Does otolith chemistry indicate diadromous lifecycles for five Australian riverine fishes?

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Abstract
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Keywords
does, fishes, chemistry, riverine, diadromous, five, lifecycles, otolith, australian, indicate

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Does otolith chemistry indicate diadromous lifecycles for five Australian riverine fishes?

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Abstract. Diadromy is an important characteristic of the lifecycle of many Australian coastal fishes, but many of these species remain poorly studied. The migratory patterns of five riverine fish species from south-eastern Australia were examined using otolith chemistry. Analyses of individual otoliths from wild-caught fishes revealed distinctive lateral variation in otolith Sr:Ca values that provide good evidence for an amphidromous lifecycle for two species: \textit{Myxus petardi} and \textit{Gobiohubius australis}. \textit{Gobiohubius cosii}, \textit{Potamalosa richmondii} and \textit{Notesthes robusta} displayed Sr:Ca patterns that indicated that these species may have more complex movements between marine and fresh water. Overall, these results provided quantitative data that supported the lifecycles previously hypothesised for most of the studied fish species. However, \textit{M. petardi}, which was thought to be catadromous, displayed Sr:Ca variations that suggested an amphidromous lifecycle, at least for the specimens examined. These results also provided further evidence to demonstrate that otolith chemistry is a useful tool for studying the movement patterns of diadromous species and this technique will be especially valuable in identifying species that are most at risk from river regulation and barriers to migration.

Additional keywords: amphidromy, barium, catadromy, ICPMS, migration, strontium.

Introduction

Migration is an important characteristic of the lifecycle of many marine and freshwater fishes (Harden Jones 1968). However, migrations have large biological and physiological costs and place fish at a heightened risk from anthropogenic impacts (Gross et al. 1988; McDowall 1988; Jonsson et al. 1999). Possibly one of the most threatened groups of migratory fishes are those that display diadromy, that is, fishes that migrate between marine and freshwater habitats to complete their lifecycle (Angermeier 1995; Jonsson et al. 1999; McDowall 1999). For example, coastal riverine fishes of south-eastern (SE) Australia inhabit an area of rapidly increasing development and population growth that results in several threats to the survival of fish in this region (Harris 1984; Faragher and Harris 1994). However, with limited information available on the lifecycle requirements of many of these fish species, assessing impacts of threats can be difficult (Harris 1984).

Future management and conservation of these migratory fishes, particularly diadromous fishes, needs to be based on a thorough understanding of their lifecycles (Jonsson et al. 1999; McDowall 1999). However, the lifecycles and migrations of diadromous fishes can be difficult to study because many species display sedentary and secretive behaviour, and may also move in small groups, during high flows or under the cover of darkness (McDowall 1988; Keith 2003; Jowett et al. 2005). Several species also have migrating larval or juvenile stages that can be difficult to detect or study in the wild owing to their small size and often unpigmented transparent bodies (McDowall 1988; Closs et al. 2003; Keith 2003; Pusey et al. 2004). Therefore, many diadromous species have lifecycles that are not easily investigated using traditional survey techniques, telemetry or fish counters.

Recent advances in analytical techniques have enabled the detection of fine-scale variations in the trace elements of fish otoliths, and have provided a new source of information on the migratory history of fishes (Elman et al. 2000; Milton et al. 2000; Gillanders 2005). Otoliths are formed from calcium carbonate or aragonite (CaCO\textsubscript{3}) that is laid in concentric rings around a central core as fish grow and is deposited at \(\sim 1-5 \mu\text{m}/\text{day}\) along the main growth axis (Markwit\textsuperscript{t}z et al. 2000). The examination of fish movements between marine and freshwaters has been possible by measuring the concentrations of strontium (Sr) in the otolith, because this element is found in higher concentrations in sea water than in fresh water (Kalish 1990; Secor and Rooker 2000; Crook et al. 2006). As a result, otolith layers formed in marine waters typically exhibit higher ratios of Sr:Ca than layers formed in fresh water (Milton et al. 2000; Crook et al. 2006).

