dried on glass, after which they were sonicated (cleaned) for 5 min and allowed to dry overnight in a laminar flow cabinet before being mounted on microscope slides. These mounted slides were again dried in a laminar flow cabinet overnight and stored in clean plastic sealable bags awaiting analysis.

The outside edges of otoliths from *A. butcheri* were sampled to ensure that the ablated material was laid down under experimental conditions. This was confirmed by prior examination of the position of the tetracycline fluorescent band. The concentration of elements (Sr, Mn, Ba, Mg, Ca) in the otolith sample was determined using a Merchantek LUV 266 Q-switched Nd:YAG UV laser microprobe (New Wave Research), with a pulse rate of 6.00 Hz and ablation spot size of 100 μm. The laser was connected to a Finnigan MAT ELEMENT HR–ICP–MS (high resolution – inductively coupled plasma – mass spectrometer; Thermo-Finnigan) with gas-flow parameters of coolant 14.00, axillary 1.55, sample 1.5, and helium 0.36 $L \cdot min^{-1}$. Ablation of the otolith material occurred inside a sealed chamber. The ablated sample was extracted from the chamber and transported to the ICP–MS, via a settlement chamber, by an argon and helium gas stream. The ablation chamber was purged for 20 s after each opening to remove any background gas or sample particle that may have contaminated further samplings (see Lahaye et al. 1997 for a detailed description of the machine setup).

Blank ablations, which consisted of measuring the sample gases without ablation, were analyzed before and after each sampling session (for approximately 120 s) to determine detection limits. A reference standard (National Institute of Standards and Technology, NIST 612) was measured after every 12 ablations to correct for low-frequency drift resulting from changes in room temperature, plasma, and electronics (Ludden et al. 1995). The analytical accuracy for the NIST standard averaged across all samples was 100% recovery for all elements; results were reproducible to within 0.0015 (Mg), 0.0003 (Mn), 0.0004 (Sr), and 0.0001 $\mu mol \cdot mol^{-1}$ (Ba). Calcium was used as an internal standard, and therefore, the actual Ca concentrations in the otoliths of each treatment level were determined using AAS (atomic absorption spectrometry); reproducibility was 1%. Samples for Ca analysis (200–600 μg) were obtained by chipping the edge of otoliths from each treatment group (described in isotopes of otolith section in the methods). Chips were diluted in 4 mL concentrated nitric acid and further diluted to 20 mL with deionized water before analysis. The concentrations of each element (Mg, Mn, Sr, Ba) were standardized to calcium by expressing as a ratio to Ca, thus giving values in concentration ratios.

Elements in water

During the rearing period, three 50-mL water samples were collected from each treatment tank, 25 mL of which was filtered through a 0.45 μm filter and acidified with 500 μL of high-grade nitric acid. Samples were refrigerated until further diluted (1:10, sample–deionized water) prior to analysis.

Water samples were analyzed using a DV ICP–AES (dual-view inductively coupled plasma – atomic emission spectrometer; Perkin-Elmer) for the analysis of Ca and Mg and a DRC ICP–MS (dynamic reaction cell inductively coupled plasma – mass spectrometer; Perkin-Elmer) for the analysis of Mn, Sr, and Ba. During the analysis, blanks (prepared in the same manner as samples but with deionized water instead of seawater), spiked blanks (elements spiked into blank sample in known quantities), and standard solutions were analyzed every 10–15 samples and used to correct for background concentrations, assess accuracy and precision of sampling and calculate concentrations in samples. Internal standards of indium (In; 5 ppb) and lutetium (Lu; 2 ppm) were used to correct for drift of ICP–MS and ICP–AES instruments, respectively. The analytical accuracy of elements averaged across all samples was 91 (Ca), 96 (Mg), 110 (Mn), 82 (Sr), and 92% (Ba), with reproducibility of <0.0001 (Ca, Mg, Sr, Ba) and 0.0036 $\mu mol \cdot mol^{-1}$ (Mn). The concentration of each element (Mg, Mn, Sr, Ba) was stan-

dardized by expressing as a ratio to calcium as these elements are likely to substitute for calcium in otoliths (Campana 1999) and the incorporation of these elements are dependent on the calcium in the water and not the absolute concentrations of the elements.

Isotopes in otoliths

Chips from the outside edges of the dorsal and ventral ends of the second otolith of each fish were collected for isotope analysis. Under a dissecting microscope, whole otoliths were imbedded in adhesive, and based on the position of the fluorescent band, a clean scalpel was used to remove chips from the outside edge so that the chips represented experimental otolith growth. Otolith chips (approximately 250 μg) were placed in a microcentrifuge tube with a 5% H_2O_2 solution and sonicated for 1 h to remove organic material. Cleaned otolith chips were washed several times with deionized water and allowed to dry overnight inside a laminar flow cabinet, after which they were stored in screw-cap plastic vials awaiting analysis.

The concentrations of carbon and oxygen isotopes in the otolith chips were determined using a Finnigan MAT 261 mass spectrometer. Blank samples, NIST standards, and bicarbonate standards were used to assess possible instrument drift. Results were reproducible to within 0.1‰ for both $\delta^{13}C$ and $\delta^{18}O$. Carbon isotope concentrations were standardized to PDB (Peedee belemnite; Epstein and Mayeda 1953) and oxygen isotope concentrations were standardized to SMOW (standard mean ocean water; Coplen 1988), both expressed as parts per thousand (‰).

Isotopes in water

During the rearing process, three water samples were collected in high density polyethylene bottles and were frozen immediately on return to the laboratory. Water samples were prepared for carbon isotope analysis by acidification with H_3PO_4 under a vacuum, followed by cryogenic purification of the gas. Water samples were prepared for oxygen isotope analysis via equilibration with CO_2 at 25°C for 24 h (Kroopnick 1974). The extracted gases for carbon and oxygen isotopes were analyzed using a Finnigan MAT 252 mass spectrometer, and standard solutions were used to assess instrumental drift. Results were reproducible to within 0.2‰ for both $\delta^{13}C$ and $\delta^{18}O$. Carbon isotope concentrations were standardized to PDB and oxygen isotope concentrations were standardized to SMOW.

