Inland Waters and Catchment Ecology

The effect of barriers on the diadromous migration of *Galaxias brevipinnis* in the Middle River, Kangaroo Island, South Australia

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EXECUTIVE SUMMARY

During 2010, the South Australian Research and Development Institute (SARDI), in conjunction with the SA Department for Water (DfW, now the Department for Environment, Water and Natural Resources) undertook a project to assess the environmental water requirements (EWR) of native fish in Kangaroo Island's Middle River. Two species of native galaxiid (*Galaxias maculatus* and *Galaxias brevipinnis*) were found to inhabit the freshwater reaches of Middle River; both with diadromous life histories that require timely oceanic connectivity and therefore specific Environmental Water Requirements (EWRs). The continuum of flow along Middle River is interrupted by two significant barriers; the natural Strepera Falls and, further upstream, the Middle River reservoir. A third species, the exotic rainbow trout (*Oncorhynchus mykiss*) was found in the reservoir and small sections of stream immediately upstream of the reservoir.

The common jollytail (*Galaxias maculatus*) occurred only below Strepera Falls (with uninterrupted oceanic access) suggesting that Strepera Falls presents an impassable barrier to upstream migration for this species. Climbing galaxias (*Galaxias brevipinnis*), however, were distributed widely throughout the Middle River catchment above and below these two barriers. Whilst specific flow bands were developed to support the migration for these species between freshwater, estuarine and marine environments (conducive to seasonal requirements), it remained unclear whether Strepera falls or the Middle River reservoir prevented the successful upstream and downstream migration of these fish. The aim of this study was to assess the extent to which Strepera Falls and the Middle River dam were blocking the inland migration of *G. brevipinnis*.

Otoliths were removed from specimens sampled from the Middle River catchment focussing on three sites along Middle River (below Strepera Falls, between the falls and Middle River reservoir and upstream of the reservoir) with additional samples taken from adjacent coastal catchments on Kangaroo Island. The assessment of otolith microchemical profiles was undertaken using laser ablation inductively coupled mass spectrometry (LA-ICPMS) at Adelaide Microscopy during October 2010.

Otolith profiling results indicated that Strepera Falls did not represent a significant barrier to upstream migrating *G. brevipinnis* under suitable flow conditions (as observed during the field study period). In contrast, there was no evidence of successful inland migration above the Middle River reservoir. It is therefore suggested that the reservoir represents an impassable

barrier for *G. brevipinnis* attempting upstream migration, thereby isolating upper catchment populations irrespective of flow provision.

Results also indicate that spawning habitats previously identified above the reservoir supported a landlocked population of *G. brevipinnis*. This landlocked population may continue to act as a source of recruits for populations downstream if non-lethal downstream passage of larvae is facilitated through the delivery of adequately timed environmental water provisions (EWPs). However, due to the presence of the Middle River reservoir, upstream populations cannot receive diadromous recruits from downstream reaches.

The restoration of fish passage to the weir wall at the Middle River reservoir is recommended to re-connect upstream and downstream populations of *G. brevipinnis.* Restoration of fish passage will optimise the benefit to population sustainability provided under EWPs to ensure that further isolation does not develop and optimise the sustainability of the species throughout the Middle River Catchment. The design and installation of a fishway, commensurate with the migratory requirements of juvenile *G. brevipinnis* should be considered for this site.

Large populations of predatory *O. mykiss* in the reservoir present another threat to the successful migration of *G. brevipinnis* to and from the upper reaches of the Middle River and control of this species may be required to facilitate safe passage for diadromous *G. brevipinnis* recruits.

1. INTRODUCTION

During 2010 the South Australian Research and Development Institute (SARDI), in conjunction with the South Australian Department for Water (DfW now the Department for Environment, Water and Natural Resources) undertook a project to assess the environmental water requirements (EWR) of native fish in Kangaroo Island's Middle River (McNeil and Fredberg 2011). Three fish species were identified; the exotic predator *Oncorhynchus mykiss* (rainbow trout) and two species of native galaxiid (*Galaxias maculatus* and *Galaxias brevipinnis*). Both species of galaxiid are known to undertake diadromous migrations (Rowe *et al*. 1992).

Diadromy involves a migration between fresh water and saltwater. Though migratory patterns vary in direction, frequency and timing, all utilise and traverse a combination of marine, estuarine and freshwater environments and rely on connectivity (McDowall 1999). For diadromous fishes, barriers to migration may be caused by physical structures (Katano *et al*. 2006) or high densities of predators (Fraser *et al*. 1995) and can also be affected by water velocity (Haro *et al*. 2004), or temperature (Powers and Osbourne 1985). All types of barriers provide very real threats to the persistence of populations. For populations of diadromous fish, barriers may result in reduced abundance or localised extinction (Gehrke *et al*. 2002), and for individuals, delay or cessation of migratory passage can result in gonad reabsorption (Bednarek 2001) or death (Powers and Osbourne 1985). This effect may be particularly harmful to italoparous fishes that die after a single spawning event. Whilst some diadromous galaxiids are known to persist in landlocked populations, it is believed that this is a sub-optimal situation (Pollard 1971) resulting in the development of genetic bottlenecks (Neville *et al.* 2006). In some species of galaxiid, permanent inland isolation has led to the evolution of resident galaxiids from a diadromous ancestral state (McDowall 2001).

Middle River is punctuated by two notable barriers, the natural Strepera Falls (7.8 km upstream of the Middle River estuary) and Middle River reservoir 3.4 km further upstream (Figure 1). For two of the three Middle River fish species (*G. maculatus* and *O. mykiss*) the impacts of the two barriers can be assessed through examination of species distribution. Populations of *G. maculatus* are confined to the stretch of river below Strepera Falls (McNeil and Fredberg 2011). This species is unable to climb the falls and therefore cannot proceed upstream of this barrier. Despite this, the species thrives in the reach below the falls, with unimpeded access to estuarine and marine environments to complete their life cycle.