However, interpretation of movement patterns using otolith chemistry relies on a strong relationship between the ambient water and otolith concentrations, and otolith chemistry can be affected by factors other than salinity, such as species, age,
temperature, food and behaviour (Kalish 1990; Radke and Shafer 1992; Secor and Rooker 2000; Buckel et al. 2004). In addition, some estuarine and fresh waters can have elevated levels of Sr (Kraus and Secor 2004; Crook et al. 2006; Limburg and Siegel 2006). As a result, barium (Ba), an element typically more available for incorporation into otoliths in fresh water compared with sea water has also been used in otolith chemistry studies (Elsdon and Gillanders 2005a, 2006; Hamer et al. 2006; Hale and Swearer 2008). Therefore, the combination of these two elements (Sr and Ba) should provide a more accurate indication of the historical record of fish movement between marine and freshwater environments (Bath et al. 2000; Elsdon and Gillanders 2005b; Crook et al. 2006). In the present study, otolith Sr : Ca and Ba : Ca for five riverine fish species from SE Australia were analysed to determine whether the movements patterns are consistent with the diadromous lifecycles that have previously been suggested for these species (Table 1).

**Materials and methods**

**Fish collection and study area**

Wild Potamalosa richmondia, Myxus petardi, Notiesthes robusta, Gobiodon australis and Gobiodon coxii were collected from five catchments of SE Australia between May 2004 and April 2006 during ad hoc netting surveys (Fig. 1, Table 2). The Hunter (catchment size 22 000 km²), Shoalhaven (7500 km²) and Clyde Rivers (2500 km²) are medium to large catchments in SE Australia. These rivers originate in deep gorges and valleys that open up into wide valleys bordered by alluvial terraces or floodplains as they approach the coast (Sussmilch 1914; Roy et al. 2001). Macquarie Rivulet (217 km²) and Hewitts Creek (16 km²) drain from the Illawarra escarpment and both have entrances that are intermittently closed to the sea. After capture, fish were immediately euthanised in an ice water slurry mix, after which the majority of specimens were frozen. Some smaller specimens were stored in ethanol before later processing.

**Otolith removal and preparation**

Sagittal otoliths were removed from the fish, cleaned, rinsed and allowed to dry on glass slides before being stored in micro-centrifuge tubes for later preparation. All sample cleaning and diluting was done using ultra-pure water. Plasticware and glassware were washed in 10% HNO₃ and rinsed several times in water before use. The otoliths were embedded in epoxy resin (EpoFix, Struers, Denmark) and sectioned transversely through the centre using a low-speed isomet diamond saw (Buehler, Lake Bluff, IL, USA) lubricated with water (Elsdon and Gillanders 2002; Crook et al. 2006). Sections of ~400 μm were then polished using 9-μm lapping film until they were ~300 μm thick. These sections were rinsed in ultra-pure water and dried on glass slides. Prepared sections were mounted on glass slides in groups of up to eight per slide with additional epoxy resin. Mounted sections were then sonicated for 5 min, rinsed and dried in a laminar-flow cabinet before being stored in clean sealable plastic bags until microchemical analyses.

**Microchemical analyses**

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was used to determine elemental concentrations
of the otoliths. Analytical equipment consisted of an ArF ultraviolet excimer laser (λ = 193 nm) coupled to an Agilent 7500 quadrupole ICPMS (Yokogawa Analytical System, Kyoto, Japan) (Eggins et al. 1998). The instrument measures several masses sequentially; 43Ca, 87Sr and 138Ba were chosen for the present study. Laser ablation of the samples was conducted using a 10-Hz pulse rate and a 32-μm diameter spot under helium and hydrogen, and the ablated material was mixed with argon carrier gas before introduction into the ICPMS. The sample cell holds a 50-mm glass microscope slide on which we had mounted up to eight otoliths. Reference standards (National Institute of Standards and Technology, NIST 612 glass) were measured regularly to calibrate the relative sensitivity of each analyte mass (Norman et al. 1996; Elsdon and Gillanders 2002, 2004; Crook et al. 2006). The standard was measured each time the sealed sample stage was opened and again after every four otoliths (mid way through each sample set). Gas background readings measured on the sample carrier gas without ablation were measured for 15 s before each analysis and subtracted from the measured intensity of each analyte mass. A continuous scan was undertaken across the otoliths from one edge to the other, passing through the core using an automated stage at a scan rate of ~0.3 mm per minute. Each analysis was normalised to the Ca content expected for pure calcite (55 wt% CaO) or measured previously in the NIST 612 glass (Norman et al. 1996) to correct for variations in ablation yield from the sample relative to the glass standard and convert the measured ion intensities to absolute concentrations in ppm. Details of these calculations are given in Norman et al. (1996). Elemental concentrations were normalised to 43Ca and data were presented as concentration ratios in units of mmol mol⁻¹ (Sr : Ca) and μmol mol⁻¹ (Ba : Ca).

