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Atypical correlation of otolith strontium : calcium and barium : calcium across a marine–freshwater life history transition of a diadromous fish

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Abstract. Variation in strontium (Sr) and barium (Ba) within otoliths is invaluable to studies of fish diadromy. Typically, otolith Sr : Ca is positively related to salinity, and the ratios of Ba and Sr to calcium (Ca) vary in opposite directions in relation to salinity. In this study of jungle perch, *Kuhlia rupestris*, otolith Sr : Ca and Ba : Ca, however, showed the same rapid increase as late-larval stages transitioned directly from a marine to freshwater environment. This transition was indicated by a microstructural check mark on otoliths at 35–45 days age. As expected ambient Sr was lower in the fresh than the marine water, however, low Ca levels (0.4 mg L^{-1}) of the freshwater resulted in the Sr : Ca being substantially higher than the marine water. Importantly, the otolith Sr : Ba ratio showed the expected pattern of a decrease from the marine to freshwater stage, illustrating that Sr : Ba provided a more reliable inference of diadromous behaviour based on prior expectations of their relationship to salinity, than did Sr : Ca. The results demonstrate that Ca variation in freshwaters can potentially be an important influence on otolith element : Ca ratios and that inferences of marine–freshwater habitat use from otolith Sr : Ca alone can be problematic without an understanding of water chemistry.

Additional keywords: catadromy, jungle perch, *Kuhlia rupestris*, larval dispersal, laser ablation.

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Introduction

Fish otoliths are metabolically inert calcium carbonate ($CaCO_3$) structures found in the inner ear of teleost fishes (Degens *et al.* 1969; Campana and Neilson 1985). Although otoliths are primarily $CaCO_3$, a variety of chemical impurities are incorporated into the otolith matrix in minor or trace quantities, which can be influenced by environmental factors, such as the physical and chemical properties of the ambient water (Campana 1999; Bath *et al.* 2000; Campana and Thorrold 2001; Elsdon and Gillanders 2004; Walther and Thorrold 2006; Hamer and Jenkins 2007; Elsdon *et al.* 2008). An important, and now relatively common application of otolith chemistry, is in the study of life history usage of, freshwater, estuarine and marine habitats (i.e. diadromy) (Kalish 1990; Secor 1992; Secor *et al.* 1995; Chesney *et al.* 1998; Secor and Rooker 2000; Gillanders 2005; Walther and Limburg 2012).

The use of otolith chemistry to study diadromy is possible because the Sr : Ca ratio of otolith carbonate is generally higher in marine than in freshwaters, related to the typically higher concentration of Sr in saline waters (Tzeng *et al.* 1997; Limburg 2001; Secor *et al.* 2001; Zimmerman 2005; Brown and Severin 2009). The otolith Ba : Ca ratio has also been useful in studies of diadromy owing to the often higher Ba incorporation in freshwater than marine environments, again linked to the typically higher ambient Ba levels in fresh than marine waters (Guay and Falkner 1998; Elsdon and Gillanders 2005*a*; Crook *et al.* 2006). Studies have also taken advantage of the complimentary nature of these two elements to study marine–freshwater habitat use. In particular, the opposite patterns of Sr and Ba incorporation into otoliths in relation to salinity have provided enhanced inferential power of marine–fresh water habitat use (McCulloch *et al.* 2005; Crook *et al.* 2006; Tabouret *et al.* 2010; Walther *et al.* 2011; Walther and Limburg 2012).

Although many studies have used otolith Sr : Ca to infer marine–freshwater habitat use, the relationships between

incorporation of Sr into otoliths and salinity can be inconsistent (Kraus and Secor 2004; Brown and Severin 2009; Walther and Limburg 2012). Variable incorporation of trace elements such as Sr and Ba into fish otoliths is influenced, not so much by salinity directly, or solely by the availability of these elements in the water, but primarily by their availability in the water relative to that of Ca (Campana 1999; Kraus and Secor 2004). Kraus and Secor (2004) demonstrated that the Sr : Ca ratios of some coastal draining rivers and creeks in the USA were actually greater than seawater, a finding supported by further review of freshwater Sr : Ca ratios by Brown and Severin (2009). Both studies indicated that simply inferring marine–freshwater habitat use from variation in otolith Sr : Ca alone without measuring, or having prior information on, the ratios of these elements in the water could be misleading. Kraus and Secor (2004) and Brown and Severin (2009), in demonstrating the potential for fish in some freshwater environments to be exposed to higher ambient Sr : Ca than their marine counterparts, also highlights the potential value of the complimentary information derived from Ba : Ca.

