INTRODUCTION

The ability to accurately trace movements of euryhaline fishes is critical to understanding their dispersal and migration patterns across freshwater, estuarine and marine interfaces. Whilst much information has been gained through traditional tagging and telemetry studies (e.g. Hindell et al. 2008, Luo et al. 2009), analysis of the chemistry of calcified structures, particularly otoliths, which can reflect environmental changes experienced by fish throughout life, has become increasingly widespread in recent years (see review by Gillanders 2005). Otoliths grow continuously throughout the life of the fish, forming daily and annual growth bands, and are composed of a calcium carbonate lattice that is not re-metabolised once deposited (Campana & Neilson 1985, Campana 1999). These 2 properties—combined with strong evidence that certain trace elements (e.g. Sr and Ba) are incorporated into otoliths in proportion with ambient water concentrations (Bath et al. 2000, Wells et al. 2003, Elsdon & Gillanders 2003, 2005a), which in turn can reflect salinity and temperature gradients (Zimmerman 2005, Martin & Wuenschel 2006)—make otoliths potentially useful as environmental recorders.

With rapid improvements in our understanding of the pathways by which elements are incorporated into otoliths and in the analytical capabilities of sampling equipment, researchers are now able to trace movement histories of individuals and connections among...
populations at increasingly fine scales (Swearer et al. 1999, Kennedy et al. 2000, Elsdon & Gillanders 2005b, Weidel et al. 2007). In complex and dynamic environments such as estuaries, however, movements by fish commonly occur across gradients of water chemistry, temperature and salinity that may vary spatially over time frames as short as a tidal cycle (Dorval & Jones 2005, Elsdon & Gillanders 2006). Furthermore, factors such as the extent of tidal ingress, the level of mixing of fluvial and marine particulates, the vertical and longitudinal location of the salt wedge, anoxia and watershed runoff can markedly influence both distribution patterns of fish (e.g. Brenner & Krumme 2007, Nicholson et al. 2008) and the distribution and abundance of trace elements throughout estuaries (Turner et al. 1981, Zwolsman & van Eck 1999). This complexity poses substantial challenges for those aiming to reconstruct environmental histories of fish in these systems based on otolith chemistry data.

A number of studies have used controlled laboratory or field experiments to assess the relationships between environmental variables and otolith elemental concentrations across salinity gradients (e.g. Bath et al. 2000, Elsdon & Gillanders 2002, 2004, Kraus & Secor 2004, Zimmerman 2005, Martin & Wuenschel 2006, Donohoe et al. 2008). Such experiments provide important information for the interpretation of otolith chemistry data from wild-caught fish, although they do not replicate the full scale of physical and chemical variability that occurs in many estuaries. Most studies have reported a strong positive relationship between otolith Sr:Ca and ambient water Sr:Ca (Bath et al. 2000, Elsdon & Gillanders 2003) and a generally positive association between water Sr:Ca and salinity (see Secor & Rooker 2000 for review, Zimmerman 2005). However, salinity may not always correlate positively with water or otolith Sr:Ca concentrations, particularly in moderate to high salinity environments (e.g. Elsdon & Gillanders 2002, Kraus & Secor 2004, Dorval et al. 2007), and freshwater Sr:Ca values have been shown to exceed marine Sr:Ca in some cases (Kraus & Secor 2004). Furthermore, Sr:Ca in water and otoliths has been shown to vary with temperature (Martin et al. 2004) or interactively with temperature and salinity (Elsdon & Gillanders 2002, 2004, Martin & Wuenschel 2006). Consequently, good knowledge of the relationships between water Sr:Ca, salinity and temperature in the study system and their respective effects on otolith Sr:Ca are required when using Sr:Ca alone to trace salinity histories of wild-caught fish.

The consistent increase in otolith Ba:Ca with water Ba:Ca (e.g. Bath et al. 2000, Wells et al. 2003), the predictable decrease in water Ba:Ca with increased salinity (e.g. Elsdon & Gillanders 2005b,c, Dorval et al. 2007) and large differences in Ba:Ca concentrations between fresh and marine waters can provide additional inference for tracing movements of fishes across the freshwater–saltwater interface. Increasingly, studies are incorporating measurement of Ba:Ca in conjunction with Sr:Ca (Crook et al. 2006, Hale & Swearer 2008) and/or other isotope ratios (e.g. Sr\(^{87,86}\); McCulloch et al. 2005, Milton et al. 2008) in order to examine movement across salinity gradients. However, recent work has shown notable complexity in Ba:Ca across otolith transects that can be difficult to interpret based on ambient Ba:Ca or salinity gradients (e.g. Elsdon & Gillanders 2005b, Crook et al. 2006, Milton et al. 2008). Such complexity might be explained by facilitation or competition in uptake between trace metals (de Vries et al. 2005) or physiological, age- or growth-related factors (Kalish 1989, 1991).