Statistical analyses

Univariate statistical techniques were used to test hypotheses relating to differences in temperature and salinity of the rearing conditions, as well as differences in fish otolith weight between treatment groups. Univariate statistical techniques were also used to test hypotheses relating to individual elemental concentration ratios (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) and concentrations of isotopes ($\delta^{13}C$ and $\delta^{18}O$) of *A. butcheri* reared under different treatment conditions. To compare the elements and isotopes among treatments in each experiment (temperature × salinity, temperature, and salinity), analyses of variance (ANOVA) were used. Temperature and salinity were treated as fixed factors and tank, a random factor, was nested within treatments. If variances were heterogeneous (Cochran's *C* test, $P < 0.05$), data were $\ln(X + 1)$ transformed, after which homogeneity of variance was always obtained. Where significant effects of treatments were detected (i.e., $P < 0.05$), means were compared using Student–Newman–Keuls (SNK) multiple-comparison tests. Backwards stepwise regression analyses were used to determine which of the variables (temperature–salinity treatment, ambient water chemistry, and otolith weight) significantly influenced the concentration of the element or isotope in the otolith. The regression analyses assume a linear relationship between the variable of interest and the concentrations in the otolith, an assumption that may not have always been met by the data.

Multivariate analysis was done for each experiment using a nonparametric multivariate analysis of variance, NP-MANOVA (Anderson 2001). The test statistic is an *F* ratio similar to that of ANOVA calculated using sum of squares. To test the null hypothesis of no-treatment effect, data were permuted by shuffling the means of each factor level into different levels for the one-way experiment, and for the two-way analysis, the means for a particular level of factor 1 and a particular level of factor 2 were permuted by shuffling these into different levels (Anderson 2001). Temperature and salinity were treated as fixed factors (all tests) for the two-way experiments and tank-nested within treatments for the one-way experiments of temperature and salinity. Euclidean distances were used as the data correspond to environmental variables (Legendre and Legendre 1998). Data were $\ln(X + 1)$ transformed as some elemental ratios and isotopes were in large concentrations relative to others. Temperature × salinity tests used permutations of residuals under a reduced model, whereas temperature-only and salinity-only tests used permutations of residuals under a full model. The number of permutations for all tests was 4999. Where significant differences were detected (i.e., $P < 0.05$), multiple pairwise comparison testing for differences among treatments were performed, with a significance level for each pairwise test of $\alpha = 0.05$. There was no correction to significance levels for the multiple tests, although it would be expected that one in 20 tests would be significant by chance alone.

Nonmetric multidimensional scaling (MDS) was used to visually represent the data. The dissimilarity matrix was calculated using Euclidean distances. Centroids of each treatment group (e.g., centroids of tanks in the salinity experiment) were plotted and stress coefficients were determined, where a stress value of less than 0.2 is acceptable and stress close to 0 indicates that the data are perfectly represented (Clarke 1993). The final MDS plots are only determined to within an arbitrary orientation, reflection, location, and scale, which explains the absence of axes scales and labels in the figures (Clarke 1993).

Results

Rearing conditions and fish growth

Temperature and salinity regimes within the treatment tanks for each experiment did not overlap throughout the duration of all experiments. Furthermore, little variation was detected in the rearing conditions of temperature and salinity between replicate tanks (Table 1). Although the elemental ratio and isotopic concentrations in the rearing water were not manip-

Table 1. Summary of the rearing conditions within treatment tanks for the three experiments.

Treatment	Tank	Temperature, °C (n = 50)	Salinity, ‰ (n = 50)	SL, mm (n = 7)	Otolith weight, mg (n = 7)
12°C, 30‰ #	1	12.81±0.11	30.24±0.16	36.10±0.56	1.02±0.02
	2	13.06±0.07	29.81±0.17	36.54±0.58	1.03±0.06
16°C, 30‰ #*†	1	15.86±0.14	30.48±0.18	38.97±0.95	1.20±0.06
	2	15.95±0.18	30.21±0.16	40.86±0.83	1.32±0.06
20°C, 30‰ #*	1	20.23±0.10	30.00±0.16	45.80±0.72	1.70±0.02
	2	20.16±0.10	29.68±0.20	43.38±0.97	1.68±0.08
24°C, 30‰ #*	1	24.12±0.10	30.27±0.20	52.03±1.09	2.58±0.10
	2	23.85±0.12	30.33±0.18	52.42±1.35	2.51±0.12
28°C, 30‰ #	1	27.93±0.09	30.38±0.20	56.01±1.85	2.94±0.24
	2	27.70±0.08	30.48±0.15	59.67±1.68	3.17±0.22
16°C, 23‰ †	1	15.44±0.01	23.06±0.05	41.84±1.19	1.46±0.10
	2	15.42±0.10	23.16±0.06	38.48±0.75	1.12±0.05
16°C, 17‰ *†	1	15.42±0.10	17.24±0.04	40.74±0.82	1.49±0.01
	2	15.77±0.08	17.05±0.05	41.60±1.37	1.45±0.02
20°C, 17‰ *	1	20.07±0.07	17.04±0.07	49.07±1.53	2.23±0.15
	2	19.99±0.08	17.02±0.07	52.34±1.03	2.43±0.11
24°C, 17‰ *	1	23.90±0.07	17.17±0.06	56.47±1.60	3.12±0.15
	2	23.89±0.06	17.05±0.05	56.83±1.46	3.17±0.14
16°C, 11‰ †	1	15.44±0.10	11.40±0.04	42.33±0.69	1.41±0.05
	2	15.41±0.01	11.40±0.03	42.58±1.13	1.41±0.07
16°C, 5‰ *†	1	15.74±0.08	5.46±0.03	42.62±0.82	1.40±0.15
	2	15.99±0.08	5.41±0.04	45.85±1.67	1.74±0.12
20°C, 5‰ *	1	20.08±0.07	5.39±0.04	50.14±0.72	2.27±0.14
	2	20.01±0.05	5.51±0.03	47.26±1.46	2.00±0.16
24°C, 5‰ *	1	23.85±0.09	5.45±0.04	58.50±1.34	2.94±0.12
	2	24.09±0.08	5.52±0.03	59.12±1.55	3.54±0.15

Note: Data are displayed as means ± standard error; #, temperature experiment; †, salinity experiment; *, temperature × salinity experiment; SL, standard length of fish.

ulated, there was some variation among tanks in several treatment groups (Table 2).

The standard length of fish and otolith weight were highly correlated (Table 1). Therefore, only one of these variables (otolith weight) was examined further. Fish that were reared in cooler temperatures had smaller otolith weights than those reared in warmer temperatures. Smaller otolith weights were also recorded for fish reared at higher salinities (single-factor experiments). There were no interactive effects between temperature and salinity on otolith weight.