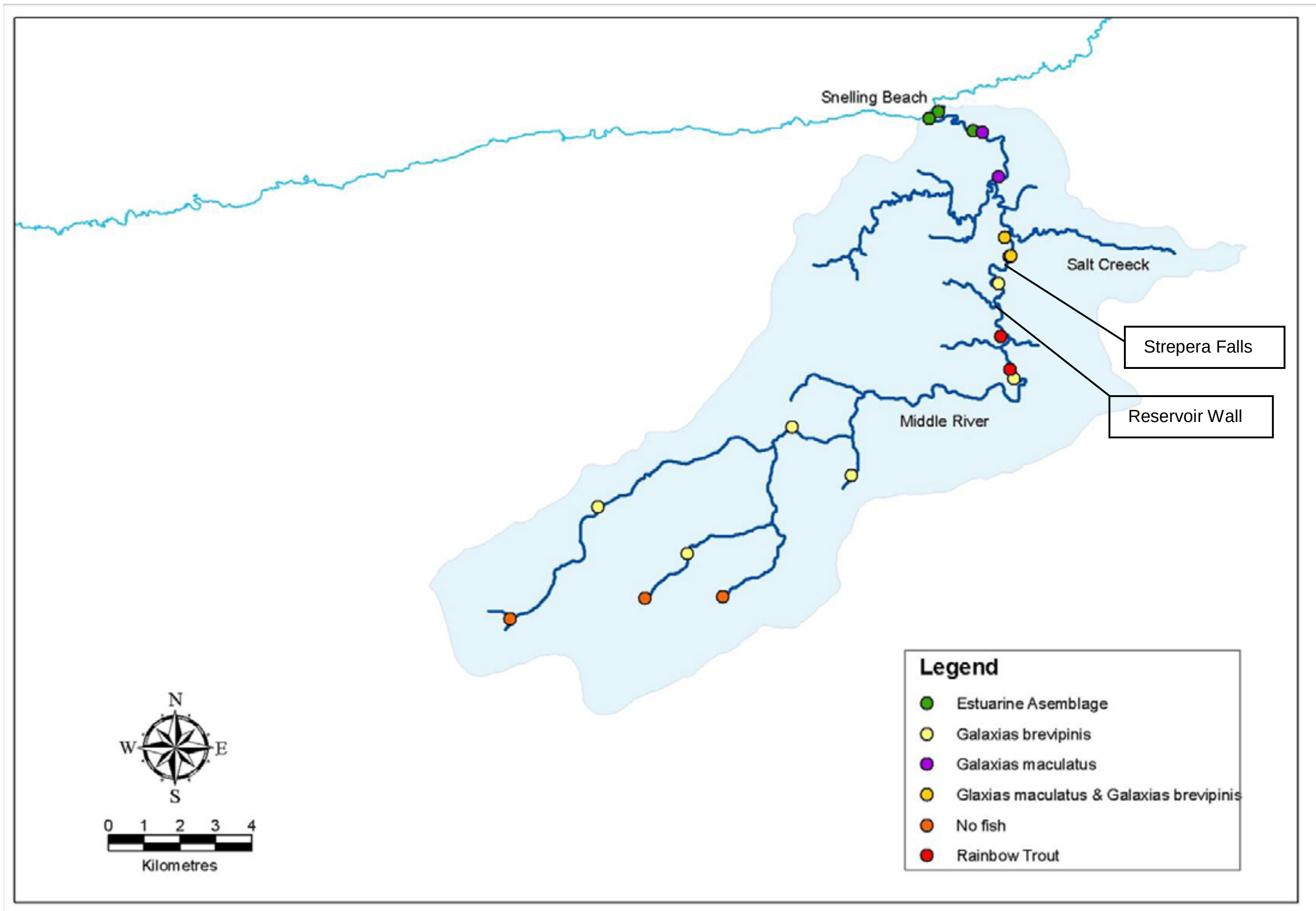

Figure 1. Fish species assemblages in the Middle River catchment during the 2009/10 fish surveys (reproduced from McNeil and Fredberg 2011).

In 2009/10, the exotic predator *O. mykiss* was restricted to the waters within and directly upstream of the reservoir (McNeil and Fredberg 2011); however, one unconfirmed report suggests *O. mykiss* was historically found immediately below the dam (JABSY 2009). This species thrives in the lentic waters of the upper catchment but appears unable to establish self-sustaining populations in the reaches below the reservoir.

The effect of these two barriers on diadromous movements of *G. brevipinnis* are less clear. Populations of *G. brevipinnis* are found above and below both Strepera Falls and the reservoir, and display polymodal length distributions within each reach. Given their distribution throughout the study area, a propensity to ascend into upper reaches of steep coastal catchments and a remarkable talent for climbing steep rocky stream sections using their broad fins to provide suction to the rock face (O'Connor and Koehn 1998), movement patterns cannot be inferred from population structure alone and other techniques must be used.

Attempts to trace the movements of small fishes are often hampered by their small size, which limits the use of traditional tagging methods such as radio and acoustic telemetry and passive inductive transponder (PIT) tagging. In addition to their small size they may be nocturnal and have highly seasonal movement patterns or cryptic life stages which may lead to unrepresentative and unreliable conclusions (Elsdon *et al*. 2008). A potential solution to characterising the movement of small fishes lies in the chemical composition of their otoliths which can allow diadromous life cycles to be accurately tracked (Campana 1999; Elsdon and Gillanders 2005a; Elsdon *et al*. 2008).

Otoliths are the inner earstones of fishes. Calcium carbonate, typically in the form of aragonite, is accreted on a daily basis onto the external surfaces of otoliths, creating a permanent record of growth (Pannella 1971). When sectioned through the core, the morphology of growth increments may be used to infer timing of hatch (Islam *et al*. 2009) and settlement (Wilson and McCormick 1999), whilst also providing insights into annual and/or seasonal growth trajectories (Berra and Aday 2004). In addition to the accretion of calcium (Ca), concentrations of trace elements like strontium (Sr) and barium (Ba) are substituted for Ca within the aragonite matrix. Otolith concentrations of Sr:Ca scale linearly with ambient Sr:Ca in the water (Bath *et al*. 2000; Kraus and Secor 2004). Oceanic Sr concentration and Sr:Ca ratios, are spatially and temporally quite stable across the world's oceans (Bowen 1956), and in general is substantially higher than that found in freshwater, though in some rare instances riverine Sr:Ca can exceed oceanic values (Kraus and Secor 2004). By measuring Sr:Ca concentrations from an otolith core to its edge, a profile is created that reflects the relative water Sr:Ca concentrations experienced throughout that fish's life. Inference on diadromous movements based solely on otolith Sr:Ca data must be treated cautiously, however, as ambient Sr:Ca (as well as Ba and Ca) have been shown to vary seasonally, weekly, daily and between tidal cycles within estuaries (Elsdon and Gillanders 2006), and Sr uptake rates may be species-specific and depend on temperature, salinity, diet, ontogenetic stage and the interactions among these variables (Elsdon and Gillanders 2004; Martin *et al*. 2004; Martin and Wuenschel 2006; Walther *et al*. 2010). Furthermore substantial time-lags from element exposure to otolith incorporation have been reported for several species (Elsdon and Gillanders 2005b; Lowe *et al*. 2009; Macdonald and Crook 2010; Engstedt *et al*. 2012) which further complicates interpretations of environmental reconstructions based on Sr:Ca data alone. This is particularly true of species that undergo rapid movements across narrow environmental gradients.

Oceanic Ba concentrations are also relatively constant, and are consistently lower than those seen in freshwater (Li and Chan 1979). Like Sr, Ba can replace Ca in the aragonitic otolith matrix, often in predictable relationships with ambient water concentrations and environmental variables (Wells *et al*. 2003; Dorval *et al*. 2007; Reis-Santos *et al.* 2013). Ba is also more bioavailable in freshwater than saltwater (Turner *et al*. 1981), resulting in marked disparity between oceanic and freshwater uptake rates into otoliths (Miller 2011). Hence, Ba:Ca may provide additional inference for examining estuarine residency times and diadromous movements (Bath *et al*. 2000; Elsdon and Gillanders 2005a), although hypersaline waters may complicate these patterns (Gillanders and Munro. 2009).

Given species-specificity in trace element uptake rates (Zimmerman *et al*. 2005; Martin and Wuenschel 2006), laboratory or field-based validation is warranted to determine elemental incorporation from surrounding water to the otolith for the species of interest (Hicks et al. 2010). However, some fishes within the same genus may display sufficiently similar elemental uptake rates to allow some generalisations to be made about diadromous movements (Hicks *et al*. 2010; Jessop *et al.* 2012; Zimmerman 2005). For example, within the Galaxiidae, otolith Sr:Ca and Ba:Ca were found to be useful in discriminating between when fish moved from freshwater to marine environments for *G. maculatus* and *G. argentus* (Hicks *et al.* 2010), with partition coefficients for a number of elements including Li, Mg, Sr and Ba being very similar for the two species across a salinity gradient encompassing 2 - 34‰. Moreover, simultaneous use of multiple trace elements and/or isotopes (e.g. δ^{13}C, δ^{18}O, Sr^{87}:Sr^{86}) that reflect the often large environmental gradients experienced by diadromous fishes has been shown to accurately trace movement histories of wild-caught fish between marine and freshwaters where validation studies were not conducted or not possible (e.g. Crook *et al.* 2006; Kerr *et al*. 2007; Milton *et al*. 2008; Tabouret *et al*. 2010).