The rate of uptake of particular elements into otoliths also warrants careful consideration. Otoliths are not in direct contact with the surrounding water, and the incorporation of ions from water to blood to endolymph to otolith is regulated by physical and physiological filters operating across branchial, intestinal and saccular epithelia (Campana 1999, Payan et al. 1999, 2002). These barriers commonly result in discrimination in elemental uptake, with lower concentrations reported in otoliths relative to the surrounding water concentrations (e.g. Elsdon & Gillanders 2003, Martin & Wuenschel 2006), although uptake of Sr and Ba above ambient levels has also been shown (Elsdon & Gillanders 2005a). The presence of physiological filters means that changes in environmental variables that affect otolith chemistry may not immediately be reflected in the otolith, but are subject to a time lag. Elsdon & Gillanders (2005c) demonstrated that ~20 d of exposure to stable environmental conditions was required before Sr:Ca in otoliths of juvenile black bream Acanthopagrus butcheri reached equilibrium. Similarly, laboratory results for juvenile largemouth bass Micropterus salmoides showed that approximately 21 d was needed for otolith Sr:Ca to reach a saturation level at salinities of 5 or 10‰ (Lowe et al. 2009). Hence, for species that undertake rapid movements between different chemical environments or across salinity gradients, a delay in uptake of any elements used to trace movements limits the spatial and temporal resolution achievable.

The present study addressed these issues by examining the response of Sr:Ca and Ba:Ca in otoliths of the euryhaline Australian bass Macquaria novemaculeata to the range of salinities typically encountered by the species in estuaries. Our specific aims were to (1) use controlled experiments to quantify relationships between salinity and Sr:Ca and Ba:Ca ratios in water and otoliths across a salinity gradient encompassing freshwater to marine values, (2) examine time lags in the
uptake of Sr:Ca and Ba:Ca following changes in salinity, and (3) compare relationships between salinity and ambient Sr:Ca and Ba:Ca derived from the laboratory experiments with results from water samples collected from coastal rivers in southern Victoria, Australia. The nature and consistency of these relationships are discussed in terms of the implications for reconstructing movements of euryhaline fishes using otolith chemistry.

**MATERIALS AND METHODS**

**Study species.** The Australian bass *Macquaria novemaculeata* (hereafter referred to as bass), a catadromous member of the family Percichthyidae, is widely distributed across coastal drainages in southeastern Australia and is an important recreational angling species. Adults of the species are thought to remain in freshwater reaches for most of the year, with a downstream migration taken to the estuary during winter to spawn (Harris 1986). Spawning occurs at salinities of >8 to 10‰ in estuaries, but has been observed in waters up to 35‰ in culture (Battaglene & Selosse 1996), and eggs hatch after 42 h under optimal conditions (i.e. 25 to 35‰, 18°C; van der Wal 1985). Relatively little is known of the development and movements of larvae and juveniles in the wild. Larvae can tolerate seawater-level salinity in captivity (Battaglene & Selosse 1996, authors’ unpubl. data) and there is evidence that at least some individuals spend time at sea during the early larval phase prior to movement back into estuaries (Trnski et al. 2005). Adults have also been captured in 11 to 17 m of water in trawls off the coast of central New South Wales, Australia (Trnski et al. 2005), suggesting that marine residency by some adults may occur at least for short periods.

**Laboratory experiments.** Juvenile (42 d old) bass were sourced from the RMIT University Marine Research facility at Lakes Entrance, Victoria, on 20 September 2006. During the first 6 wk of life, the fish were housed in 1000 l tanks held at a constant 16°C and 27‰. Approximately 50 fish were transported to the aquarium facility at the Arthur Rylah Institute for Environmental Research (ARI) and kept in the original hatchery water for 8 d. During this period fish were fed daily with rotifers *Brachionus plicatilis*.

To examine the response of Sr:Ca and Ba:Ca in bass otoliths to changes in salinity, 3 experimental salinity treatments (5, 20 and 38‰) were selected to reflect the likely range of salinities in estuaries. A total of 200 l of full-strength seawater (38‰) was collected from Seaspray Beach (38° 22’ S, 147° 27’ E) on 25 September 2006 and transported immediately to the ARI aquarium. Upon arrival, 100 l of the water was separated and diluted with ARI aquarium water (0.5‰) to 5 and 21‰, while the remaining 100 l was maintained at 38‰. After 8 d, fish were randomly allocated to one of six 20 l aerated glass tanks containing the 3 salinity treatments, with 2 replicate tanks per treatment (Table 1). Fish were maintained in static water under these conditions for 72 d. Salinity levels and water temperatures in each tank were recorded every second day and kept constant throughout the experimental period (Table 1). A one-third volume water change using aquarium water and seawater collected from Seaspray Beach was conducted every 2 to 3 d to remove waste and detritus and maintain water quality. All fish were fed daily with equal amounts of *Artemia* sp. nauplii.