Two-factor experiment

Significant interactive effects of temperature and salinity were detected for Sr:Ca, Ba:Ca, $\delta^{13}C$, and $\delta^{18}O$ concentrations in fish otoliths (Table 3; Fig. 1), and a significant effect of temperature was also detected for Mg:Ca. There were no interactive effects of temperature and salinity for Mg:Ca and Mn:Ca, although differences were detected between tanks for both Mg:Ca and Mn:Ca. The concentration ratio of Sr:Ca in otoliths increased with increasing temperature (from 16, 20, 24°C), with salinity having no effect at 16°C but significant effects at higher temperatures (SNK tests; Fig. 1*c*). The concentration ratio of Ba:Ca in otoliths also increased with increasing temperature from 16 to 20°C but did not increase between 20 and 24°C, with the exception of 30‰ (SNK tests; Fig. 1*d*). The effect of salinity on $\delta^{13}C$ and $\delta^{18}O$ isotopes in otoliths increased with increasing temperature, with significant differences detected between salinities at each temperature (SNK tests; Figs. 1*e*, 1*f*).

Backwards stepwise regression performed on Sr:Ca, Ba:Ca, $\delta^{13}C$, and $\delta^{18}O$ indicated that of the variables (temperature, salinity, ambient water chemistry, and otolith weight), only temperature and salinity influenced Sr:Ca, Ba:Ca, and $\delta^{13}C$ concentrations in otoliths, whereas temperature, ambient $\delta^{18}O$ water concentration, and otolith weight influenced $\delta^{18}O$ concentration in otoliths.

NP-MANOVA detected an interactive effect of temperature and salinity on the elemental fingerprints of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca in otoliths (Table 4; Fig. 2*a*) and on the isotopic fingerprints of $\delta^{13}C$ and $\delta^{18}O$ in otoliths (Table 4; Fig. 2*b*). A posteriori pairwise comparisons revealed that the elemental and isotopic compositions in otoliths of fish reared under different temperatures were significantly different from each other, but that there was greater variation among salinities as temperature increased, resulting in a significant interaction effect.

Temperature experiment at 30‰

A significant effect of temperature was detected for Sr:Ca, Ba:Ca, $\delta^{13}C$, and $\delta^{18}O$ in fish otoliths, with no significant effects detected for Mg:Ca and Mn:Ca (Table 5*a*; Fig. 3). Despite ANOVA detecting a significant difference for Sr:Ca, the post hoc SNK test initially failed to determine which treatment differed; thus, the pooling of replicate tank data

Table 2. Summary of the water conditions within treatment tank for the three experiments.

		Trace elements								Isotopes	
Treatment	Tank	Mg (n = 3)	Mg:Ca (n = 3)	Mn (n = 3)	Mn:Ca (n = 3)	Sr (n = 3)	Sr:Ca (n = 3)	Ba (n = 3)	Ba:Ca (n = 3)	$\delta^{18}O$ (n = 3)	$\delta^{13}C$ (n = 3)
12°C, 30‰ #	1	286.67±3.33	1.23±0.00	1.36±0.19	2.37±0.32	6.23±0.03	6.79±0.04	6.83±0.13	4.75±0.09	3.30±0.10	13.55±0.35
	2	290.00±0.00	1.14±0.00	2.10±0.26	3.65±0.46	6.23±0.03	6.79±0.04	6.80±0.06	4.72±0.04	3.12±0.16	13.96±0.18
16°C, 30‰ #*†	1	290.00±0.00	1.14±0.00	2.17±0.14	3.76±0.25	6.23±0.03	6.79±0.04	7.10±0.10	4.93±0.07	3.41±0.04	11.80±0.65
	2	283.33±6.66	1.20±0.05	1.97±0.19	3.66±0.14	5.63±0.47	6.59±0.13	6.07±0.60	4.51±0.17	3.02±0.03	11.86±0.45
20°C, 30‰ #*	1	290.00±0.00	1.13±0.00	2.37±0.28	4.11±0.49	6.20±0.00	6.75±0.00	7.40±0.15	5.14±0.10	3.43±0.05	8.82±1.84
	2	286.67±3.33	1.13±0.01	2.33±0.32	4.05±0.57	6.20±0.00	6.75±0.00	6.97±0.09	4.84±0.06	3.24±0.08	12.41±0.63
24°C, 30‰ #*	1	290.00±0.00	1.34±0.00	3.13±0.37	5.44±0.64	6.27±0.03	6.83±0.04	7.30±0.12	5.07±0.08	3.09±0.40	11.19±0.24
	2	290.00±0.00	1.14±0.00	2.97±0.20	5.15±0.35	6.20±0.00	6.75±0.00	7.10±0.06	4.93±0.04	3.34±0.07	11.19±0.30
28°C, 30‰ #	1	290.00±0.00	1.11±0.00	3.03±0.18	5.15±0.30	6.40±0.00	6.81±0.00	7.63±0.15	5.18±0.10	3.24±0.28	11.45±0.34
	2	290.00±0.00	1.11±0.00	3.00±0.18	4.92±0.35	6.40±0.00	6.81±0.00	7.48±0.08	5.09±0.07	3.58±0.14	12.49±0.26
16°C, 30‰ †	1	260.00±0.00	1.35±0.01	2.27±0.42	5.21±0.93	4.43±0.03	6.40±0.06	5.27±0.12	4.86±0.16	3.03±0.29	9.48±0.33
	2	260.00±0.00	1.35±0.01	2.20±0.21	5.06±0.44	4.43±0.03	6.40±0.06	5.03±0.12	4.64±0.06	2.64±0.30	7.88±0.34
16°C, 17‰*†	1	240.00±0.00	1.65±0.00	1.77±0.07	5.37±0.20	3.20±0.00	6.10±0.00	4.07±0.09	4.94±0.11	2.15±0.26	9.50±1.46
	2	240.00±0.00	1.65±0.00	2.20±0.12	6.69±0.35	3.23±0.03	6.16±0.06	3.87±0.15	4.70±0.18	2.48±0.13	7.89±0.60
20°C, 17‰ *	1	240.00±0.00	1.60±0.02	1.47±0.09	4.35±0.30	3.37±0.03	6.24±0.02	3.93±0.03	4.66±0.10	2.65±0.33	10.47±1.13
	2	236.66±3.33	1.63±0.02	2.73±0.07	8.31±0.10	3.23±0.12	6.16±0.10	4.10±0.26	4.98±0.21	2.52±0.17	10.22±0.51
24°C, 17‰ *	1	240.00±0.00	1.65±0.00	2.30±0.25	6.99±0.76	3.23±0.03	6.16±0.06	4.37±0.03	5.31±0.04	2.58±0.15	8.23±1.18
	2	240.00±0.00	1.65±0.00	2.65±0.09	8.10±0.36	3.30±0.00	6.29±0.00	4.60±0.12	5.67±0.16	2.37±0.23	9.02±0.83
16°C, 11‰ †	1	193.33±6.67	2.13±0.07	1.47±0.18	7.11±0.56	1.97±0.13	6.00±0.01	2.60±0.21	5.05±0.12	2.05±0.05	7.47±2.20
	2	196.67±3.33	2.12±0.08	1.00±0.00	4.76±0.10	2.10±0.00	6.27±0.13	2.60±0.06	4.95±0.15	1.64±0.29	6.59±0.45
16°C, 5‰ *†	1	120.00±0.00	2.87±0.04	1.20±0.10	12.66±0.86	0.93±0.01	6.14±0.02	1.63±0.20	6.89±0.76	2.37±0.77	4.52±1.05
	2	120.00±0.00	2.90±0.04	1.03±0.03	11.04±0.40	0.92±0.01	6.16±0.02	1.33±0.03	5.70±0.17	1.21±0.13	5.77±0.52
20°C, 5‰ *	1	126.67±3.33	2.85±0.01	1.20±0.01	11.90±0.74	0.98±0.02	6.11±0.01	1.67±0.13	6.61±0.40	1.48±0.15	6.52±1.26
	2	130.00±0.00	1.62±0.05	1.00±0.00	9.78±0.16	0.97±0.01	6.05±0.05	1.97±0.37	7.72±1.55	1.35±0.15	5.95±0.73
24°C, 5‰ *	1	130.00±0.00	2.85±0.07	1.63±0.29	15.96±3.18	0.99±0.01	5.99±0.07	2.50±0.06	9.69±0.04	1.68±0.18	6.68±0.63
	2	130.00±0.00	2.83±0.05	2.37±0.32	22.95±3.54	0.99±0.01	5.99±0.06	2.53±0.13	9.76±0.37	1.44±0.15	4.03±1.54