In this study, otolith Sr:Ca and Ba:Ca profiles were used to explore the life history variability in coastal populations of *G. brevipinnis* by examining populations above and below natural and artificial barriers in Middle River. The aim was to determine what proportion of fish were diadromous and what proportion were freshwater residents within each population and to determine the impacts of each barrier on migration patterns in those populations.

2. METHODS

2.1. Study area

Middle River is situated on the north coast of Kangaroo Island, approximately 50 km West of Kingscote, South Australia. The Middle River is punctuated by two notable barriers, the natural Strepera Falls and artificial Middle River reservoir (Figure 1). Strepera Falls is approximately 7.8 km upstream of the estuary mouth. It is a steep waterfall with a number of near vertical drops (Figure 2) punctuated by small pools that may provide resting places for ascending galaxiids. Wet sections are present for much of the year, providing surfactant potential and biofilm growth which may assist with climbing.

Figure 2. Steeper sections of the Strepera falls that may provide a barrier to fish migration for all but the most exceptional climbers (such as *G. brevipinnis*).

The Middle River reservoir (Figure 3) lies a further 2.4 km upstream of Strepera Falls. It has a capacity of 540 ML and constitutes Kangaroo Island's only public water supply. The water level in the reservoir drops by several metres over the summer months resulting in autumn flows being captured by the reservoir rather than continuing downstream to replenish pools. The reservoir functions in a fill and spill capacity with over-dam flow occurring in winter every year since the dam's construction in 1968. The spill usually commences in early to mid-winter and continues into early spring but is considerably reduced during drought years (SA Water 2009).

Figure 3. Middle River reservoir at high water levels during 2009. Picture coutesy of Kumar Savadamuthu (DfW)

The spillway (Figure 4) presents a near vertical barrier that is likely to obstruct fish passage both upstream and downstream. It contains two sections, the first with a drop of ~1 m in height from the wall onto a concrete spillway apron (Figure 5a) and the second section consists of a further drop of ~0.6 m from the apron onto the bedrock of the spillway channel (Figure 5b). During low reservoir levels, the upstream side of the spillway wall presents a complete barrier to upstream and downstream passage with a drop of ~2 m from the spillway wall to the reservoir (Figure 6).

Figure 4. Panorama of the spillway at Middle River reservoir during full reservoir levels. Picture courtesy of Kumar Savadamuthu (DfW)

Figure 5. a) Spillway wall step barrier onto concrete apron and b) from apron onto bedrock channel. Picture coutesy of Kumar Savadamuthu (DfW)

Figure 6. Weir wall at Middle River reservoir viewed from upstream reservoir area. Any restorative works would need to cater for fish passage under lowered reservoir levels such as these that occurred during the *G. brevipinnis* spawning period in 2010.

2.1. Study species

The climbing galaxias (*G. brevipinnis*) is endemic to New Zealand and south eastern Australia and is Australia's largest galaxiid (Allen *et al*. 2003). Renowned for its ability to traverse near vertical wet surfaces, this species is often able to colonise habitats in upper catchments that are inaccessible to other species. Observations show *G. brevipinnis* spawns in the Middle River during autumn (McNeil and Fredberg 2011) which is consistent with observation from other parts of southern Australia (McNeil and Hammer 2007) and New Zealand (McDowall and Fulton

1996; Allibone and Caskey 2000). During autumn, banks adjacent to resident areas become inundated. Eggs are laid in aggregations deposited on vegetation and in interstitial spaces in substrates which are exposed as the waters recede. Subsequent high flows stimulate hatch, carrying larvae downstream (O'Connor and Koehn 1998). Following a period of marine residency, juveniles migrate upstream during late spring flows to settle (Mathews *et al.* 2005). Anthropogenic barriers to migration are now common across their range (Koehn and O'Conner 1990) placing diadromy under increasing pressure. This species has also been recorded existing in self-sustaining, landlocked populations with all life stages completed in freshwater (McDowall and Fulton 1996).

2.1. Site selection and sampling

A total of 73 *G. brevipinnis* were collected for study. During December 2009, the Middle River was sampled at three sites; below Strepera Falls (*n* = 14), below reservoir (*n* = 7), and above reservoir (*n* = 9). Three control rivers were also sampled at this time; the Lower Rocky River (*n* = 9), Western River (South Branch) (*n* = 11) and North West River (*n* = 10). A second sample was collected from the Middle River below Strepera Falls during June 2010 (*n* = 13).

Table 1. Study fish were collected from four catchments on Kangaroo Island.

Site	Catchment	Date Sampled	Distance from the Ocean(km)	(*n*)
Below Strepera Falls (Dec)	Middle River	Dec-09	7.8	14
Below Strepera Falls (Jun)	Middle River	Jun-10	7.8	13
Below Reservoir	Middle River	Jun-10	11.4	7
Above Reservoir	Middle River	Jun-10	13.4	9
Lower Rocky River	Breakneck River	Jun-10	3.5	9
South Branch	Western River	Jun-10	13.8	11
North West River	Stun'Sail Boom River	Jun-10	33.2	10

In each of the three control rivers (Western, North West and Rocky rivers) a single 'control' site was selected. Control sites were located such that no significant barriers to fish movement occurred between the sampling location and the ocean. Each control site was selected to be a similar distance from the ocean to a significant reach of the Middle River. In this way 'Lower

Rocky River' control site represents a near-coastal situation which is comparable to the reach below Strepera Falls; 'South Branch' is a similar distance inland to the Middle River reservoir and 'North West River' is comparable to the uppermost reaches of the Middle River catchment (upstream of the reservoir).

Fish were collected using fyke nets (3 m leader, 2 m funnel, 3 mm mesh) and box-style bait traps. Individuals were identified to species and target fish were euthanised in an ice slurry prior to being placed into 95% ethanol solution.

2.1. Otolith preparation

Fish were measured (total length in mm) then their sagittal otoliths were removed, washed thoroughly in ultrapure water and allowed to air dry in eppendorf tubes. Each of the 29 fish collected from the Middle River during June sampling were further dissected and gonadal somatic index calculated. One sagittal otolith from each fish was prepared for analysis by embedding in EpoFix™ (Struers) resin spiked with indium at a concentration of 30 ppm. A low-speed diamond saw was used to transversely section each otolith to a thickness of 500 µm encompassing the otolith core. The saw was lubricated with ultrapure water during sectioning. Otolith sections were carefully polished to a depth of 200 µm using 50, 20 and 3 µm lapping film, exposing the core. Polished sections were mounted onto glass slides using crystal bond, spiked with indium at a concentration of 200 ppm. Indium spiking allows easy identification of resin during laser analysis. Each slide, containing between fifteen and twenty otolith sections, was cleaned and sonicated in ultrapure water, then dried in a laminar flow cabinet.

2.2. Chemical analysis

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was used to analyse a longitudinal chemical profile across the length of each sectioned otolith, passing through the core. Instrument settings are detailed in Table 2. Prior to commencement, two 320 second calibration transects were taken each day from a Microanalytical Carbonate Standard Block 3 (MACS-3).

Each otolith analysis was preceded by thirty seconds of ambient measurements, to allow calculation of background element counts. Profiles across the entire otolith were carried out by continuously moving the laser across the surface of the otolith at a speed of 3 µm/s continuing into the indium-spiked resin for a further 30 seconds. Ablations occurred inside a sealed, argon-filled chamber and gases were extracted to the Agilent 7500CS mass spectrometer via a smoothing manifold. The ablation chamber was purged with argon gas for 30 seconds between each profile to remove traces of background gases (Berra and Aday 2004). Otolith profiles were conducted in blocks of ten with two NIST (National Institute of Standards and Technology) 612 transects conducted before and after each block and prior to commencement of new slides.