On Day 72 of the experiment, all fish from Tanks 1, 3 and 5 were removed from their tanks, killed by immersion in an ice slurry, measured for total length (TL, ±1 mm) and kept frozen. At this time, all fish from Tanks 2, 4 and 6 were also removed, measured for TL and immersed in a 0.025% alizarin red S (ARS) solution for 10 min to provide an otolith mark (Crook et al. 2007), before being transferred to 1000 l tanks each filled with ARI aquarium water (0.5‰). This provided a fourth salinity treatment of 0.5‰, representing typical

<table>
<thead>
<tr>
<th>Salinity treatment (‰)</th>
<th>Tank</th>
<th>n</th>
<th>TL (mm) 72 d</th>
<th>TL (mm) 122 d</th>
<th>Salinity (%)</th>
<th>Temperature (°C)</th>
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<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>17.8 ± 0.37</td>
<td>5.55 ± 0.09 (28)</td>
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<td>17.71 ± 0.42</td>
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<td>17.86 ± 0.46</td>
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<td>38</td>
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<td>6</td>
<td>17.71 ± 0.42</td>
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<td>17.86 ± 0.46</td>
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<td>20.67 ± 0.49</td>
<td>0.5 ± 0.004 (22)</td>
<td>20.75 ± 0.04 (22)</td>
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Table 1. *Macquaria novemaculeata*. Summary of fish numbers (n), total length (TL) after 72 and 122 d and experimental conditions in each tank. Data are means ± SE. For salinity and temperature data, the number of measurements upon which means are calculated is shown in parentheses. Blank cells: not applicable.
salinities experienced by bass living in freshwater. These tanks (Tanks 7, 8 and 9; Table 1) were maintained on a recirculation system fed by the same water source used to dilute the seawater, with fish taken from a particular salinity treatment allocated a tank containing only fish from that treatment. Fish were held under these conditions for a further 50 d with the feeding regime kept constant. Fish were then killed by immersion in an ice slurry, measured for TL and frozen as before (Table 1).

**Otolith preparation and analysis.** Sagittal otoliths from all frozen samples were dissected under a stereo microscope, cleaned of adhering tissue, rinsed in Milli-Q water (Millipore) and stored dry in 0.5 ml polypropylene microtubes. One sagitta from each fish was mounted whole (proximal surface downwards) on a glass slide in thermoplastic glue (Crystalbond™), then polished to the level of the primordium using 5 and 3 µm lapping films and 0.5 µm alumina slurry. The samples were then rinsed in Milli-Q water and air-dried overnight in a class 100 laminar flow cabinet at room temperature.

Otoliths were analysed using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). We used a Resonetics LPX120i ArF 193 nm excimer laser system (Resonetics) coupled to an Agilent 7500s ICP-MS (Agilent Technologies) located at Australian National University, Canberra, Australia. The procedures for transect analysis followed those of Crook et al. (2008) with some minor modifications. Briefly, slides were placed in the sample cell and the otoliths visualised with a 400× objective lens and a video imaging system. Each otolith was ablated along a transect from the ventral to the dorsal margin through the core using a 6 × 100 µm rectangular ablation slit (see Fig. 1). The laser was operated at 120 mJ energy and pulsed at 10 Hz with a scan rate of ~2 µm s⁻¹, resulting in an ablation depth of ~3 µm along the transects. The otoliths were analysed for several elements including ⁴³Ca, ⁵⁵Mn, ⁸⁸Sr and ¹³⁸Ba, and Ca was measured for use as an internal standard to correct for variation in ablation yield among samples. Data reduction and processing was done offline using Excel spreadsheets. To reduce the noise along transects, raw counts for Mn, Sr, Ba and Ca were averaged using a 9-point running mean, and then further smoothed using a 9-point running median (Sinclair et al. 1998). This was followed by subtraction of background ion counts from otolith counts and normalisation of each element to ⁴³Ca using an external calibration standard (National Institute of Standards Technology, NIST 612) which was analysed after every 10 otolith samples. Data from each otolith were expressed as element:Ca ratios (i.e. Sr:Ca, Ba:Ca). Measurement precision (% relative standard deviation [RSD]) was determined based on analyses

Fig. 1. *Macquaria novemaculeata*. A representative juvenile Australian bass sagittal otolith after marking with alizarin red S (ARS). (a) Image taken with a stereomicroscope under unfiltered white light showing the position of the 100 µm wide laser ablation track running from the ventral to the dorsal otolith margin through the core. A: anterior; P: posterior; V: ventral; D: dorsal. (b) Image taken under a TXR filter set, showing the position of the ARS mark and the regions of the otolith accreted when fish were kept in the hatchery (27‰), when exposed to either the 5, 21 or 38‰ treatments, and after transfer to the 0.5‰ treatment (i.e. post-ARS marking). Scale bar = 50 µm
distinct peaks of $^{55}$Mn at the primordium of the bass. Macdonald et al. 2008). Similarly, we found the otolith core (e.g. Brophy et al. 2004, Ruttenberg et al. reported marked enrichment of particular elements at 2.5% for Ba:Ca. Several authors have now run concurrently with the otolith samples. Mean % RSD across all NIST 610 samples was 2.4% for Sr:Ca and 2.5% for Ba:Ca. Several authors have now reported marked enrichment of particular elements at the otolith core (e.g. Brophy et al. 2004, Ruttenberg et al. 2005, Macdonald et al. 2008). Similarly, we found distinct peaks of $^{55}$Mn at the primordium of the bass otoliths, which provided a marker for the core during the transect analysis and data reduction process.