Finally, whereas in many cases variation in Sr : Ca and Ba : Ca in otoliths will be more influenced by the amount of Sr and Ba in the water as opposed to Ca, the amount of Ca in the water could also have an important influence on otolith element : Ca ratios in situations where ambient Ca shows high variation. Calcium concentrations of marine waters are typically much higher and more consistent than freshwaters (i.e. 400 mg L^{-1} marine, $<$100 mg L^{-1} freshwater), but Ca concentrations of freshwaters can be highly variable, potentially reaching very low levels, e.g. $<$1–20 mg L^{-1} (Bayly 1964; Hem 1985; Tims 1986; Dalesman and Lukowiak 2010). Despite this known high variation, the role of ambient Ca variation in influencing variation in otolith chemistry has received limited attention.

This study involves a remnant population of the jungle perch, *Kuhlia rupestris*, on Fraser Island, a world heritage listed sand island of 184 000 ha (UNESCO 2001) situated off the coast of Queensland (Qld), Australia (Fig. 1). Jungle perch are the largest member of the *Kuhliidae* family and are found throughout the Indo-Pacific region, including eastern Australia from northern New South Wales (NSW) to Cape York Peninsula (Fig. 1) (Pusey *et al.* 2004; Feutry *et al.* 2013). They are thought to be catadromous across their range, although adult migration to marine waters may not occur in all systems, can reach up to 450 mm length, 2–3 kg in weight and 15 years of age (Merrick and Schmida 1984; Herbert and Peeters 1995). Jungle perch distribution has been considerably reduced since the 1960s with populations in southern Qld now considered depleted and or under threat owing to loss of habitat and construction of weirs and dams that have created barriers to migration (Hutchison *et al.* 2002).

In this study we use otolith life history profiles of Sr : Ca, Ba : Ca and Sr : Ba to investigate the marine–freshwater habitat transition of larval–early juvenile jungle perch in Wyuna Creek, Fraser Island (Fig. 1). This creek lacks an estuary and the only time when seawater intrusion into this stream may occur is during the highest lunar spring tides and storm surge events. As a consequence, movement of jungle perch life stages between the marine environment and this stream would involve a rapid transition between marine and freshwater. Further, Wyuna Creek, like numerous other perched lakes and streams on Fraser Island, has exceptionally low dissolved ionic concentrations, in particular Ca (Bayly 1964; Arthington *et al.* 1990). This system provided an opportunity to both investigate diadromy of jungle perch and compare the reliability of the otolith Sr : Ca, Ba : Ca and Sr : Ba ratios for inferring diadromous behaviour under an extreme and rapid water chemistry and salinity transition.

Materials and methods

Sample collection

As a result of the highly vulnerable nature of the Fraser Island jungle perch population, samples for this study were obtained opportunistically from unintended mortalities ($n = 9$) during an independent electrofishing (Smith-Root Inc. LR-24 back pack electrofisher) and fyke net survey in the lower reaches of Wyuna Creek in 2008–2009.

Otolith preparation and chemical analysis

Sagittal otoliths were extracted and prepared by embedding in epoxy resin (Struers Epofix) and polishing to the core in the transverse plane using aluminium oxide lapping films lubricated with Milli-Q water. Polished sections were sonicated in Milli-Q water for 5 min and liberally rinsed in Milli-Q water before drying in a laminar flow and storage in plastic containers. Polished otolith sections displayed clear daily micro-increment structures (i.e. Feutry *et al.* 2012*b*) and were aged by counting the daily increments under a magnification of ×400 immersed in

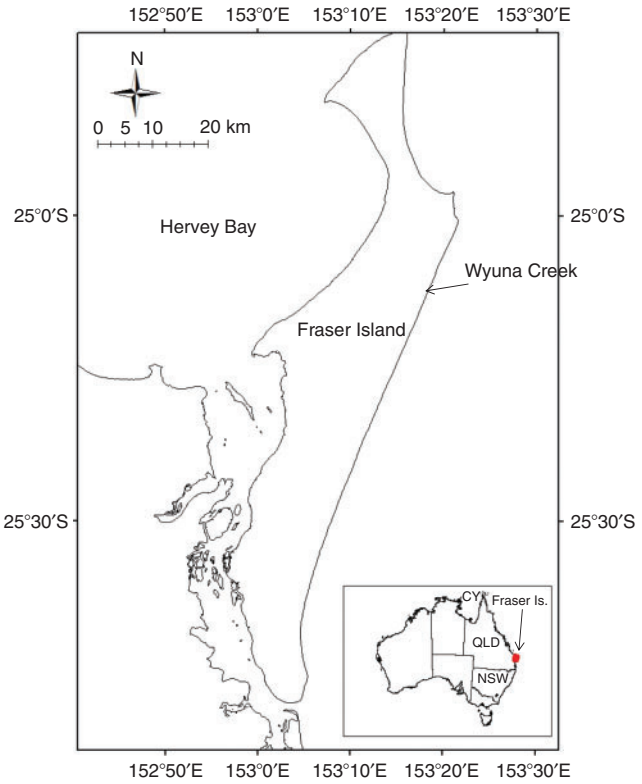

Fig. 1. Map showing the Wyuna Creek sampling area on Fraser Island, Qld, Australia (inset). CY, Cape York Peninsula; NSW, New South Wales; Qld, Queensland.