Table 2. Details of Laser ablation inductively coupled mass spectrometer (LA-ICPMS) operating parameters during otolith profile analysis.

Parameter	**Description**
Laser	
Model	Newave UP-213
Wavelength	213nm
Mode	Q-switched
Frequency	5 Hz
Spot size	30µm
Laser Power	70%
Beam energy	0.1-0.15mJ
Laser scan rate	3um.s^{-1}
Carrier Gas	Ar (0.9 L.min^{-1})
ICP-MS	
Model	Agilent 7500CS
Optional gas	He (55-60%)
Cone	Pt
Dwell times	^{44}Ca and ^{115}In (50ms)
	^{43}Ca (100ms)
	^{24}Mg, ^{55}Mn, ^{88}Sr and ^{138}Ba (200ms)
Detection modes	Pulse and Analogue

2.3. Statistical analysis

The GLITTER software package (www.glitter-gemoc.com) was used to visualise profiles and to identify the start and completion of each otolith analysis. Profiles were observed for a palendromic centrepoint signifying the otolith core. NIST612 standards were used to observe machine drift across each day of sampling (Bath *et al*. 2000). From this, regression analysis was used to provide individual time-corrected calibration for each otolith profile. Data reduction was carried out using spreadsheets to remove background counts and produce eleven point running mean and running medians for each of the seven elements measured. Profiles were time corrected using the NIST612 drift curve calculated for the day of sampling. Once smoothed, otolith profiles were standardised to Ca43 (as the internal standard) (Kraus and Secor 2004) and visualised. Standardised Mn:Ca profiles were used to check the position of the otolith core as core Mn is known to be elevated in a wide range of marine and freshwater species (Bowen 1956).

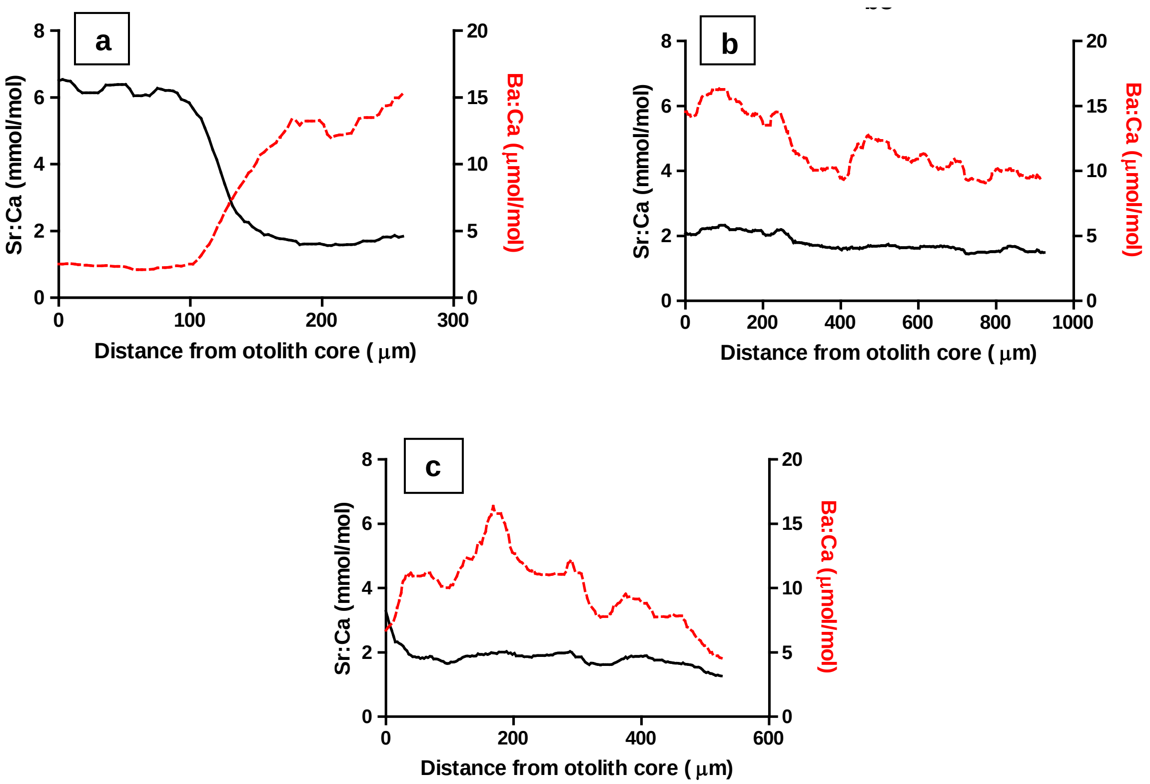

Figure 7. Sr:Ca and Ba:Ca plots displaying; a) a migratory morph with an oceanic chemical signature at the otolith core, b) a freshwater resident, and c) an unclear otolith that cannot be unabiguously interpreted.

2.1. Classifying migratory history

Normalised Sr:Ca and Ba:Ca profiles were visualised graphically (Appendices A - G) and examined for unambiguous diadromous or freshwater resident signatures (Figure 6a and b) then assigned either a ‘migratory’ (Figure 7a) or ‘resident’ (Figure 7b) morph. Individuals assigned as ‘migratory’ displayed profiles consistent with a period of juvenile ocean residence

(Sr:Ca ≥ 4 mmol/mol (Hicks *et al*. 2010), Ba:Ca < 2 µmol/mol near the otolith core) and a subsequent return to freshwater (Sr:Ca ≡ 2 mmol/mol, Ba:Ca > 10 µmol/mol) (Figure 7a). ‘Resident’ individuals showed no evidence of ocean residence and had remained in freshwater throughout their lifetime (Sr:Ca ≡ 2 mmol/mol, Ba:Ca > 10 µmol/mol through the entire profile) (Figure 7b). Profiles with any level of ambiguity were assigned as ‘unclear’ (Figure 7c). These individuals displayed trends in Sr:Ca and Ba:Ca that were difficult to interpret, with no clear evidence of ocean or freshwater residence.

3. RESULTS

3.1. Middle River

The reach below Strepera Falls was sampled on two occasions, the first in December 2009 (*n* = 14) and the second in June 2010 (*n* = 13) (Table 2). The proportion of migratory morphs varied between the two dates. In December 2009, 87% of the fish collected had undergone a juvenile period of oceanic residence before undertaking a diadromous migration into freshwater whereas in June 2010 only 54% of the fish were found to be migrants (Figure 8, Appendix A and B).