Following transect analysis, each otolith was photographed using a stereomicroscope (Model MZ16 F, Leica) coupled to image analysis software (Infinity Capture 3.5.1, Lumenera, and Image Pro Express 5.0.1.26, Media Cybernetics). Photographs were taken under unfiltered white light and a TXR filter (Leica), which was used to detect the ARS marks. This allowed us to relate the position of the otolith core and ARS mark to the corresponding time-resolved elemental concentrations along the transects. Additionally, as we knew the period of time (50 d) that the fish were kept at the 0.5% salinity treatment and the distance from the ARS mark to the dorsal otolith margin, we were able to estimate the mean otolith growth rate per day post-marking for each fish and standardise plots of Sr:Ca and Ba:Ca values for each individual against the estimated days post-marking.

Standardising the data in this way makes the assumption that daily otolith growth for an individual fish was relatively stable during the last 50 d of the experiment. To test this assumption, we examined a subset of the otoliths (n = 6, 2 from each of the 0.5% tanks) to validate the formation of daily increments and estimate variability in increment widths in the last 50 d of otolith growth. We examined increments accreted post-ARS marking along the dorsoventral axis immediately anterior to the ablation transect using image analysis software (Infinity Capture 3.5.1, Lumenera, and Image Pro Express 5.0.1.26, Media Cybernetics). Importantly, we found little variability in increment width distances within each of the 6 otoliths over the last 50 d of the experiment. Mean increment width distances ranged from 1.535 to 1.902 µm, with low errors around all estimates (SE range = 0.018 to 0.029 µm).

**Water chemistry and salinity.** Water samples were collected from each tank on 3 separate occasions during the laboratory experiments (Tanks 1 to 6: Days 2, 34 and 71; Tanks 7 to 9: Day 73, 94 and 122) to measure ambient Sr:Ca and Ba:Ca concentrations in each salinity treatment. Samples were collected in 250 ml acid-washed polypropylene bottles, filtered through a 0.45 µm filter, acidified with re-distilled nitric acid and then analysed with a high-resolution ICP-MS at the National Measurement Institute, Sydney, Australia. Surface water samples were also collected from the estuarine and freshwater reaches of 7 coastal rivers across Victoria, Australia (see Fig. 5), and from the marine environment immediately adjacent to the river mouths, to compare field and laboratory relationships between salinity and ambient Sr:Ca and Ba:Ca. Samples were collected in 250 ml acid-washed polypropylene bottles at a depth of ~5 cm beneath the water surface. Bottles were capped immediately and kept refrigerated at 4°C for transport to the laboratory. Samples were processed and analysed using identical procedures to those described above. All rivers except the Tarwin River were sampled once during summer in either 2004 or 2005, with the Bunyip, Tambo and Barwon Rivers re-sampled during winter 2006. The Tarwin River was sampled twice during winter 2006. Salinity was recorded at the time of collection.

Simple mixing models were developed for each river to predict the relationship between Sr:Ca, Ba:Ca and salinity and to test these predictions against the empirical data from each river. Using the measured marine water values of Sr:Ca, Ba:Ca and salinity and those from the most upstream freshwater sites as end members, Sr:Ca and Ba:Ca concentrations were estimated across the range of 100% marine water to 0% marine water at 0.1% dilution increments. Mixing curves developed from these models were plotted against the empirical Sr:Ca and Ba:Ca values from river water samples.

**Data selection and statistical analysis.** Changes in fish TL among salinity treatments at Day 72 of the experiment were assessed with a nested 2-way ANOVA, with the random factor tank nested within each fixed salinity treatment. Variation in TL among 0.5% tanks at the conclusion of the experiment was examined with a 1-way ANOVA. Otolith data were selected from a 3 × 18 × 100 µm portion of each otolith accreted immediately prior to the ARS marking (or at the dorsal otolith margin for fish from Tanks 1, 3 and 5) to represent ~10 d of otolith growth during the period that each fish was exposed to either the 5, 21 or 38% salinity treatments. For ARS-marked fish, data from the same amount of otolith material (3 × 18 × 100 µm) were selected at the dorsal margin of each otolith to represent the time of exposure to the 0.5% treatment. Non-linear and linear regression were used to relate ambient salinity and measured values of Sr:Ca and Ba:Ca in the otoliths and the tank water. After specifying a base function, we determined values of the model parameters that minimized the residual sum of squares. Differences in Sr:Ca and Ba:Ca in the otoliths and tank water among the 0.5, 5, 21 and 38% salinity treatments were examined using 1-way ANOVAs or 2-way nested ANOVAs, with salinity a fixed factor and tank a random factor nested within each salinity treatment. Data were natural log(x + 1)-transformed where