Movements between fresh and salt water were inferred from the patterns in Sr : Ca and Ba : Ca from the full otolith scans. Interpretation of this data was aided through a preliminary experiment that examined the uptake of Sr and Ba into otoliths of the study species under aquaria conditions (Miles 2007).
regions of the otoliths, relating to larval and juvenile growth, as well as the outer regions relating to subadult and adult growth were then compared. To smooth the data points, Sr : Ca and Ba : Ca values were averaged for 5-µm increments of otolith material.

**Statistical analyses**

To further investigate the lifecycle of *M. petardi*, mean Sr : Ca and Ba : Ca values in the central region of the otolith relating to larval and juvenile growth were compared with those on the outer region of the otolith (which corresponds to adult growth) across all specimens using Student t-tests. All statistical analyses were undertaken in SPSS ver. 10.0 (SPSS Inc, Chicago, IL, USA).

**Results**

**Otolith chemistry of wild-caught fish**

Otolith Sr : Ca transects for *P. richmondia* typically showed a series of peaks from the otolith core towards the edge (Fig. 2a, b). Ba : Ca showed variable patterns, with a series of high and low values that were mostly unrelated to Sr : Ca values. However, in some otoliths, the Ba : Ca values declined with the first peak in Sr : Ca (Fig. 2a, b). The highly variable values for both ratios and the consistent peaks and troughs of Sr : Ca for all fish across all catchments suggested that the *P. richmondia* examined in this study had inhabited waters with a variety of salinities.

Otolith Sr : Ca in the inner regions of *M. petardi* otoliths were considerably higher than those in the outer region (Fig. 2c, d). The high Sr : Ca values towards the core were mirrored by lower Ba : Ca values in this region of the otolith. The mean Sr : Ca values from the inner region of *M. petardi* otoliths across all fish from all catchments were significantly higher than those from the outer region (*t*-test, *t* = 98.6, *P* < 0.001, Fig. 3). Ba : Ca displayed the opposite trends, where significantly lower levels were found in the central region (*t* = 123.8, *P* < 0.001, Fig. 3). In addition, *M. petardi* juveniles <150 mm showed elevated Sr : Ca and lowered Ba : Ca levels across most of the otolith, which may suggest that these fish had only recently entered fresh water (Fig. 2e). Overall, the consistent peaks of Sr : Ca values in the region of the otolith relating to larval/juvenile growth and the lower values during adult growth suggest that early life is spent in higher salinity waters and the remainder of life is spent in fresh water. These patterns suggest that *M. petardi* examined in the present study displayed an amphidromous lifecycle.

The transects for Sr : Ca values in *N. robusta* otoliths showed a highly variable pattern, with several peaks and troughs from the inner region of the otolith to the edge (Fig. 2f, g). The transects for Ba : Ca values were also highly variable between otoliths and few discernible patterns were observed (Fig. 2f, g). Further, there appeared to be no consistent pattern in otolith chemistry as a result of the collection river. The highly variable values of Sr : Ca alone would seem to suggest that the specimens examined in this study had inhabited waters with a variety of salinities.