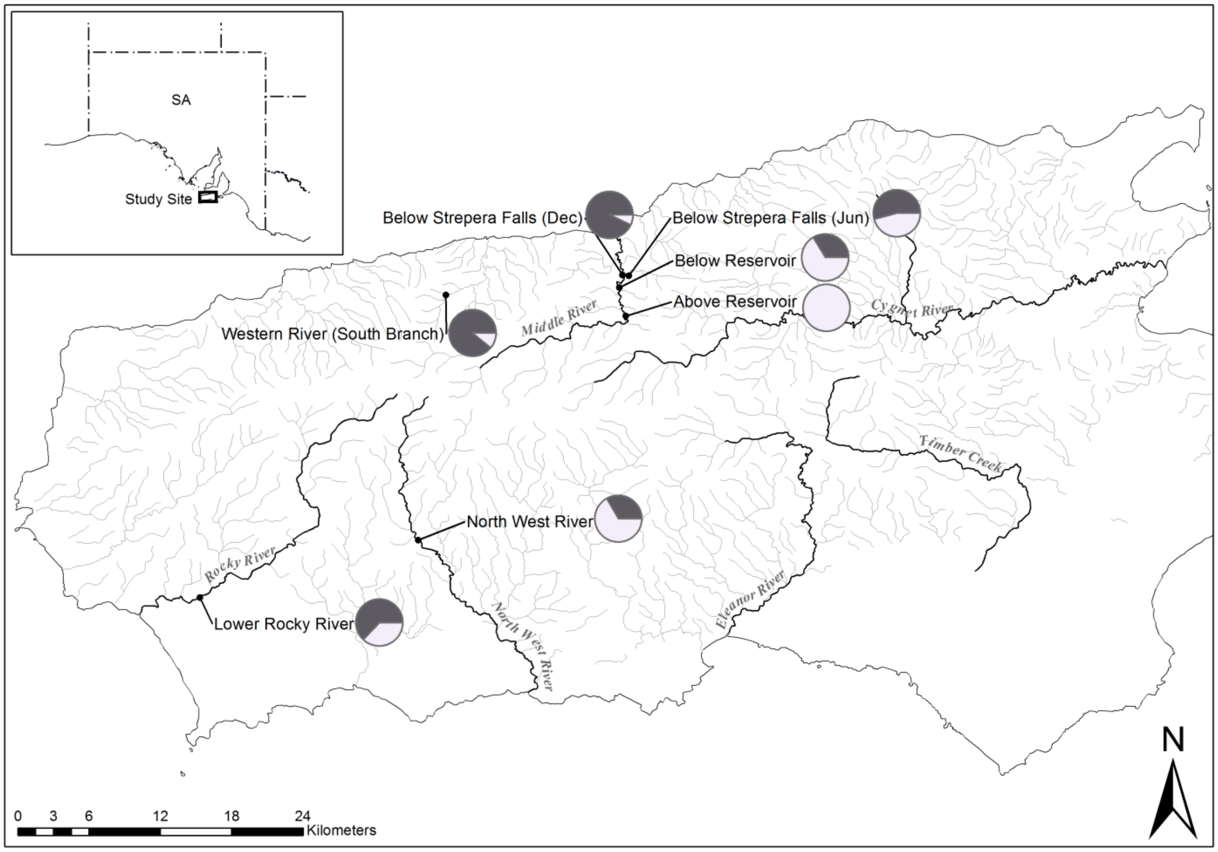

Figure 8. Bars represent the proportion of Migrant (black) and Resident (grey) *Galaxias brevipinnis* observed at each Kangaroo Island study site. The only site that contained purely resident morphs was above the Middle River reservoir.

The otolith profiles of seven fish collected in June 2010 were analysed from the site between Strepera Falls and Middle River reservoir (Below Reservoir) (Figure 8). The majority of these fish (57%) remained resident within the stream, however, 29% of the fish had undergone migration between saltwater and freshwater environments (Table 3, Appendix C). One of the eight fish displayed an intermediate profile and was assigned an “unclear” morph.

Above the Middle River reservoir nine fish, collected in June 2009 were examined and 100% of these fish displayed a freshwater resident profile (Table 3, Figure 8, Appendix D).

Table 3. A total of 73 otolith profiles were measured from seven sampling events spread across four Kangaroo Island rivers. Five profiles were assigned an unclear morph.

Site	(*n*)	Length Range(mm)	Mean Length (mm)	Migratory (%)	Resident (%)	Unclear (%)
Below Strepera Falls (Dec)	14	39 - 117	61	93	7	0
Below Strepera Falls (Jun)	13	53 - 103	76	54	46	0
Below Reservoir	7	142 - 198	166	29	57	14
Above Reservoir	9	111 - 150	135	0	100	0
Lower Rocky River	9	83 - 180	123	56	33	11
Western River (South Branch)	11	45 - 150	91	73	9	18
North West River	10	79 - 160	130	30	60	10

3.2. Control rivers

Nine otolith profiles were examined from the lower Rocky River site (3.5 km inland). The majority of fish examined displayed a migratory morph (56%), while 33% of fish examined were freshwater residents (Figure 8, Appendix E). One profile was considered unclear (Table 3). Further inland (13.8 km) and adjacent to the Middle River, the Western River’s South Branch displayed one of the highest proportions of diadromous fish (73%). Of the 11 individuals observed, only one was a freshwater resident, however two profiles were assigned as unclear (Figure 8, Appendix F). The furthest inland site examined, North West River, contained 30% diadromous morphs, despite being 33.2 km from the ocean. Of the remaining fish, 60% were freshwater residents and one individual was unclear (Figure 8, Appendix G).

4. DISCUSSION

The highest migratory proportions of *G. brevipinnis* were observed in December 2009 below Strepera Falls. At this time, 93% of the fish collected had undergone a period of juvenile oceanic residence. In Victorian rivers, *G. brevipinnis* commence upstream migration predominantly during spring (O'Connor and Koehn 1998) but may enter rivers from September to December (Jung *et al.* 2009). This pattern doesn’t differ between rivers despite their variable properties (Moser *et al.* 1991). Diadromous *G. brevipinnis* cohorts travel upstream at a speed of 100–400m.day^{-1} during estuarine transition (Jung *et al.* 2009). At these speeds, a cohort entering Middle River estuary would reach Strepera Falls approximately 30 days after entering the estuary, and as migration occurs between September and December, diadromous juveniles would have likely been accumulating below Strepera Falls during December sampling. The presence of these trends in the Middle River was strongly supported by otolith profile analysis which confirmed that 93% of the fish present were migrants and was further reflected in the small mean length (61 mm) of these fish.

By June 2010, the proportion of diadromous individuals below the falls had dropped to 54%. This value is a more reasonable representation of steady state proportions and matches almost precisely the lowest control site on the Rocky River (56%). The loss of diadromous individuals from immediately below Strepera Falls reflects losses through upstream and downstream emigration and mortality, including that related to unsuccessful passage of fish through the falls (Powers and Osbourne 1985).

Above Strepera Falls and below the reservoir, 33% of the population had undertaken diadromy. Evidence of diadromy within this population proves that, under favourable conditions (such as those seen during spring 2009), Strepera Falls is a passable barrier to upstream migrating juvenile *G. brevipinnis*. The difficulty of passage over the falls appears to be significant as migratory proportions at this site were the lowest observed. The only other site with 33% migration was the North West River site which was 21 km further inland.

Above the Middle River reservoir, otolith analysis identified no migratory morphs. This result suggests that no *G. brevipinnis* were able to move upstream past the dam wall during the spring 2009 migratory period. This implies that the dam wall constitutes an impassable barrier to upstream migration under the flow conditions seen between August and December 2009.

4.1. Relevance to catchment and water management

The data suggests that the Middle River reservoir represents a significant barrier to the upstream migration of juvenile *G. brevipinnis*. Furthermore, the data also suggests that Strepera Falls, although a much higher structure than the weir wall, does not present a barrier to diadromy for the *G. brevipinnis* population, although it may restrict upstream movement of a large proportion of individuals within the population. This result highlights the climbing ability of this species when faced with natural migration barriers such as cascades, waterfalls or shallow riffles and stream reaches. Passage may be facilitated by the presence of a wetted rock face, covered with a fine layer of biofilm which provides not only roughness and structure, but also surfactant potential to assist fins with gripping and forming suction against the rock during climbing.