(n = 11) of a secondary reference standard (NIST 610) run concurrently with the otolith samples. Mean % RSD across all NIST 610 samples was 2.4% for Sr:Ca and 2.5% for Ba:Ca. Several authors have now reported marked enrichment of particular elements at the otolith core (e.g. Brophy et al. 2004, Ruttenberg et al. 2005, Macdonald et al. 2008). Similarly, we found distinct peaks of $^{55}$Mn at the primordium of the bass otoliths, which provided a marker for the core during the transect analysis and data reduction process.
necessary to meet parametric assumptions of normality and homogeneity of variances among groups, and Tukey’s Honestly Significant Difference (HSD) tests were used to compare group means when a significant \((p < 0.05)\) ANOVA result was obtained. To compare uptake rates of Sr and Ba from the water to the otolith among salinity treatments, partition coefficients \((D_i\) see Morse & Bender 1990) were calculated for each fish in each tank for a given trace metal \((Me)\):

\[
D_{Me} = \frac{Me:Ca_{otolith}}{Me:Ca_{water}}
\]

and a mean value was derived for each salinity treatment. These values were then plotted against measured salinity in the tank waters and compared with a 1-way ANOVA with salinity as a fixed factor. Linear regression was used to examine relationships between the empirical river water values of Sr:Ca and Ba:Ca, and those predicted from the mixing models. Data for the high and low salinity end-members in each river were excluded from these analyses, as these are both empirical values and form the upper and lower bounds to the models. All statistical analyses were conducted in SYSTAT version 10 (Systat Software). Means are presented ±SE.

RESULTS

Tank conditions

Water temperature was relatively stable across all tanks and treatments during the experimental period, and salinity levels remained constant within a given treatment (Table 1). There was no effect of salinity on fish length at Day 72 of the experiment, and no differences between tanks within the same treatment (2-way nested ANOVA, salinity: \(F_{2, 30} = 0.216, p = 0.807\); tank: \(F_{3, 30} = 0.308, p = 0.820\)). However, obvious darkening of body pigmentation was evident in most individuals maintained at the lowest salinities (i.e. 0.5 and 5‰). Mean TL at the end of 72 d across all treatments and tanks was 17.67 ± 0.18 mm. There was no significant variation in TL among the 0.5‰ tanks at the end of the experiment (1-way ANOVA, \(F_{2,17} = 0.189, p = 0.830\)).

Water and otolith chemistry: tank experiments

The ambient Sr:Ca concentration in the rearing tanks increased non-linearly with salinity, with the greatest rate of change occurring between 0.5 and 5‰ (Fig. 2a). At 0.5‰, the mean Sr:Ca concentration across all water samples \((n = 9)\) was 1.89 ± 0.01 mmol mol\(^{-1}\), rising to 6.83 ± 0.13 mmol mol\(^{-1}\) at 5‰ \((n = 6)\). At salinities >5‰, Sr:Ca variation was relatively small, with maximum values observed at 21‰ \((8.79 ± 0.08\) mmol mol\(^{-1}\), \(n = 6\); Fig. 2a). There were no differences in Sr:Ca observed between tanks for all salinity treatments tested (2-way nested ANOVA, \(F_{3,12} = 0.498, p = 0.691\)).

A negative logarithmic function best described the relationship between water Ba:Ca and salinity in the rearing tanks (Fig. 2a). Most notable was the large decrease in Ba:Ca concentration from the 0.5‰ \((512.48 ± 5.40\) µmol mol\(^{-1}\), \(n = 9\)) to the 5‰ treatment \((265.07 ± 1.08\) µmol mol \(^{-1}\), \(n = 6\)), coinciding with a marked increase in Sr:Ca. A more gradual drop in Ba:Ca was evident from 5 to 38‰ (Fig. 2a). The minimum Ba:Ca \((11.70 ± 0.14\) µmol mol\(^{-1}\), \(n = 6\)) occurred at the highest salinity tested (38‰), and again there were no differences between tanks within each salinity treatment (2-way nested ANOVA, \(F_{3,12} = 0.009, p = 0.999\)).

\[
\begin{align*}
  y & = 1.614 (0.149 SE) log(x) + 3.338 (0.356 SE) \quad R^2 = 0.944, p < 0.001 \\
  y & = -119.06 (3.719 SE) log(x) + 434.54 (8.907 SE) \quad R^2 = 0.993, p < 0.001 \\
  y & = 0.449 (0.039 SE) log(x) + 1.380 (0.093 SE) \quad R^2 = 0.950, p < 0.001 \\
  y & = 13.82 (0.849 SE) x - 0.63 (0.079 SE) \quad R^2 = 0.984, p < 0.001
\end{align*}
\]
The general nature of the relationships between salinity and otolith Sr:Ca and Ba:Ca reflected the patterns observed for the water chemistry data. Relatively little variation in Sr:Ca concentration was found among the original 5, 21 and 38‰ treatments, with a small but statistically significant increase from ~2 to ~3 mmol mol\(^{-1}\) between 5 and 38‰ (2-way nested ANOVA, \(F_{2, 30} = 51.599, p < 0.001;\) Tukey’s HSD, \(p = 0.001\)). Similar to the water chemistry results, there was a marked decrease in otolith Sr:Ca to 1.06 ± 0.08 mmol mol\(^{-1}\) (n = 20 fish pooled across tanks) when bass were transferred to the 0.5‰ treatments (Fig. 2b). This drop in Sr:Ca corresponded with an abrupt elevation in otolith Ba:Ca from <~4 µmol mol\(^{-1}\) in the 5 to 38‰ treatments to 22.62 ± 4.10 µmol mol\(^{-1}\) (n = 20) at 0.5‰. Otolith Ba:Ca was significantly higher at 5‰ than at either of the 2 higher salinity treatments (2-way nested ANOVA, \(F_{2, 30} = 16.740, p < 0.001;\) Tukey’s HSD, 21‰: \(p = 0.001;\) 38‰: \(p < 0.001\)), with little difference observed between 21 and 38‰ (Tukey’s HSD, \(p = 0.221\)). There was no difference in otolith Sr:Ca or Ba:Ca observed among tanks within each of the 5, 21 or 38‰ treatments (Sr:Ca: 2-way nested ANOVA, \(F_{3, 30} = 0.501, p = 0.685;\) Ba:Ca: \(F_{3, 30} = 0.975, p = 0.418\)), and no difference in otolith Sr:Ca (1-way ANOVA, \(F_{2, 17} = 1.53, p = 0.245\)) and Ba:Ca (1-way ANOVA, \(F_{2, 17} = 0.119, p = 0.888\)) among the 0.5‰ tanks at the end of the 50 d experiment. However, individual bass within the same tank sometimes showed substantial variation in the final values of otolith Sr:Ca and, in particular, Ba:Ca after 50 d (see Figs. 2b, 3b & 4).