Otolith Sr : Ca transects for *G. australis* typically showed elevated levels in the central region and lowered levels on the outer region. The first peak was situated in the middle of the central region and the second occurred after a short band of lower Sr : Ca values (Fig. 2h, i). Patterns were consistent for all fish from all catchments. For example, the transects for *G. australis* are fish from Hewitts Creek (Fig. 2h) and the Shoalhaven River (Fig. 2i). The lower ratios in the central region may indicate a period of freshwater residency (or downstream drift) before entering saline waters. These values were mirrored by Ba : Ca values and together strongly suggest that early stages of the lifecycle are spent in waters with elevated salinities. These patterns are consistent with amphidromy. The transects for Sr : Ca values in the limited number of *G. coxi* otoliths examined in this study displayed similar patterns to *G. australis*; however, the levels of Sr : Ca relating to larval/juvenile were proportionally much smaller than the corresponding levels in *G. australis* (Fig. 2j). These results suggest that some portion of the early life might be spent in waters with elevated salinities.

**Discussion**

The results from this research provide new data on the movement patterns of five fish species from SE Australia. In general, the Sr : Ca values determined using LA-ICP-MS demonstrated differences within the otoliths of all species examined and indicated that all of these species probably exhibit residency in both marine and freshwater environments at some stage of their lifecycle (Table 3). Many of these patterns could be consistent with patterns of diadromy reported elsewhere (David et al. 2004; McCulloch et al. 2005; Crook et al. 2006, 2008).

The correlation between Sr : Ca and Ba : Ca was often poor in the outer region of the otolith, which made interpreting movements difficult for some species (i.e. *P. richmondia* and *N. robusta*). Similar results have been reported elsewhere for diadromous fish, where Ba : Ca has been difficult to interpret or relate to ambient salinity (Crook et al. 2006; Hale and Swearer 2008). Crook et al. (2006) also reported difficulties in interpreting the outer edges of otoliths from Australian grayling *Prototroctes maraena* (Günther, 1864) and they suggested that this may have been the result of movement into estuaries or between tributaries. The present study did not examine the water chemistry of the collection rivers; however, the relationships between Sr and Ba and salinity in river waters have been quantified in other SE Australian streams (Crook et al. 2006, 2008). Despite some high Sr concentrations in ambient fresh waters, which were also reflected in the otolith chemistry, the otolith Sr : Ca patterns that demonstrate diadromy remained consistent (Crook et al. 2006). It was also found that otolith chemistry patterns suggesting diadromy were consistent across all catchments for the species examined.

The apparent movements between fresh water and salt water found in this study for *P. richmondia* might support previous reports of catadromy for this species (Merrick and Schmida 1984; Koeln and O’Connor 1990; Briggs and McDowall 1996). Pigeon (1989) suggested that the lifecycle of this species involved adult fish undertaking annual winter downstream migrations from freshwater to marine areas to spawn. Juveniles are then thought to migrate back upstream after a period of development in marine environments (Pigeon 1989). After spending the winter in marine waters, *P. richmondia* adults also migrate back upstream. The series of peaks and troughs in otolith Sr : Ca values (Fig. 2a, b), the number of which fits within the known longevity of this species and the likely age of the specimens (Pigeon 1989), supports the hypothesis of an annual migration of
Fig. 2. Sr: Ca (mmol mol$^{-1}$) and Ba: Ca (μmol mol$^{-1}$) values along transects across ooliths of representative subsets of P. richmondi (a, b), M. petardi (c, d, e), N. robusta (f, g), G. australis (h, i) and G. coxii (j). (e) and (f) represent the data for juvenile fish. Scales for both axes vary for each fish.
Does otolith chemistry indicate diadromy?

P. richmonda between marine and fresh water. However, interpreting migratory patterns of catadromous or euryhaline species such as P. richmonda from otolith chemistry may be difficult because the downstream migration into marine waters occurs during the winter months. This means that potentially less otolith material is deposited and fine-scale variations may be difficult to detect (Radtke and Shaffer 1992; Secor and Rooker 2000; Elsdon and Gillanders 2002). Many fish movements also coincide with flood events and it is recommended that future studies should consider examining the water chemistry over long time frames in several flow conditions.