Milli-Q water. All otoliths displayed a prominent microstructural check mark which was assigned an age of formation (Fig. 2). This check mark is thought to be related to the transition from the marine to freshwater environment (see Feutry *et al.* 2012*a*).

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS, Laser – New Wave UP213, ICP-MS – Thermo Scientific Element 2, Department of Environment and Primary Industries, Queenscliff, Vic, Australia) was used to obtain life history element : Ca profiles from the core to the margin of otolith sections along the ventral axis (Fig. 2). LA settings were: beam diameter ~25 µm, fluence 10 J cm^{-2}, and repetition rate 6 Hz, with 2 µm s^{-1} stage movement along the transect path.

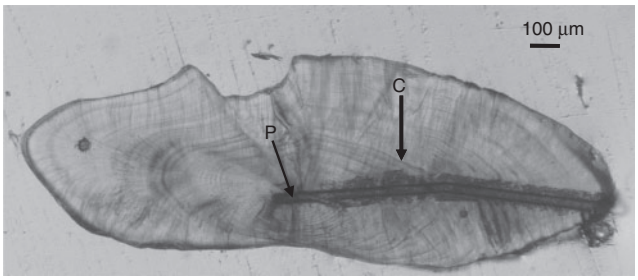

Fig. 2. Image of transverse section of a jungle perch otolith showing the laser ablation sampling trench and the otolith check mark. C, check mark; P, primordium.

Ablation occurred in helium which was mixed with argon for injection to the plasma. The ICP-MS measured the isotopes ^{88}Sr, ^{138}Ba and ^{43}Ca, which was used as the internal standard to adjust for variation in ablation yield. The Ca concentration of otolith matrix was 38.8% by weight (Yoshinaga *et al.* 2000). Blanks were obtained by analysing sample gases for 30 ICP-MS scans of the selected isotopes before sample ablation, and the averages of the blank counts were subtracted from the sample counts before calibration. Calibration was achieved with the National Institute of Standards (NIST) 612 certified reference pellet (Lahaye *et al.* 1997). Precision and accuracy were assessed by analysing the NIST 612 as an unknown sample with the actual concentrations as reported in Pearce *et al.* (1997), and an in house pressed pellet of the certified otolith reference powder FEBS-1 (National Research Council, Canada). The NIST 612 was analysed by continuous transects of 70 isotope scans, with 30 scans of blank gases before ablation. Average (% ± s.d.) recovery from eight analyses of the NIST 612 as an unknown were Sr 101 ± 8 and Ba 102 ± 5. Average relative standard deviation (RSD, %) (±s.d.) for the eight NIST 612 transects (i.e. 70 isotope scans each) was Sr 13 ± 4, Ba 13 ± 5. Average detection limits based on three times the standard deviation of the blank gases adjusted for ablation yield (Lahaye *et al.* 1997) were Sr : Ca 3.5 and Ba : Ca 0.05 µmol mol^{-1}.

For comparison, we had four samples (Table 1) repeat analysed along an adjacent laser transect path at another laboratory (IPREM/LCABIE, Université de Pau et des Pays de

Table 1. (*a*) Sample details for the nine jungle perch, including summary data for otolith Sr : Ca and Ba : Ca before (marine stage) and after (freshwater stage) otolith check formation; (*b*) physico-chemical water parameters for Wyuna Creek and the adjacent marine waters

Repeat analysis values are given in bold below the initial analysis value. Note: value for Ba of 5 µg L^{-1} used to calculate Ba : Ca for the marine water