A strong positive linear relationship was found between otolith Sr:Ca and water Sr:Ca (Fig. 3a), and a positive exponential relationship was observed between Ba:Ca in otoliths and water (Fig. 3b). Examination of the partition coefficients for each element illustrates that the uptake of both Sr and Ba into the juvenile bass otoliths changed across the salinity gradient, with uptake maximised at the lowest and highest salinities examined (Fig. 3c). Uptake of Sr was greatest at 0.5‰ (mean = 0.56 ± 0.044) and lower at 5 and 21‰ (\(D_{Sr} = -0.30\)), with an increase to ~0.38 at 38‰. Ba uptake was highest at 38‰ (\(D_{Ba} = 0.119 ± 0.019\)) and varied substantially among individuals within the same tank, as demonstrated by the large error values (Fig. 3c). The rate of uptake declined with salinity to 0.047 ± 0.003 at 21‰ and 0.016 ± 0.002 at 5‰. Like \(D_{Sr}\), there was an increase in \(D_{Ba}\) at 0.5‰ to 0.044 ± 0.008 with substantial individual variation in uptake rates after transfer to the 0.5‰ treatment.

Examination of the otolith transect data from bass transferred from the 5, 21 and 38‰ treatments to the 0.5‰ treatment revealed complex and variable re-
sponses in both elemental ratios, but for Ba:Ca in particular (Fig. 4). For all individuals tested, a distinct time lag was apparent before the concentration of each elemental ratio reached a stable level (Fig. 4). With regard to Sr:Ca, although the final concentrations at Day 50 were relatively consistent among the 3 tanks, there was some within-tank variability, most notably when fish were transferred from 38 to 0.5‰ (Fig. 4c). Additionally, Sr:Ca did not reach a stable level in the otolith for at least 20 d after bass were transferred to the 0.5‰ treatment, with some individuals taking >30 d and one ~40 d (see Fig. 4a). A similar lag effect was observed with the uptake of Ba:Ca, although again some individuals responded more quickly than others, both within and among tanks (e.g. Fig. 4a,b). It typically took between ~10 and ~30 d after transfer to the 0.5% treatment for a stable Ba:Ca value to be reached. Some otolith transects were characterised by large Ba:Ca peaks reaching ~90 µmol mol–1 before concentrations decreased to a more stable value (e.g. Fig. 4b,c), whilst transects of other bass exposed to identical experimental conditions within the same tank displayed no major peaks and a more gradual increase in Ba:Ca before reaching a stable value (see Fig. 4c). Importantly, the equilibrium value where Ba:Ca concentration became stable varied widely among individuals in the same tank.

**River water chemistry**

The mixing curves derived from our models provided a good fit to the empirical data collected in each of the 7 rivers (Figs. 5 & 6). The relationships between salinity and water Sr:Ca and Ba:Ca appear to follow a similar non-linear pattern to those observed in the laboratory experiments, with relatively small variation in Sr:Ca and Ba:Ca above a threshold salinity value of ~5‰. The general nature of these relationships was consistent for all water samples collected regardless of the collection season (see Fig. 5); however, Sr:Ca values were substantially lower during winter in the 3 rivers where both summer and winter collections were made (Bunyip, Tambo and Barwon; Fig. 5).