Conversely, the dry and homogenous vertical surface of the reservoir wall may present a surface incommensurate with climbing requirements. As such, restorative interventions are highly recommended to restore fish passage at the reservoir wall. This could be achieved by installing a fishway at the site, to restore passage to the isolated and landlocked populations upstream. At present, *G. brevipinnis* populations in the Middle River have been split into two isolated populations, one obligatory landlocked population and one capable of facultative diadromy. Restoration of fish passage at the reservoir is a simple solution to restore genetic linkage at the metapopulation scale, with likely benefits to the resilience and long term viability of the population. This would reduce their vulnerability to events such as drought, climate change and water extraction.

In the Mount Lofty Ranges where large impassable barriers (such as reservoirs) are now abundant, *G. brevipinnis* populations are almost exclusively restricted to landlocked, upper catchment reaches (McNeil *et al.* 2011a). In recent years only a few juveniles have been caught in the Onkaparinga, Torrens, Gawler or Myponga Rivers between reservoirs and the sea (McNeil and Hammer 2007; McNeil *et al.* 2011b). This pattern matches that observed in other coastal catchments across south eastern Australia such as Tallowa Dam on the Shoalhaven River in New South Wales, where *G. brevipinnis* populations are now only found above the dam wall (Gehrke *et al.* 2002).

It is possible that in these systems, a similar split in *G. brevipinnis* populations occurred following the construction of reservoir walls. The resultant drying of downstream reaches as catchment flows were collected to fill dams, may have resulted in the demise of coastally confined individuals. The impact of this change in life history on landlocked upstream populations is unknown, but it is possible that the relative scarcity of this species in the Mount Lofty Ranges is related to the inability to complete diadromous migrations within the population.

4.2. Importance of fish migration

To clarify the study of fish migration the terms obligate and facultative have been applied from the study of entomology and ornithology (Dingle 1996; Holland *et al.* 2006; Terrill and Able 1988). They are applied to populations to describe if migration always occurs (obligate) or if migration is in response to conditional stimuli (facultative) (Dingle and Drake 2007). The concept of obligate migration is under increasing scrutiny as otolith chemical analysis allows accurate description of migratory proportions to be observed. Both in Australia (Miles 2007; Crook *et al.* 2008; Mathwin 2010) and internationally (Daverat *et al.* 2006; Tsunagawa and Arai 2008) otolith studies to examine migration are increasingly finding that diadromous fish display a range of contrasting migratory phenotypes and that obligate migration is less common than previously thought.

Below Strepera Falls, Middle River flows freely to the ocean. Despite this, *G. brevipinnis* populations collected below Strepera Falls in June 2010 displayed 46% resident morphs. This form of migration, where a proportion of the population remains resident whilst the remainder undergo migration is probably the most common form of migration (Chapman *et al.* 2012) and is termed partial migration (Jahn *et al.* 2004). This study is the first record that regardless of river morphology, *G. brevipinnis* display partial migration. Although the conditional stimuli to migrate are not understood for this species, it is likely that migration in *G. brevipinnis* is a facultative behaviour. The stimuli to migrate are probably a combination of genetic (Snyder 1991) and environmental controls (Wysujack *et al.* 2009).

The majority of diadromy work to date has focussed on species of commercial importance. In northern hemisphere salmonid species, diadromy allows migrants to access oceanic waters with higher productivity (Tsukamoto *et al.* 2002) resulting in greater size on return (Jonsson and Jonsson 1993) and greater reproductive fitness (Elliott 1984; 1988; 1989). In this way diadromous migrants have been shown to provide a disproportionately high genetic contribution to the following generation. Supporting timely passage in partially migratory species not only supports fresh population inputs but is essential to avoid severe genetic bottlenecks in resident populations (Neville *et al.* 2006).

EWPs developed for the Middle River have highlighted the importance of providing flow bands that enable fish to migrate between the ocean and upper catchment reaches. The identification of the reservoir as a barrier to movement highlights the need for complementary works (installation of fish passage) to optimise the benefit of flow provisions to meet migratory environmental water targets. The combined impact of restoring flow regime and restoring fish passage will aid the long term viability of native fish populations in the Middle River catchment.

4.3. Possible limitations of the approach

Rates of element inclusion into otoliths are known to vary with ontogeny, water temperature, diet (Walther *et al*. 2010) and salinity (Hicks *et al*. 2010). Due to potential confounding it is recommended that species-specific validation is undertaken to allow for robust interpretation of otolith microchemistry (Elsdon *et al*. 2008). The completion of manipulated validation experiments was beyond both the budget and timeframe of this study and situational controls were required. The inclusion of landlocked populations in the analysis provided a field-based validation of the freshwater Sr:Ca and Ba:Ca values for the species. We considered that substantial departures from these values along the otolith transect (with reference to the seawater values reported for *G. maculatus* and *G. argentus* by Hicks *et al.* 2010) can be interpreted as time spent in estuarine or marine waters, even though a critical value for estuary/marine residence for *G. brevipinnis* was not determined experimentally.

Without undertaking laboratory experiments that consider the many potential drivers of Sr:Ca and Ba:Ca uptake into otoliths, fine-scale movement patterns within rivers and estuaries are not reliably traced from Sr:Ca and Ba:Ca otolith profiles, and even then interpretation is difficult (Macdonald and Crook 2010). As this study cannot track movements within freshwater, it is unable to determine if Strepera Falls or the Middle River reservoir were acting as barriers to downstream movement. The potential exists for future work to examine variability in natal sections of the otolith core to search for characteristic geographic variation in microchemical signatures, for example looking at Sr^{87}:Sr^{86} isotope ratios of water chemistry in different reaches (Kennedy *et al.* 2000). If these could be determined then complex directional movement patterns could be constructed that would inform both upstream and downstream dispersal patterns within the Middle River.

Determining the capacity for upper Middle River fish populations to emigrate downstream based on the current data is contingent on their ability to survive the vertical drops associated with the reservoir and Strepera Falls. To date, little work has been carried out on downstream migration although one study was able to determine that overshot weirs result in lower rates of larval mortality than undershot weirs (Baumgartner *et al.* 2006). This is encouraging as the reservoir is an overshot structure and galaxiid emigration is most likely to occur during spring when the Middle River reservoir is typically flowing. As rates of downstream emigration are unknown a precautionary approach is advisable. If we assume nonlethal passage is possible then this is best supported with EWPs that ensure late autumn flows over the weir. Survival rates down natural falls are also little quantified but EWPs that support downstream movement past the reservoir will also support downstream passage over Strepera Falls, if it exists.

While the design of this study is unable to quantify the extent to which upper catchment populations act as sources for downstream populations, it is important to note that the installation of structures to support upstream passage is likely to also support downstream passage.

4.4. Rainbow trout

Rainbow trout (*O. mykiss*) exist in large populations through the Middle River reservoir and the tributary streams immediately above it (McNeil and Fredberg 2011). In Middle River *O. mykiss* dominate their range excluding native fishes from these habitats, a trend common across their South Australian range (McNeil *et al.* 2011a). This species has a diet that generally includes small fishes (Allen *et al.* 2003) and is one of the key introduced salmoniids implicated in the global decline of galaxiid fishes (McDowall 2006).

Any passage between the upper and mid-sections of the Middle River must pass both the spillway and the predator dominated reservoir directly above it. For migrating *G. brevipinnis* this includes both downstream movement of larvae or possibly spawning adults during autumn and the subsequent upstream movement of juveniles during spring. In effect, the population of predators in the reservoir forms another ‘biological’ barrier to diadromous migration in the

Middle River. For this reason it is suggested that any installation of a fish ladder be coupled with some level of *O. mykiss* control in the reservoir itself.