(*a*) Otoliths

Sample ID	TL (mm)	Estimated age (days)	Marine stage			Freshwater stage		
			Sr : Ca (mmol mol^{-1})	Ba : Ca (µmol mol^{-1})	Sr : Ba	Sr : Ca (mmol mol^{-1})	Ba : Ca (µmol mol^{-1})	Sr : Ba
411	22	41	2.35	2.33	1008.6	5.16	598.2	8.62
508	49	113	2.88	2.25	1280	7.87	571.3	13.77
508 repeat analysis			**1.96**	**1.56**	**1256.4**	**5.46**	**513.7**	**10.63**
500	41	100	2.14	1.96	1091.8	6.53	716.1	9.12
500 repeat analysis			**2.09**	**1.85**	**1129.7**	**6.39**	**741.2**	**8.62**
497	37	97	2.36	1.53	1542.5	8.68	1106.4	7.85
492	45	118	2.83	1.38	2050.7	7.08	446.6	15.85
492 repeat analysis			**2.16**	**1.41**	**1531.9**	**5.14**	**414.9**	**12.38**
486	45	104	2.79	2.86	975.5	7.65	689.8	11.1
486 repeat analysis			**2.1**	**2.87**	**731.7**	**5.28**	**553.8**	**9.53**
324	82	209	2.84	1.83	1551.9	7.51	961.1	7.8
323	95	291	2.47	11.97	206.3	9.03	984.8	9.2
322	88	246	2.34	3.73	627.3	8.01	883.1	9.1
Mean (s.d.)			2.52 (0.25)	3.71 (3.15)	1148.3 (543.3)	7.51 (1.16)	773.1 (221.2)	10.26 (2.80)

(*b*) Water

	Salinity	T (°C)	pH	Ca (mg L^{-1})	Sr (mg L^{-1})	Ba (µg L^{-1})	Sr : Ca (mmol mol^{-1})	Ba : Ca (µmol mol^{-1})	Sr : Ba
Marine – Fraser Island surf zone	33.6	27.6	8.8	160	3.10	<5	8.84	9.10	620
Marine – literature				406	7.64	5.5	8.59	3.98	1389
Fresh – Wyuna Creek	0.1	22.3	6.3	0.41	0.01	9.1	12.25	6466.51	1.1

l'Adour, Pau, France) with a femtosecond laser ablation system (Alfamet, Novalase SA – Amplitude Systemes, Canejan, France) connected to an Elan DRC II ICP-MS (Perkin Elmer, Waltham, MA, USA). These samples were analysed as described in Feutry *et al.* (2011) and the calibration methods applied were consistent with the initial analysis described above (i.e. use of NIST612).

Water chemistry

During the last sampling trip of the electofishing and fyke net survey in August 2009 salinity, temperature, dissolved oxygen and pH were measured in the mid water column in Wyuna Creek ~200 m upstream from the mouth, and in the adjacent marine waters (i.e. surf zone, ~100 m from shore) ~200 m from the mouth of the creek. Water parameters were measured with YSI 556MPS (YSI Inc.) multiprobe. A water sample was also collected in the mid water column. The water was collected directly into acid leached (10% HNO_3) and washed (4 times with Milli-Q water) high-density polyethylene (HDPE) bottles. Sub-samples of the collected water were then filtered through 0.45 µm membrane syringe filters into new (acid leached and rinsed) HDPE bottles, acidified with ultrapure HNO_3 and frozen until analysis. Sub-samples of marine water were then diluted 10 times with Milli-Q water and analysed for Ba concentration by ICP-MS (Perkin Elmer Elan DRC II), and for Ca and Sr by ICP-AES (Varian 730-ES). The Wyuna Creek water samples were not diluted owing to low dissolved solids and ionic concentrations and were analysed for Sr and Ba by ICP-MS and for Ca by ICP-AES. Yttrium (2 µg L^{-1}) was added as an internal standard to correct for drift. Recovery of Ca, Sr and Ba from laboratory standards analysed as unknowns was 97, 95 and 105%. Matrix enhancement or suppression effects for the marine water samples were corrected from analyses of unspiked and spiked duplicates (Ca, Sr and Ba, High-Purity Standards) of seawater quality control samples (i.e. USEPA method 6020A), and the Yttrium internal standard included in all samples. Water samples were analysed by the National Measurement Institute, Sydney, NSW, Australia, method NT2–47.

Data analysis

To reduce noise in the LA-ICP-MS data, a three point running median followed by three point moving average was applied to the resolved element : Ca ratio data. Resolved element : Ca data and the Sr : Ba ratio were plotted as continuous life history profiles from the core to the otolith margin. Distance along the profiles for each ICP-MS measurement (i.e. scan) was determined by the speed of the laser stage movement and the time period for an individual scan of the selected isotopes. Indicators for 20-day periods of age are included on graphical presentations of the element : Ca profiles by measuring these intervals along the ablation trenches under a compound microsope at ×400 magnification. For graphical presentations Ba : Ca and Sr : Ba were displayed with $\log_{10}$ transformation owing to the order of magnitude variation.