**DISCUSSION**

The present study examined the response of Sr:Ca and Ba:Ca in water and otoliths across the full range of salinities commonly experienced by euryhaline
fishes. In the analyses of otolith and water chemistry from our laboratory experiments, in addition to the water samples collected from 7 coastal rivers, we found strong, consistent non-linear relationships between salinity, Sr:Ca and Ba:Ca, with the greatest rate of change in elemental concentrations occurring at salinities <5‰. The nature of these relationships appears to depend greatly on the inclusion of the lowest salinity treatment at 0.5‰. Positive logarithmic responses in water and otolith Sr:Ca with increasing salinity have been reported previously, but only in studies that have incorporated low (i.e. <2‰) salinity levels (e.g. Kraus & Secor 2004, Zimmerman 2005, Kerr et al. 2007). In higher salinity environments, laboratory and field-
based studies have described linear relationships (e.g. Martin & Wuenschel 2006) or no relationship (Elsdon & Gillanders 2005a, Dorval et al. 2007) between otolith and/or water Sr:Ca and salinity. When data for 0.5‰ were removed from our analyses, the association between otolith Sr:Ca and salinity adhered to a weakly positive linear relationship (linear regression, Sr:Ca$_{otolith} = 0.028$ (salinity) + 2.206, $R^2 = 0.927$, $p < 0.01$). Variation in both water and otolith Sr:Ca was minimal between 5 and 38‰, suggesting that movements of bass between estuarine and marine environments may not be resolvable using otolith Sr:Ca alone. High Ba:Ca values such as those measured in our aquarium and river water samples (i.e. >500 µmol mol$^{-1}$) have generally only been reported in entirely freshwater systems exhibiting very low salinities (e.g. Wells et al. 2003, Gibson-Reinemer et al. 2009). However, in the few studies that have examined variation in water Ba:Ca across a large salinity gradient (e.g. Elsdon & Gillanders 2005a, Crook et al. 2006, 2008, Martin & Wuenschel 2006), a negative non-linear relationship is apparent. The negative correlation between otolith Ba:Ca and salinity has been well documented in field studies in estuaries, but the nature of the relationship has been shown to vary from linear (Elsdon & Gillanders 2005b) to exponential (Dorval et al. 2007) depending on the species examined. By contrast, in their work on elemental uptake in gray snapper $Lutjanus$ $grieseus$, Martin & Wuenschel (2006) observed no discernable relationship between otolith Ba:Ca and salinity across salinities of 5 to 45‰, despite reporting a clear negative exponential trend in water Ba:Ca with increasing salinity. Our data suggest a decreasing non-linear relationship for both otolith Ba:Ca in bass and water Ba:Ca as salinity increases, with otolith Ba:Ca responding more gradually than Sr:Ca to equivalent salinity changes.

Like several previous laboratory studies (e.g. Bath et al. 2000, Elsdon & Gillanders 2003, Martin et al. 2004), we observed a strong positive linear relationship between otolith Sr:Ca and water Sr:Ca in our experimental tanks. The relationship between partition coefficients for Sr ($D_{Sr}$) and salinity was not linear, with the maximum $D_{Sr}$ observed at our lowest salinity treatment (0.5‰) and only minor differences between 5 and 38‰. A similar pattern was reported by Zimmerman (2005), who found that $D_{Sr}$ in 5 species of salmonids was highest at the lowest salinity tested (0.1‰), declined to a minimum value at the next highest salinity (6.3‰) and gradually increased thereafter (see also Elsdon & Gillanders 2005a). Several other studies that have examined uptake rates of Sr and Ba have related $D$ to ambient water concentrations, observing, in general, greater uptake of Sr:Ca and Ba:Ca at lower ambient concentrations (e.g. Bath et al. 2000, de Vries et al. 2005, Elsdon & Gillanders 2005a). Our data for Sr:Ca showed a similar pattern, with $D_{Sr}$ reaching a maximum at the lowest ambient concentrations measured (~2 mmol mol$^{-1}$), dropping substantially at ~7 mmol mol$^{-1}$ (as salinity increased from 0.5 to 5‰) then remaining relatively stable at higher ambient concentrations. These results may reflect differential pathways of Sr uptake into the blood plasma between freshwater (branchial uptake) and marine (intestinal uptake) environments (Olsson et al. 1998), with subsequent effects on endolymph and otolith concentrations and/or regulation of Sr$^{2+}$ and Ca$^{2+}$ activity within the endolymph (Payan et al. 2002). Data on ionic concentrations and activity levels in the endolymph would be required to directly test these hypotheses.

The positive relationship between otolith and water Ba:Ca was best described by an exponential equation, which differs somewhat from the logarithmic (Elsdon & Gillanders 2005b) or linear (Bath et al. 2000, Wells et