5. CONCLUSION

Significantly, this is the first observation that *G. brevipinnis* display distinct migratory and resident morphs irrespective of connectivity. This species is utilising multiple migratory strategies simultaneously and successful management must support both strategies. This will require an EWP that maintains both migratory passage and permanent refugia.

When wet, Strepera Falls is a passable obstacle to the inland migration of *G. brevipinnis* in Middle River. The difficulty of upstream passage is likely to act as a biological filter allowing only small numbers of the fittest individuals to pass. Although the local proportion of migratory morphs may be low, these individuals are likely to contribute disproportionately to the following generation. By supporting this behaviour the fitness of the partially migrating local populations is also supported. This may be done by providing spring flows that are sufficient to maintain a wetted rock wall at Strepera falls.

In contrast, the reservoir is an impassable barrier to inland migration. This isolates fish populations above the reservoir leaving *G. brevipinnis* populations in the upper catchment vulnerable to local extinction and severe genetic bottlenecks (Neville 2006).

The techniques used in this study cannot determine if Strepera Falls or the reservoir are barriers to downstream movement. No study has looked at fish survival rates over natural falls but, if we assume that fish are capable of surviving downstream passage then this behaviour can be supported through EWPs that ensure late autumn flows. Evaluation of this could potentially use Sr isotope studies to characterise river reaches and determine natal origins of *G. brevipinnis* migrants.

Again emigration from above the reservoir remains possible only if emigrants could survive the fall. While downstream movements through weirs can be lethal, mortality relates closely to the type of weir, with undershot weirs causing much higher mortalities than overshot weirs (Baumgartner *et al.* 2006) such as present in Middle River. It is therefore possible that populations above the reservoir are acting as recruitment sources for sites lower in the catchment.

A third ‘biological’ barrier may exist above the reservoir in the form of the introduced predator *O. mykiss* as currently migrant *G. brevipinnis* attempting to pass the reservoir must also pass the *O. mykiss* populations resident therein.

A best practice management strategy for native fishes in the Middle River could be achieved by combining scientifically robust EWPs, the installation of a fish ladder at the Middle River reservoir and implementing invasive fish control measures.

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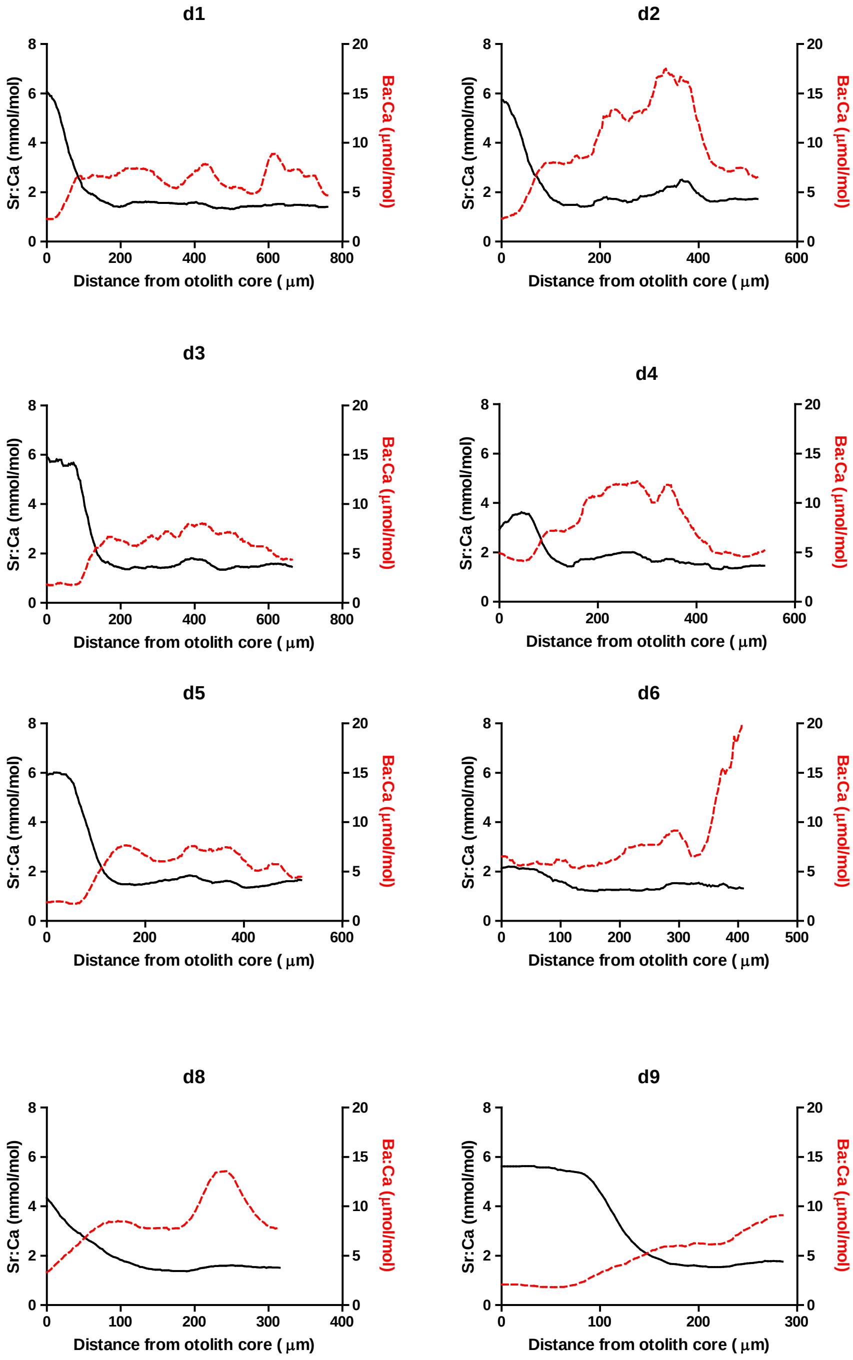
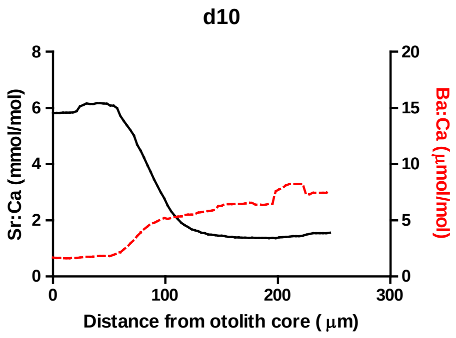
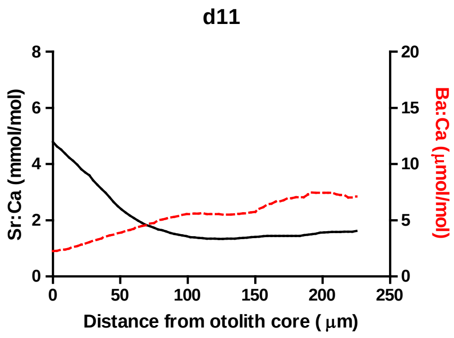
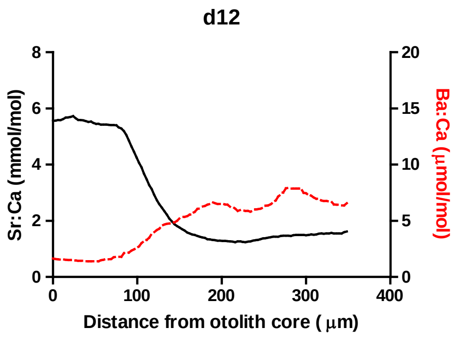
APPENDIX A

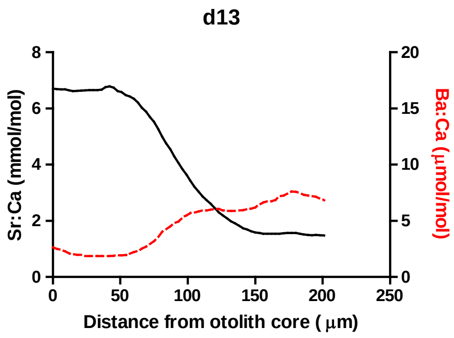
Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 14 fish sampled from below Strepera Falls during December on the Middle River. Plot d6 was assigned a resident morph. The 13 remaining plots were assigned as migrant morphs.