Partition coefficients (D_{ME}) (Morse and Bender 1990) were calculated by dividing the mean element : calcium (Me : Ca) ratios measured in individual otoliths before (marine) and after (freshwater) the otolith check mark (Fig. 2) and associated transition of element : Ca values, by the Me : Ca ratio measured in the surf zone (marine) and Wyuna Creek (freshwater) water samples. Further, because we could not access water samples from further offshore beyond the surf zone, and larvae may have spent a portion of their marine dispersal beyond the surf zone we also calculated D_{ME} values for typical surface marine water Ca (406 mg L^{-1}), Sr (7.6 mg L^{-1}) and Ba (5.5 µg L^{-1}) values (Bernat *et al.* 1972; Hamer *et al.* 2006; Brown and Severin 2009).

$$D_{ME} = (\text{Me : Ca Otolith}) \div (\text{Me : Ca Water})$$

The partition coefficients indicate the level of discrimination against incorporation of elemental species from the water to the otolith ($D_{ME} = 1$ indicates no discrimination, $D_{ME} = 0$ indicates complete discrimination). Variation in partition coefficients for elemental species, across life stages, and physico-chemical conditions, can indicate factors that may affect elemental discrimination from the water into otoliths, and therefore the ability to extrapolate regression coefficients between ambient water chemistry and otolith chemisty (Campana 1999; Wells *et al.* 2003; Walther *et al.* 2010).

Results

Daily ageing

The length and age estimates of the nine jungle perch sampled in Wyuna Creek ranged from 22 to 95 mm TL and 41 to 291 days (Table 1). All nine otoliths displayed clear check marks (Fig. 2) at similar distances (i.e. 250–350 µm) from the primordium corresponding to between 35 and 45 days of age (Fig. 3).

Otolith chemistry

All otoliths showed similar Sr : Ca and Ba : Ca profiles characterised by lower values of both ratios from the core to the check mark, followed by an abrupt increase in both ratios starting at the point where the laser beam traversed the check mark (Fig. 3). This increase generally peaked after ~30 µm (i.e. equivalent to the beam diameter) of laser traverse from the point at which the leading edge of the laser beam met the check zone.

Relative to the variation between the pre- and post-check otolith material, Sr : Ca values before check mark formation were relatively stable for individual fish, with mean values ranging between 2.14 and 2.88 mmol mol^{-1} (Table 1*a*, Fig. 3). A similar result was observed for Ba : Ca in the pre-check region, with mean values ranging between 1.38 and 11.97 µmol mol^{-1} although there was a pattern of higher Ba : Ca in the otolith core region of most samples, and for some a similar increase just before the check mark (Table 1*a*, Fig. 3, note: log scale used for displaying Ba : Ca variation). Post-check formation Sr : Ca ratios increased by 2–3 times the pre-check values with mean values among fish ranging between 5.16 and 9.03 mmol mol^{-1} (Table 1*a*, Fig. 3). Ba : Ca increased by over two orders of magnitude after the check mark with mean values among fish ranging from 446 to 1106 µmol mol^{-1} (Table 1*a*, Fig. 3). The levels of both Sr : Ca and Ba : Ca remained relatively stable post-check formation (Fig. 3). The four samples that were repeat analysed at the two laboratories using different ICP-MS and LA instruments showed similar levels of the element : Ca ratios and differences between the marine and freshwater stages