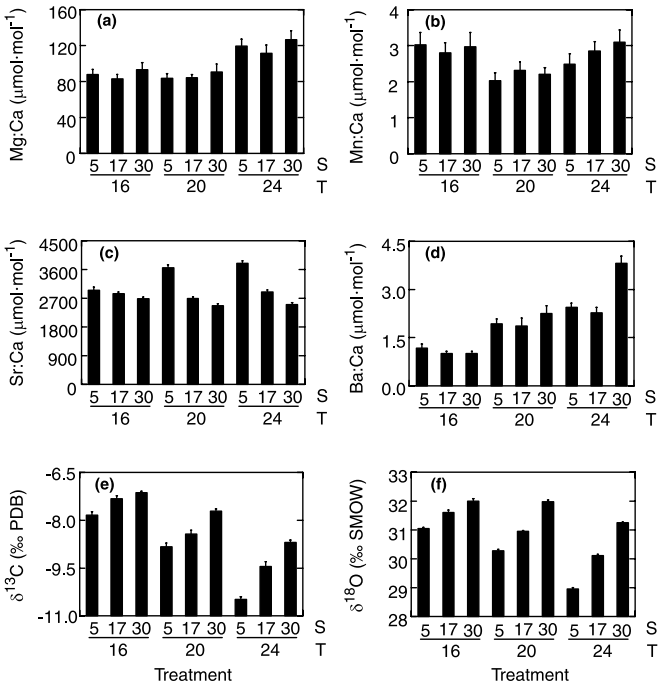
Note: Data are displayed as means ± standard error, where errors of 0.00 represent identical replicate samples; #, temperature experiment; †, salinity experiment; *, temperature × salinity experiment. Units: Mg, Sr ($mg \cdot L^{-1}$); Mn, Ba ($\mu g \cdot L^{-1}$); Mg:Ca ($mol \cdot mol^{-1}$); Mn:Ca and Ba:Ca ($\mu mol \cdot mol^{-1}$); Sr:Ca ($mmol \cdot mol^{-1}$); $\delta^{13}C$ and $\delta^{18}O$ (‰).

Table 3. Results of analysis of variance (ANOVA) comparing the elemental ratio and isotopic concentrations in otoliths among temperature (T) and salinity (S) treatments (two-way manipulation).

Source	df	MS Mg	MS Mn	MS Sr	MS Ba	MS $\delta^{13}C$	MS $\delta^{18}O$
Temperature	2	14 552.72**	6.775	593 845.24**	33.279***	47.799***	22.918***
Salinity	2	1 222.28	0.627	9 306 157.87***	4.802**	15.229***	28.637***
Tank (T × S)	9	1 391.99*	3.616***	71 662.77	0.468	0.164	0.079*
T × S	4	89.44	0.570	1 293 878.98***	2.922*	1.223**	1.665***
Residual	108	699.64	1.002	68 390.14	0.424	0.135	0.036

Note: Cochran's *C* tests were used to test homogeneity of variance; all tests were nonsignificant. For this table, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 1. Mean concentrations (± standard error (SE)) of (*a*) Mg, (*b*) Mn, (*c*) Sr, (*d*) Ba, (*e*) $\delta^{13}C$, and (*f*) $\delta^{18}O$ in otoliths of black bream (*Acanthopagrus butcheri*) reared under experimental treatments of temperature and salinity (two-way manipulations). S, salinity of the rearing water in ‰; T, temperature of the rearing water in °C. Carbon isotope concentrations are standardized to Peedee belemnite (PDB) and oxygen isotope concentrations are standardized to standard mean ocean water (SMOW).



was done to increase the power of the test and to allow for differences to be determined (Underwood 1999). This analysis was achieved by pooling the mean squares of the interaction term with the residual (Winer et al. 1991). The concentration ratio of Sr:Ca in otoliths was significantly greater at low and high temperatures (12 and 16°C and 28°C, respectively) compared with intermediate temperatures (20 and 24°C), where the concentration ratio was lower (SNK tests; Fig. 3*c*). Significant differences were observed for Ba:Ca for the 16, 20, and 24°C treatments, where Ba:Ca concentration ratio increased with temperature but the upper two (24 and 28°C) and lower two (12 and 16°C) treatments showed no significant differences (SNK tests; Fig. 3*d*). The concentration of $\delta^{13}C$ decreased as temperature increased, with significant differences found among the four higher temperatures (16, 20, 24, 28°C) and no difference detected between 12 and 16°C (SNK tests; Fig. 3*e*). Significant differences were observed for $\delta^{18}O$ between 12 and 16°C and again between 20, 24, and 28°C, with a general trend for a decrease in $\delta^{18}O$ with increasing temperature (no differences were detected between 12 and 20°C treatments and again between 16 and 20°C treatments) (SNK tests; Fig. 3*f*).