The apparent multiple movements by N. robusta between fresh water and salt water is contradictory to some previous reports that suggested the life cycle is completed in fresh water (Pollard and Parker 1996). The results from the present study are consistent with catadromy, which was also suggested by Pusey et al. (2004) after they collected gravid females and small juveniles from estuarine areas. As a result, it seems unlikely that this is an exclusively freshwater species, but if the records of juveniles occurring above barriers prove to be correct, it is possible that landlocked populations (i.e. where diadromy is abandoned for an exclusively freshwater lifecycle) could exist or that diadromy is facultative.

Otolith chemistry for G. australis and G. coxii displayed Sr:Ca and Ba:Ca patterns that support their previously suggested amphidromous and marginally amphidromous lifecycles (Koehn and O’Connor 1990; Larson and Hoese 1996; Pusey et al. 2004). This includes a marine larval stage in G. australis followed by upstream migrations by juveniles into freshwater habitats where the remainder of their lifecycle is completed (Koehn and O’Connor 1990; Larson and Hoese 1996; Pusey et al. 2004). G. australis juveniles have been collected in estuarine areas and small fish observed recruiting back into freshwater in spring (Larson and Hoese 1996; Pusey et al. 2004; Miles 2007). The present study also indicated that juveniles of G. coxii recruit from brackish or estuarine water into fresh water (Koehn and O’Connor 1990; Larson and Hoese 1996; Pusey et al. 2004). The reduced amplitude in Sr:Ca peaks for G. coxii compared with those observed for the closely related G. australis may reflect recruitment from waters with lower salinities. This would suggest that this species could be ‘marginally’ amphidromous. Further research is required to determine the significance of these differences in Sr:Ca between these two species. The lower Sr:Ca values found between the peaks around the otolith core in these species might reflect the downstream larval drift period in fresh water before they enter the marine environment (Fig. 2h, i, j). Landlocking has been observed for the related common bully, Gobiomorphus cotidianus (McDowall, 1975), in New Zealand (Closs et al. 2003); however, no evidence of landlocking was recorded in the populations of Gobiomorphus examined in this study. Diadromy is further supported because there are no records of G. australis and G. coxii forming landlocked populations, despite the large number of barriers in coastal catchments in the range of these species (Harris 1984).

Otolith chemistry for M. petardi (Fig. 2) strongly suggested an amphidromous lifecycle, rather then the previously assumed catadromous lifecycle. There appears to be very little published data supporting catadromy in this species. The main evidence for a catadromous lifecycle emanates from Humphrey (1979), where ripe fish were recorded in brackish water after flood events in the Macleay River. As a result, spawning was thought to occur in waters with salinities of 5 to 15. This hypothesis is not supported by the present study, which indicates that after a period in marine waters as juveniles, the remainder of life is spent in fresh water. The fish recorded by Humphrey (1979) in brackish water could reflect downstream displacement during a flood event, and not active migration. In addition, ripe and spent fish were collected in fresh water during a recent study (Miles 2007) and the otolith chemistry of the spent fish displayed no sign of recent movement into salt water (see Fig. 2d). The apparent lack of adults from surveys in several estuarine studies (e.g. West and Walford 2000) could further support that there is no active downstream migration of adults to marine areas for spawning.

Before this study, very little data existed on the lifecycle and movement patterns of these five riverine species. Otolith chemistry has proven to be a useful tool in providing new data for these species. The movement patterns indicated by otolith chemistry in the present study highlight the need to maintain and improve the connectivity in riverine environments. This is especially important in eastern Australia because there are over 290 dams and weirs in coastal streams between southern Queensland and northern Victoria that form major barriers to fish migration (Harris 1984). Given the widespread regulation of rivers in many regions of the world, it is important that the lifecycles of migratory fishes
are better understood. Otolith chemistry is likely to play a key role in providing this new information.

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References


New Zealand Department of Conservation, Wellington.


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Markwitz, A., Grambole, D., Hermann, F., Trompetter, W. J., Dioso, T., et al. (2000). Reliable micro-measurement of strontium is the key to cracking
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