![Graph](image_url)
from higher salinities to the lowest salinity treatment (0.5%). Despite the controlled chemical environment, stable temperature regime and standardised diet, distinct individual variability was observed in relation to both the magnitude and timing of Ba:Ca uptake. Most notably, some fish exhibited very large Ba:Ca peaks immediately following transfer into the low salinity treatment before concentrations returned to lower and more stable values towards the end of the experiment. These results may help explain the findings of several field-based studies that have reported large peaks in Ba:Ca associated with movements from the sea into lower salinity environments (e.g. Crook et al. 2006, Hale & Swearer 2008). Otolith Sr:Ca responded more predictably in relation to the final equilibrium values reached after transfer to 0.5‰. However, some variability in these values within tanks was still apparent, and the timing of the drop in Sr:Ca also differed substantially among individuals in the same tank. The reasons for such individual variability are unclear. Previous work on Sr incorporation in marine Australian salmon Arripis trutta, blue grenadier Macrourus novaezelandiae and bearded rock cod Pseudophycis barbatus points to physiological factors related to changes in blood plasma and endolymph chemistry, otolith growth rates and reproductive investment as the major determinants of $D_{\text{Ba}}$ in these species (Kalish 1989, 1991). Furthermore, changes in an individual’s ability to osmoregulate as they grow or acclimate to different salinity environments (e.g. Jensen et al. 1998, Herrera et al. 2009) may also affect transfer of ions such as Sr$^{2+}$ across gills and other epithelial membranes into the blood, which in turn may influence endolymph and otolith Sr composition (Kalish 1991). It is possible that juvenile bass within the same tank had different osmoregulatory and physiological responses to the transfer to the lowest salinity treatment in our experiment, which may explain some of the variation we observed. Although recent work has suggested that Sr and Ba incorporation is driven primarily by environmental factors of water chemistry, salinity and temperature in other species (e.g. Elsdon & Gillanders 2004, Walther & Thorrold 2006), further research into the processes controlling ion transport from water to the otolith (e.g. Payan et al. 2002, Melancon et al. 2009) may help elucidate the roles of ontogeny and physiology in driving elemental uptake across salinity gradients.

Regardless of the mechanisms at play, our findings of substantial and variable time lags in the uptake of Sr:Ca and Ba:Ca and large individual differences in equilibrium values at the conclusion of the experiment have obvious potential implications for reconstructing the salinity histories of bass and other euryhaline species using these elements. For fish that undertake
rapid movements across salinity gradients within estuaries, the presence of time lags means that the residence time in a given salinity environment may be too short for a chemical signal representing ambient salinity to be accurately reflected in the otolith. Consequently, particular Sr:Ca or Ba:Ca values could either represent equilibrium with the ambient water or transitional values at some unknown stage within a time lag period. Distinguishing the difference between these possibilities is very difficult without subsidiary information (e.g. detailed water chemistry and fish movement data). Furthermore, our data suggest that even if fish remain in a stable salinity environment for long enough to reach equilibrium, individual variation in the magnitude of uptake may hinder accurate reconstructions of environmental histories across small salinity gradients, particularly at higher salinities (>5‰) where water Sr:Ca and Ba:Ca are relatively invariant. Despite these issues, large changes in otolith Sr:Ca and Ba:Ca observed for fish transferred between 0.5‰ and the higher salinity treatments suggest that movements at coarser scales, such as between freshwater and marine environments or where salinity and temperature gradients are stable for extended periods, may be resolvable using these 2 elements either alone or in combination.

To illustrate the difficulties often faced when interpreting otolith chemistry data from wild-caught fish, we have included Sr:Ca and Ba:Ca transect data from the otoliths of 3 adult Australian bass captured in the Albert River, Victoria, Australia (Fig. 7). The complex nature of the data is evident, with the 3 fish showing variable patterns in Sr:Ca, marked cycling in Ba:Ca, particularly in the outer otolith growth regions, and no consistent positive or negative relationships between Sr:Ca and Ba:Ca across the transects. So how should we interpret these patterns? Do the inverse relationships between Sr:Ca and Ba:Ca near to the otolith core reflect estuarine and/or marine residence as juveniles? Does the cycling of Ba:Ca (Fig. 7b,c) represent movements between freshwater and the estuary as adults? What does it mean when Sr:Ca and Ba:Ca appear positively correlated? Can we define when an actual movement across a salinity gradient has occurred, compared with a change in salinity at the same location? Without intimate knowledge of the salinity, temperature and ambient elemental concentration gradients in the Albert River over the lifetimes of these bass, answering such questions with any confidence is not possible. Furthermore, as elemental uptake rates may shift with ontogeny (de Pontual et al. 2003), the relationships derived between salinity and ambient and otolith elemental concentrations for juvenile bass cannot necessarily be extrapolated to older fish without first assessing any age-related effects.

The complex and variable nature of both the movements of euryhaline fishes and the environments they inhabit continues to pose major challenges for accurately retracing environmental histories in these systems using otolith chemistry methods. Whilst otolith Sr:Ca and Ba:Ca have clearly proved useful as indicators of movement by fish across coarse salinity gradients (e.g. Elsdon & Gillanders 2005b, McCulloch et al. 2005, Crook et al. 2006), the complexity of the data as observed in the present study suggests that the addition of alternative chemical markers to augment the measurement of trace element concentrations may aid interpretation at finer scales. Recent advances in the analytical capacities of sampling equipment have allowed researchers to explore the
use of other elements and isotopes (e.g. $\delta^{13}$C, $\delta^{18}$O, $\delta^{34}$S, $\delta^{87}$Sr and $\delta^{86}$Sr) to track dispersal, migratory and thermal histories at fine scales (Kennedy et al. 1997, Weber et al. 2002, Milton & Chenery 2005, Whitledge et al. 2006, Kerr et al. 2007, Weidel et al. 2007). By examining the mechanisms controlling incorporation of these markers into otoliths and considering the potential limitations of each with regard to the species of interest, it may be possible to trace the transition between freshwater, estuarine and marine environments for euryhaline species with greater confidence and accuracy.

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