Backwards stepwise regression for Sr:Ca and Ba:Ca indicated that of the variables (temperature, ambient water chemistry, and otolith weight), only temperature influenced the Sr:Ca and Ba:Ca concentration ratios in otoliths. Backwards stepwise regression analysis performed on $\delta^{13}C$ and $\delta^{18}O$ isotopes indicated that temperature, ambient $\delta^{13}C$ water concentration, and otolith weight influenced the concentration of $\delta^{13}C$ in otoliths, whereas otolith weight influenced $\delta^{18}O$ concentration in otoliths.

NP-MANOVA detected a significant effect of temperature on the elemental fingerprints of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca in otoliths (Table 4; Fig. 2*c*) and the isotopic fingerprints of $\delta^{13}C$ and $\delta^{18}O$ (Table 4; Fig. 2*d*). A posteriori pairwise comparisons revealed differences in the elemental fingerprints between 16, 20, and 24°C, although the two lower (12 and 16°C) and two higher (24 and 28°C) treatments showed no significant differences (Fig. 2*c*). A posteriori pairwise comparisons revealed no difference in the isotopic fingerprints between 12 and 16°C, whereas significant differences were observed at higher temperatures (Fig. 2*d*).

Salinity experiment at 16°C

There were no significant effects of salinity on the concentration ratios of any elements in the fish otoliths (Table 5*b*). Significant tank effects were recorded for Mg:Ca, Mn:Ca, and Ba:Ca, indicating variation between replicate treatment tanks (Table 5*b*; Fig. 4). A significant effect of salinity was detected for $\delta^{13}C$ and $\delta^{18}O$ (Table 5*b*; Figs. 4*e*, 4*f*). Backwards stepwise regression performed on carbon and oxygen isotopes indicated that of the variables (salinity, ambient water chemistry, and otolith weight), only salinity influenced the concentrations of $\delta^{13}C$ and $\delta^{18}O$ in otoliths.

Nonparametric analysis of variance did not detect any significant effect of salinity on the elemental fingerprints in otoliths (Table 4; Fig. 2*e*), although a significant tank effect was detected, again suggesting variability between replicate tanks. A multidimensional scaling plot of element centroids from each tank demonstrates the similarity among treatment groups and shows the high degree of variability between replicate tanks (Fig. 2*e*). A significant effect of salinity on the isotopic fingerprints of $\delta^{13}C$ and $\delta^{18}O$ in otoliths was detected (Table 4; Fig. 2*f*). A posteriori pairwise comparisons revealed differences in the isotopic fingerprints between the low salinities (5 and 11‰) and high salinities (17, 23, 30‰)

Table 4. Results of nonparametric multivariate analysis of variance (NP-MANOVA) comparing multi-element and -isotope fingerprints in otoliths among treatments of the temperature and salinity experiment and the temperature and the salinity experiments alone.

Experiment	Source	df	MS elements	MS isotopes
Temperature × salinity (two-way)	Temperature	2	5.720***	2.870***
	Salinity	2	1.436***	1.057***
	Temperature × salinity	4	0.364*	0.185***
	Residual	117	0.196	0.019
Temperature (one-way)	Temperature	4	3.191***	0.893***
	Tank (temperature)	5	0.277	0.005
	Residual	60	0.202	0.008
Salinity (one-way)	Salinity	4	0.153	0.129***
	Tank (salinity)	5	0.693**	0.002
	Residual	60	0.194	0.010

Note: All data were transformed to $\ln(X + 1)$ before analysis. For this table, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(Fig. 2*f*), seen with an increased concentration of $\delta^{13}C$ and $\delta^{18}O$ with increasing salinity.

Discussion

Interactive effects of temperature and salinity

Temperature and salinity interacted to influence Sr:Ca, Ba:Ca, $\delta^{13}C$, and $\delta^{18}O$ (and the combined elemental and isotopic fingerprints) in black bream otoliths. The influence of salinity on the elemental ratio and isotopic concentrations also generally increased with increasing temperature. Interactive effects of temperature and salinity on the elemental ratio and isotopic concentrations in otoliths have rarely been reported. Secor et al. (1995) detected a significant interactive effect of temperature and salinity on Sr concentration, but the biological significance of this is difficult to interpret because of the unnaturally low Sr concentrations in the artificial seawater used in their experiment.

The results of the present study suggest that the choice of temperature and salinity treatments may explain why the interactive effects of these factors have not been detected in previous experiments. A strong interactive effect between temperature and salinity on Sr:Ca was detected when comparing higher (i.e., 20 and 24°C) with lower temperatures (i.e., 16°C), but comparison of the two high temperatures (20 and 24°C) did not yield this interaction. Thus, the treatments chosen by Tzeng (1996) of two high temperatures (23 and 28°C) and by Chesney et al. (1998) of three high salinities (20, 26, and 33.4‰) may not have been broad enough to detect interactive effects on elemental concentrations. In other studies, interactive effects of temperature and salinity on elements in otoliths were not detected as they were not analyzed to detect such effects (Kawakami et al. 1998) or because of the loss of all fish from one treatment, reducing the experiment to a single-factor design (Fowler et al. 1995*a*, 1995*b*).