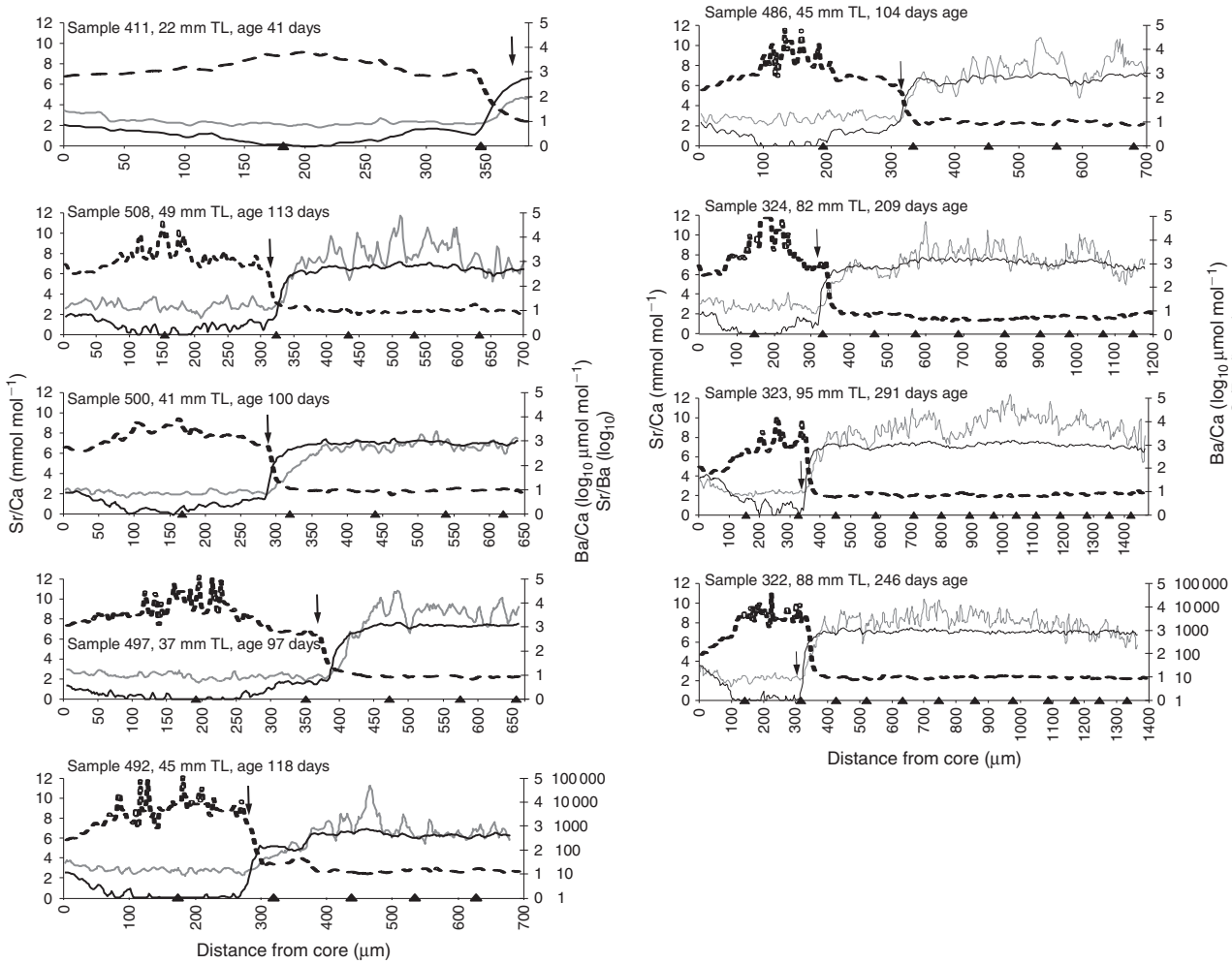

Fig. 3. Otolith Sr : Ca (grey line), Ba : Ca (black line) and Sr : Ba (dashed line) profiles for nine jungle perch sampled in Wyuna Creek. Vertical arrow indicates position of otolith check mark, triangles indicate 20-day age intervals. Length and age indicated for each sample. Note: log scale used to display Sr : Ba and Ba : Ca data to allow resolution of variation before and after otolith check formation.

(Table 1*a*). The Sr : Ba ratio showed a major reduction immediately after the check mark, upon entry to freshwater. Prior to the check mark, the Sr : Ba ratio showed some variation with most profiles increasing from the core to 100 µm out from the core before dropping at the check mark (Fig. 3).

Water chemistry

Concentration of Sr was ~300 times lower in the freshwater (salinity = 0.1) Wyuna Creek compared with adjacent surf zone (salinity = 33.6), whereas Ba concentration was approximately two times higher in the Wyuna Creek than in the surf zone (Table 1*b*). Despite the unusually low Ca values for the marine surf zone (160 mg L^{-1}), the Ca concentration was still ~400 times higher than in the freshwater of Wyuna Creek (Table 1*b*). The surf zone water was slightly alkaline (pH = 8.8), whereas the Wyuna Creek water was slightly acidic (pH = 6.3) (Table 1*b*). The low Ca concentration of the Wyuna Creek freshwater meant that both the Sr : Ca and Ba : Ca ratios were higher in the freshwater than the marine water, and for Ba : Ca this difference was extreme (i.e. Ba : Ca was ~700 times higher in the Wyuna Creek water) (Table 1*b*). Further, the Sr : Ca ratio was similar for both the surf zone water sample and the literature values for typical marine surface waters (Table 1*b*).

Partition coefficients – D_{ME}

Using the values for the surf zone water sample, the marine life stage (i.e. before the otolith check) had similar mean D_{ME} for Sr and Ba of 0.29 and 0.25 respectively, but for the freshwater stage (post-check formation) D_{Sr} doubled to a mean of 0.61 whereas mean D_{Ba} decreased by half to 0.12 (Table 2). Using the typical (literature) values of Sr, Ba and Ca for marine waters D_{Sr} for the marine life stage was unchanged at 0.29, whereas D_{Ba} was higher at 0.62 (Table 2).