Effects of temperature

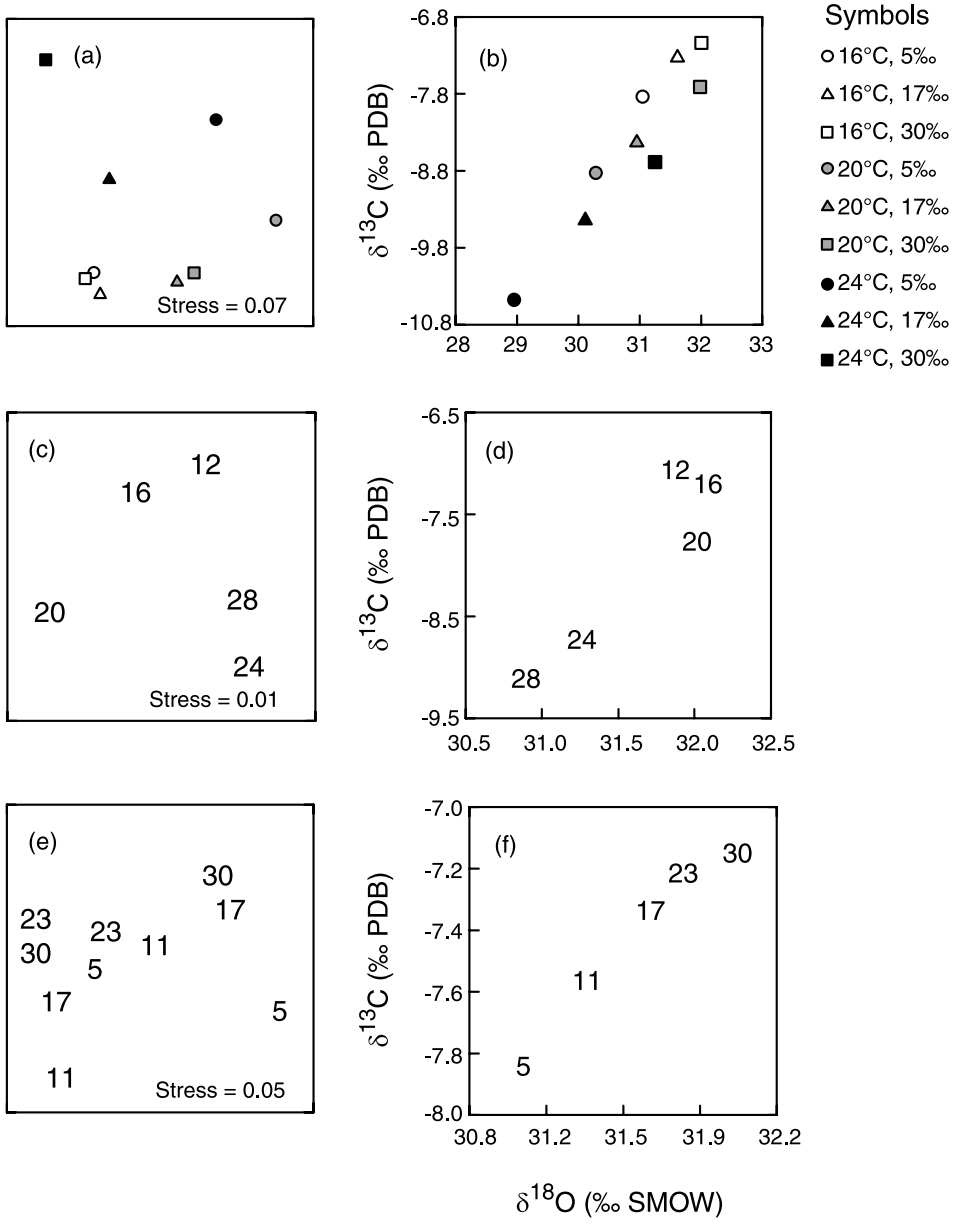
The single-factor experiments in the current study revealed that the concentration ratio of the elements Sr:Ca and Ba:Ca and concentrations of isotopes $\delta^{13}C$ and $\delta^{18}O$ were significantly affected by temperature, whereas Mg:Ca and Mn:Ca concentration ratios were not. Furthermore, the combined elemental fingerprint of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca and the combined isotopic fingerprint of $\delta^{13}C$ and $\delta^{18}O$ both showed significant effects owing to temperature. The concentration ratio of Sr:Ca in otoliths was greater at the two extreme temperature treatments (12 and 28°C), whereas the concentration ratio of Ba:Ca increased with increasing temperature. Both $\delta^{13}C$ and $\delta^{18}O$ concentrations decreased with increasing temperature, although the trends were nonlinear.

The result that Sr:Ca concentration ratio in otoliths was greater at low (12°C) and high (28°C) temperatures and was reduced at mid-range temperatures (20–24°C) may help to explain the variation of results reported in several independent experiments. Experiments that have detected a positive effect on Sr with temperature reared fish at high temperatures (i.e., 20°C and above) (Hoff and Fuiman 1993, 1995; Bath et al. 2000), whereas those that detected negative effects reared fish at low temperatures (i.e., 17°C and below) (Townsend et al. 1992, 1995). However, there are experiments that do not conform to these trends, such as Kalish (1989), who detected a slight increase in Sr:Ca with temperature using treatments of 13, 16, 19, and 22°C. In addition, Tzeng (1996) and Kawakami et al. (1998) detected no effect of temperature (23 and 28°C and 12, 17, 22, and 27°C, respectively) on otolith Sr:Ca. However, both experiments that detected no effect used the same species of eel, *Anguilla japonica*, and it is possible that temperature may influence the concentration ratio of Sr:Ca differently in different species. If previous studies had used broad ranges of temperatures, then the results of the literature may be more consistent. We recommend that future experiments focus on a broad range of temperatures, or at least the entire range experienced by the species, and be designed to test for interactions between environmental factors.

The concentration ratio of Ba in otoliths increased with temperature in the current study, which is in contrast to the nonsignificant effect of temperature on barium concentration ratio detected by previous studies (e.g., Fowler et al. 1995*a*, 1995*b*; Bath et al. 2000). The greatest effect was over the temperature range of 16 to 24°C, and no effect was detected between the highest temperatures of 24°C and 28°C. The limited temperature ranges investigated by both Fowler et al. (1995*a*, 1995*b*) and Bath et al. (2000) of 20 and 25°C may explain why they did not detect an effect.

The nonsignificant effect of temperature on Mn:Ca con-

Fig. 2. Plots summarizing the variation in elemental and isotopic fingerprints of otoliths from black bream (*Acanthopagrus butcheri*) reared under experimental treatments. Nonmetric multidimensional scaling (MDS) ordinations comparing centroids of treatment groups of (*a*) temperature and salinity (two-way) manipulations (stress = 0.07), (*c*) temperature manipulations (stress = 0.01), (*e*) salinity manipulations (stress = 0.05); these plots are only determined to within an arbitrary orientation, reflection, location, and scale, which explains the absence of axis scales and labels in the figures. Plots of carbon versus oxygen isotope concentration comparing the means of each treatment group of (*b*) temperature and salinity (two-way) manipulations, (*d*) temperature manipulations, and (*f*) salinity manipulations. In *c* and *d*, numbers = temperature (°C), and in *e* and *f*, numbers = salinity (‰).



tradicts the only other experimental results for manganese. Fowler et al. (1995*a*) found a reduction in Mn concentration at higher temperatures using solution-based analysis. In the current experiment, variability in Mn concentration of otoliths between replicate tanks of each treatment was high, and differences between treatments were not detected. Experiments investigating the effects of temperature on Mg have shown both significant effects (Fowler et al. 1995*a*, 1995*b*), with Mg concentration decreasing with increasing temperature, and nonsignificant effects (Hoff and Fuiman 1995). In the current experiment, Mg:Ca was highly variable in concentration between otoliths from individual fish in each tank, as well as between replicate tanks of each treatment. Thus, the large variations in Mn:Ca and Mg:Ca concentration ratios detected in the current study suggest that these particular elements may not be applicable for use in otolith microchemistry studies involving temperature and salinity, in contrast to Sr:Ca and Ba:Ca, which yield clearer results.

The linear relationship between $\delta^{13}C$ and $\delta^{18}O$ and temperature has previously been described with some degree of

Table 5. Analysis of variance (ANOVA) comparing the elemental ratio and isotopic concentrations in otoliths among (*a*) temperature treatments (T) and (*b*) salinity treatments (S).

	Source	df	MS Mg	MS Mn	MS Sr	MS Ba	MS $\delta^{13}C$	MS $\delta^{18}O$
(*a*)	Temperature	4	4610.270	2.694	173 649.503**	26.227***	0.145***	3.735***
	Tank (T)	5	1582.526	2.427	32 664.084	0.055	0.001	0.030
	Residual	60	940.244	1.401	37 975.092	0.519	0.001	0.070
(*b*)	Salinity	4	254.772	0.841	0.024	0.027	1.124***	1.976**
	Tank (S)	5	1989.666**	5.530**	0.008	0.076*	0.017	0.093
	Residual	60	559.783	1.338	0.009	0.029	0.079	0.047

Note: Cochran's *C* tests were used to test homogeneity of variance. All tests were nonsignificant except $\delta^{13}C$ (temperature), which was transformed to $\ln(X + 1)$. For this table, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 3. Mean concentrations (± standard error (SE)) of (*a*) Mg, (*b*) Mn, (*c*) Sr, (*d*) Ba, (*e*) $\delta^{13}C$, and (*f*) $\delta^{18}O$ in otoliths of black bream (*Acanthopagrus butcheri*) reared under experimental treatments of temperature. Carbon isotope concentrations are standardized to Peedee belemnite (PDB) and oxygen isotope concentrations are standardized to standard mean ocean water (SMOW). Graphs with letters above bars indicate similarity between treatments; graphs with n/s indicate that there are no significant effects between treatments.

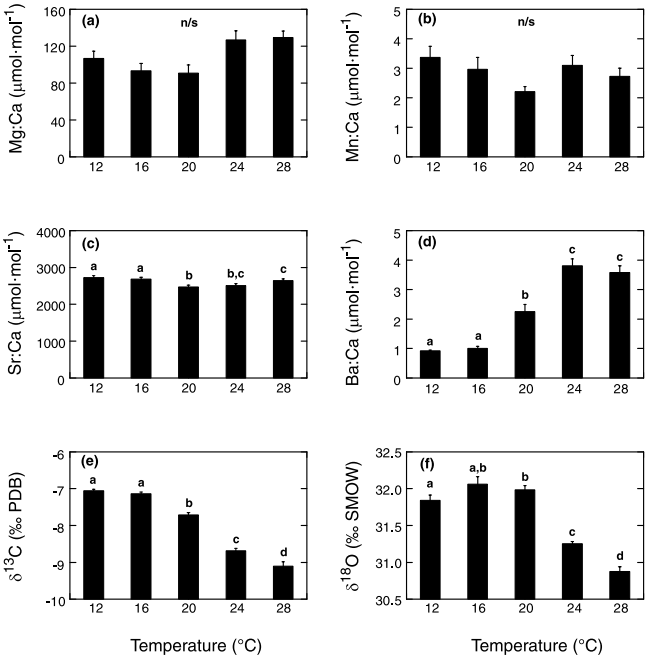
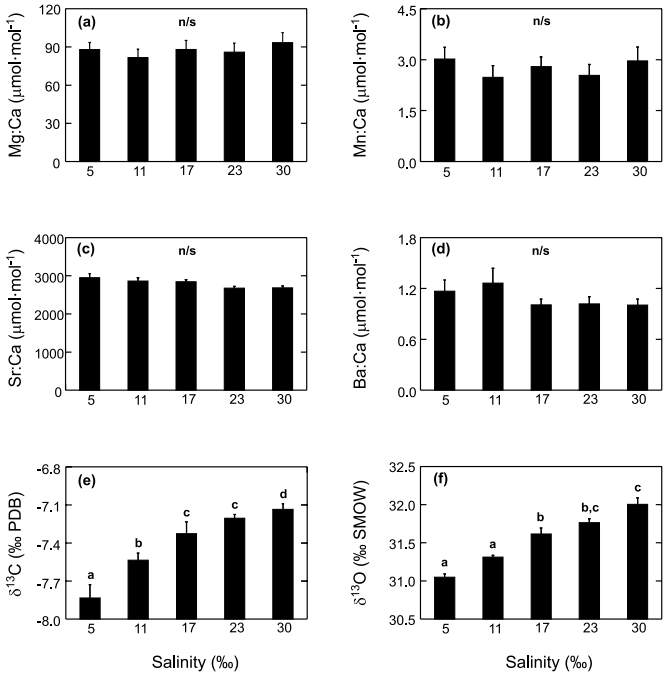


Fig. 4. Mean concentrations (± standard error (SE)) of (*a*) Mg, (*b*) Mn, (*c*) Sr, (*d*) Ba, (*e*) $\delta^{13}C$, and (*f*) $\delta^{18}O$ in otoliths of black bream (*Acanthopagrus butcheri*) reared under experimental treatments of salinity. Carbon isotope concentrations are standardized to Peedee belemnite (PDB) and oxygen isotope concentrations are standardized to standard mean ocean water (SMOW). Graphs with letters above bars indicate similarity between treatments; graphs with n/s indicate that there are no significant effects between treatments.



variation (see Kalish 1991*b*) and has formed the basis of many predictive equations (Kalish 1991*a*, 1991*b*; Thorrold et al. 1997) extensively used in fisheries ecology (e.g., Edmonds et al. 1999) and palaeoecology (e.g., Patterson 1999) for reconstructing past temperatures. The results of the current study support the use of such equations, although the relationships detected in this study were not always linear and the magnitude of the relationship changed with salinity. Our work gives some insight into why slight variations of this equation may occur. Previous studies by Kalish (1991*a*), who reared fish at 38.4‰, and Thorrold et al. (1997), who reared fish at 30‰, showed variations in the $\delta^{18}O$–temperature relationship that may have been due to salinity. Slight variations of the equation may also be due to rearing temperature, for example, Kalish (1991*a*) reared fish at 13, 16, 19, and 22°C and Thorrold et al. (1997) reared fish at 18, 20.5, 22.5, and 25°C.