Discussion

This study provides an example where rapid migration of fish early life stages from a marine to a freshwater environment resulted in a rapid and sustained increase in both otolith Sr : Ca and Ba : Ca. The higher Sr : Ca and Ba : Ca of otolith

Table 2. Partition co-efficients (D_{ME}) for Sr and Ba before (marine stage) and after (freshwater stage) otolith check formation
Marine stage D_{ME} are calculated for both the Fraser Island surf zone and literature marine water Sr : Ca and Ba : Ca values (Table 1)

Habitat	Partition co-efficient (D_{ME}) (mean ±s.d.)	
	D_{Sr}	D_{Ba}
Marine stage – Fraser Island surf zone	0.29 (0.03)	0.25 (0.08)[A]
Marine stage – literature	0.29	0.62
Freshwater stage	0.61 (0.09)	0.12 (0.03)

[A]Excludes one high Ba : Ca marine stage outlier value (sample 323).

material deposited in the freshwater stage was consistent with their higher element : Ca ratios in the freshwater than the adjacent marine waters. However, Sr levels in the freshwater were, as expected considerably lower than in the marine water. The higher Sr : Ca, and exceptionally high Ba : Ca in the freshwater, were largely driven by the extremely low Ca levels in the freshwater. The increase in the Sr : Ca and Ba : Ca ratios immediately at the otolith check mark indicates that the changes in water chemistry exposure were rapidly recorded in the otolith (i.e. days), perhaps more rapidly than reported by previous studies for juvenile fish (i.e. >10 days; Elsdon and Gillanders 2005*b*; Yokouchi *et al.* 2011). The apparent rapid increase in otolith Ba : Ca and Sr : Ca may relate to a rapid and significant increase in branchial uptake of metals upon exposure to the very low ambient Ca (soft water) environment of Wyuna Creek (Campana 1999; Uchida *et al.* 2002).

The Sr : Ba ratio showed a clear pattern across all fish of a sharp decrease from the marine to the freshwater stage. The pattern of higher otolith Sr : Ba in marine versus freshwater has been demonstrated for marine–freshwater migrations in other species, and is consistent with expected changes in Sr and Ba levels between fresh and marine waters (McCulloch *et al.* 2005; Walther *et al.* 2011). The Sr : Ba ratio may be a more reliable indicator of marine–freshwater migrations, particularly when Ca levels of the freshwater may be low or highly variable, and water chemistry data on element : Ca ratios are unavailable.

Kraus and Secor (2004) and Brown and Severin (2009) document a range of Sr : Ca values from <1 to >19 mmol mol^{-1} for coastal draining rivers, creeks and lakes. Although most freshwaters are likely to have Sr : Ca ratios in the range of 1–6 mmol mol^{-1} (Kraus and Secor 2004; Clarke *et al.* 2007; Brown and Severin 2009), the Sr : Ca value for Wyuna Creek of 12.25 mmol mol^{-1}, is not necessarily unusual. The value for the near shore marine waters of 8.8 mmol mol^{-1} was typical of marine waters (Bruland 1983; Kraus and Secor 2004; Brown and Severin 2009). Whereas marine water typically has Ca levels of around 400 mg L^{-1} (Gianguzza *et al.* 2002), the Ca concentration of freshwater can vary greatly depending on catchment geology (i.e. age and composition of the underlying bedrock and soil), temperature, pH, carbon dioxide levels, biological processes and the proportion of stream flow derived from ground water and surface runoff or rainfall (Hem 1985). Ca concentrations of natural freshwaters can vary from <1 (i.e. rainwater) to over 1000 mg L^{-1} (i.e. long-residence time ground waters and limestone catchments) (Hem 1985; Tims 1986; Drever 1997). This variation will be an important, but perhaps underappreciated influence on variation in otolith element : Ca ratios in freshwaters.

In the case of Fraser Island's streams and perched lakes, and other coastal draining streams on the adjacent mainland 'sandy' region, the exceptionally low levels of Ca ions (typically <0.8 mg L^{-1}) are as a result of the low input of terrigenous ions from catchments composed largely of silica sands (Bayly 1964; Hawkins *et al.* 1988; Larsen and Cox 2011; M. Hutchison, unpubl. data). It was also notable that the Ca and Sr levels of the seawater sample taken off the beach at Fraser Island were also considerably lower than expected for marine water. Although, because both elements were similarly low, the Sr : Ca ratio remained consistent with typical marine waters. Salinity was also slightly lower than marine waters, and the low Ca and Sr may have been partly related to the influence of ground water seepage on the near shore water chemistry, although further water sampling is required to confirm this.

Whereas the otolith Ba : Ca values measured for the marine stage where typical for marine fish (Campana 1999), the values measured for the freshwater stage of jungle perch were relatively high (i.e. 500–1000 μmol mol^{-1}) compared with observations for other freshwater fish species (Campana 1999). In other studies of jungle perch otolith chemistry from Pacific island insular streams the freshwater otolith stages have displayed Ba : Ca ratios of 100–200 μmol mol^{-1} (Feutry *et al.* 2011; Feutry *et al.* 2012*a*). The Ba : Ca levels of the Wyuna Creek water were extremely high (>6000 μmol mol^{-1}), consistent with the exceptionally high Ba : Ca values in the freshwater otolith stage. Previous studies have shown clear positive relationship between Ba : Ca in otolith and ambient water (Bath *et al.* 2000; Elsdon and Gillanders 2003; Wells *et al.* 2003; Miller 2009, 2011; Webb *et al.* 2012). Laboratory studies of Ba : Ca incorporation into otoliths, however, have typically involved exposures to water with Ba : Ca ratios less than 250 μmol mol^{-1} (i.e. Bath *et al.* 2000; Elsdon and Gillanders 2003). Importantly, the example presented here for jungle perch demonstrates that the link between otolith Ba : Ca and water Ba : Ca is consistently positive, albeit with variable partition coefficient, at very high ambient Ba : Ca values, driven largely by low calcium as opposed to high ambient Ba levels.

Although the Ba : Ca ratio was high in the freshwater otolith stage, the partition coefficient (D_{ME}) for Ba was actually lower than in the marine stage, indicating greater discrimination against otolith Ba incorporation in the low Ca freshwater environment. In contrast the opposite was observed for the D_{Sr}. Previous studies have indicated that otolith partition coefficients for Sr and Ba may vary depending on salinity, temperature, element : Ca ratios of the ambient water and growth rates (Bath *et al.* 2000; Elsdon and Gillanders 2003; Wells *et al.* 2003; Dorval *et al.* 2007; Miller 2009; DiMaria *et al.* 2010; Macdonald and Crook 2010; Walther *et al.* 2010). Similar to our observations for jungle perch, Macdonald and Crook (2010) showed for Australian bass (*Macquaria novemaculeata*) that D_{Sr} and D_{Ba} were maximised at lower and higher salinities respectively. Zimmerman (2005) also showed for five salmonid species, that although D_{Sr} increased with increasing salinity from 6 to 33, it was actually highest at a salinity of 0.1 (freshwater). Consistent

with our data for jungle perch, Miller (2009) also showed for black rockfish (*Sebastes melanops*) that D_{Ba} decreased with a decrease in temperature, and Elsdon and Gillanders (2003) showed that at marine salinities, D_{Ba} decreased with higher Ba : Ca of the ambient water, but contrary to our observations D_{Sr} decreased with increasing ambient Sr : Ca. The influence of the reduced pH between Wyuna Creek and the adjacent marine water on elemental discrimination between water and the otoliths is unclear. Although reduced pH may lead to increased accumulation of some trace metals in fish (Bury *et al.* 2003; Jezierska and Witeska 2006), the study by Munday *et al.* (2011) suggests the effect of pH variation on otolith chemistry may be limited. Further work on pH influences on otolith chemistry is warranted.

Previous studies of jungle perch reproductive biology have suggested that sperm motility is low in freshwater and that for maximum fertilisation success spawning must take place at the saline reaches of estuaries or in the sea (Hogan and Nicholson 1987). Our data showed elevated Ba : Ca in the otolith core region, but levels were still an order of magnitude less than occurred post-otolith check formation. This may indicate a maternal effect on otolith Ba : Ca or be related to growth, ontogeny or metabolism (Chittaro *et al.* 2006; Thorrold *et al.* 2006; Hamer and Jenkins 2007; Walther *et al.* 2010; Miller 2011) rather than early larval life spent in freshwater. It may also indicate that the first part of the dispersal phase is spent close to shore where ambient Ba : Ca ratios may be higher, than offshore waters. This may also explain why some fish showed a small increase in Ba : Ca just before the check formation. Spawning events are likely to be very concise and may happen between one and three nights over the new and full moon cycles when adults can access marine waters during spring tides. The consistent length of the marine larval phase may be related to the timing of jungle perch spawning with the lunar spring tides, the requirement for larvae to use spring tides to re-enter insular streams, and the need for larvae to reach a certain stage of ontogeny and size to allow them to make the marine–freshwater transition.

Conclusions

This study illustrates the value of otolith chemistry to understanding of diadromy, but cautions against the routine interpretation of diadromy from otolith Sr : Ca variation under the assumption that Sr : Ca in otoliths has a consistent positive relationship with salinity. The use of both Sr and Ba are recommended for studies of diadromy, along with understanding of water chemistry. The inferences from otolith Sr : Ca and Ba : Ca, despite the variation in ratios being similar, indicate that the Wyuna Creek population of jungle perch is catadromous, with a marine larvae phase of 5–6 weeks before recruitment into freshwater. The replenishment of the jungle perch population in Wyuna Creek, and more generally on other Fraser Island insular spring fed creeks, depends on the ability of larval stages to access the creeks on extreme high tides, and as such will be highly sensitive to tide and sea conditions. Changes to beach morphology that affect this process of marine–freshwater connectivity will have a major influence on the annual recruitment of these populations.

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