Biological factors such as kinetics may be the underlying reason as to why temperature effects on otolith chemistry are detected (Kalish 1989). Kinetic effects in otoliths are thought to manifest as changes in the proteinaceous compounds surrounding the otolith (e.g., Brown and Severin 1999). These proteins are likely to be indirectly affected by temperature and hence may result in changes to the otolith crystal morphology from the normal aragonite structure to vaterite, which has been shown to affect uptake of elements into otoliths (e.g., Brown and Severin 1999). Greater knowledge of influence of kinetics on otolith microchemistry may lead to a more de-

tailed explanation as to why otolith microchemistry changes with temperature treatments.

Effects of salinity

The concentration ratios of individual elements in otoliths of *A. butcheri* showed no relationship with salinity, whereas $\delta^{13}C$ and $\delta^{18}O$ concentrations displayed a strong positive relationship. Similarly, the combined elemental fingerprint of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca displayed no trends with salinity; however, the isotopic fingerprints of $\delta^{13}C$ and $\delta^{18}O$ had a positive relationship with salinity. When temperature was constant, the concentrations of $\delta^{13}C$ and $\delta^{18}O$ in otoliths appeared to be primarily due to an effect of rearing-water salinity.

The results from the single-factor salinity experiment agree with prior experiments that found no effects of salinity on Sr and Mg (Hoff and Fuiman 1995; Chesney et al. 1998) and Mn and Ba (Fowler et al. 1995*b*) in otoliths. However, there were significant salinity effects detected for Sr and Ba at 20 and 24°C in the two-factor experiment. Thus, the temperature at which the single-factor experiment was done in this study (16°C) appeared to be too low to detect any salinity effects. The results of Tzeng (1996) seem to support this claim, with differences in Sr:Ca detected among salinity treatments at high temperatures (22–23 and 27–28°C), although Fowler et al. (1995*b*), Hoff and Fuiman (1995), and Chesney et al. (1998), who also reared fish at high temperatures (20°C and above), found no salinity effects on elemental concentrations in otoliths. Therefore, the ability to draw reliable conclusions regarding the effect of salinity alone on otolith elemental concentration ratios is impeded by the variable results reported between studies, and hence further investigation is required. The effects of salinity on isotopic concentrations in fish otoliths have not been previously investigated, and further independent analyses are required for their validation.

Other factors influencing otolith microchemistry

Although it is necessary to determine the influence of temperature and salinity on otolith microchemistry if otoliths are to be used to reconstruct migratory patterns or temperature and salinity histories of fish, it is equally important to recognize factors other than these that may affect the incorporation of elements and isotopes into otoliths. The microchemistry of the surrounding water has been demonstrated to influence the concentrations of elements and isotopes in otoliths (e.g., Thorrold et al. 1997; Bath et al. 2000; Milton and Chenery 2001). In the current study, although the concentration of isotopes in otoliths was influenced by that of the water, this effect was minimal compared with that of temperature and salinity. The fact that elemental concentration ratios in otoliths were not affected by that of the water may be explained by the smaller range in elemental concentration ratios among treatment groups compared with studies such as that of Bath et al. (2000), who found significant effects of water chemistry.

Biological factors, such as metabolism, kinetics, and ontogeny, are thought to influence otolith microchemistry (Kalish 1989). Sadovy and Severin (1994) reported that the concentration ratio of Sr:Ca in fish otoliths was inversely related to the growth rate of the fish. In the current study, fish growth (as indicated by otolith weight) was positively affected by rearing temperature. There was also a relationship between both carbon and oxygen isotopic concentration in the otolith and otolith weight. The relative differences in fish growth rates between treatments cannot be ruled out as a factor influencing otolith microchemistry. Similarly, concentrations of Sr, Ba, and Mg in otoliths have been shown to vary with ontogeny (Fowler et al. 1995*b*). Ontogenetic factors are unlikely to have affected otolith microchemistry in the current study as all of the fish were the same age and from the same brood stock.

Applications

The ability to reconstruct aspects of the life history of a fish (e.g., migratory patterns) using otoliths relies largely on predictive responses of otolith microchemistry to ambient temperature and salinity. Kafemann et al. (2000) and Secor et al. (2001) have both used the concentration ratio of Sr:Ca in fish otoliths to reconstruct migratory patterns based on salinity profiles of the water. These studies collected fish from different salinity environments (e.g., freshwater and brackish) and, after analysis of the otoliths, have charted possible migratory patterns of the fish along large rivers (up to 250 km; Secor et al. 2001) varying in salinity from brackish to oceanic. Similarly, Edmonds et al. (1999) reconstructed the salinity histories of fish from a large bay based on isotopic concentrations in otoliths. It is logical to assume that coastal regions such as rivers and bays may vary in both temperature and salinity simultaneously. Thus, the reconstruction of migratory patterns based solely on the salinity effects on otolith microchemistry (without taking into account possible interactions of salinity with temperature) may result in incorrect interpretations of fish migratory patterns. Conversely, in areas where only temperature or salinity vary, such as in open oceans where salinity may remain relatively constant over large areas yet temperature changes markedly, the use of single-factor reconstructions of migratory patterns may be more reliable.

In conclusion, differences in elemental and isotopic concentrations of otoliths appear to be strongly affected by both water temperature and salinity. Importantly, these results suggest that although single-factor tests of environmental processes provide quantitative information if their effects are independent, they cannot predict the interactive effects of multiple factors. This point is especially significant where variables are not independent of one another, as for temperature and salinity of seawater. The results presented here suggest that otolith microchemistry may be a useful tool for studying and reconstructing the temperature and salinity life histories of fish. The potential applications include accurate predictions of fish migratory patterns.

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