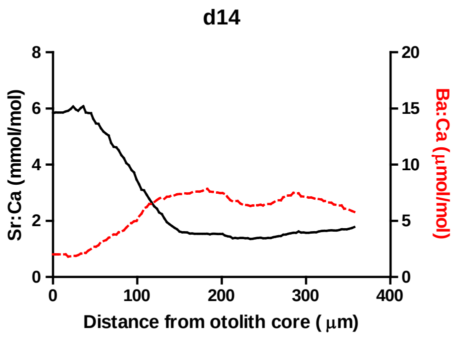


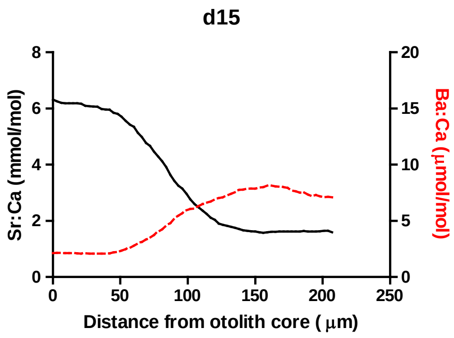

d10
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300


d11
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
50
100
150
200
250


d12
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
400


d13
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
50
100
150
200
250

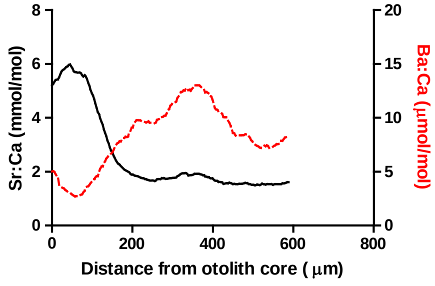

d14
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
400


d15
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
50
100
150
200
250

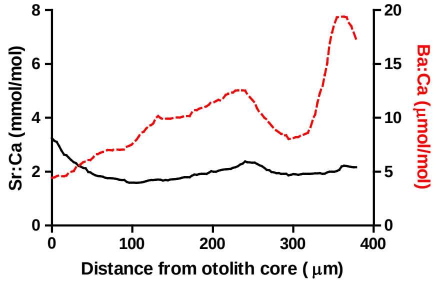
APPENDIX B

Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 13 fish sampled from the Middle River site Below Strepera Falls during June 2010. Plots 7, 8, 11, 12 and 13 were assigned resident morphs. The remaining 7 plots were assigned considered migrant morphs.

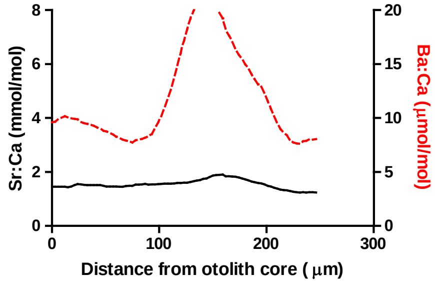
1



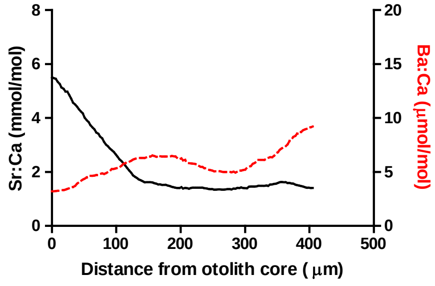
3



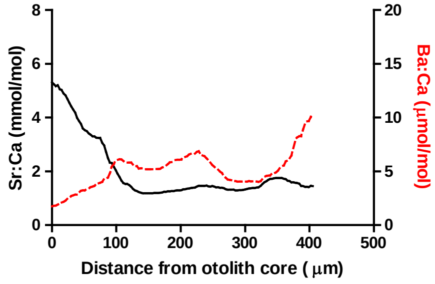
4



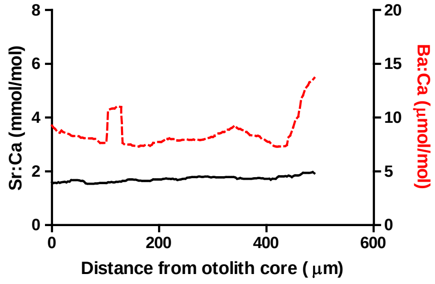
5



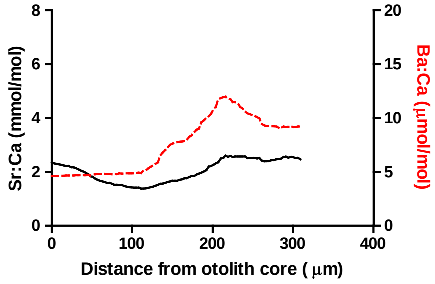
6



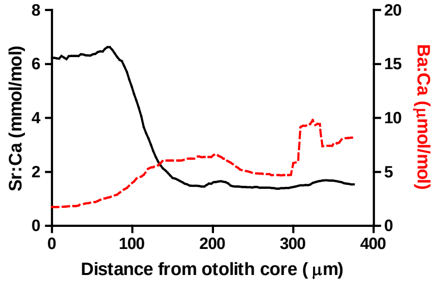
7



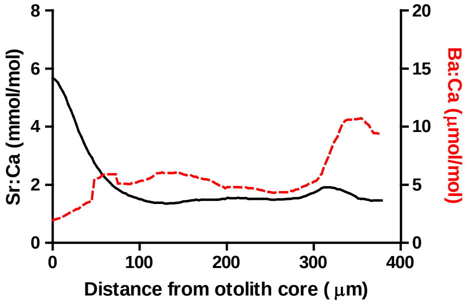
8



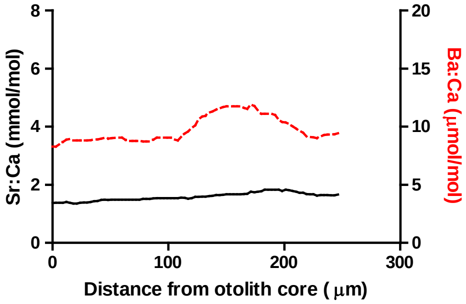
9



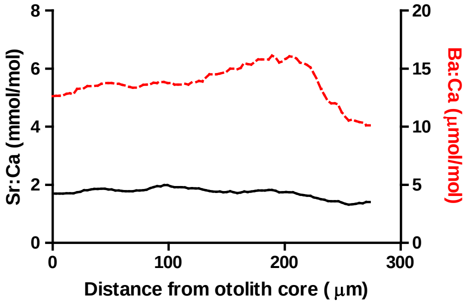
10


Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
400

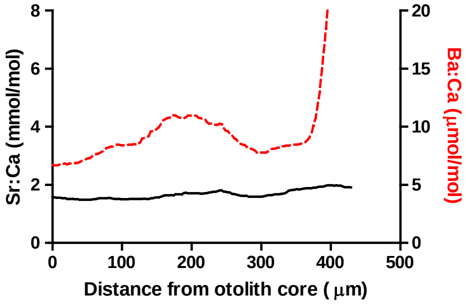
11


Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300

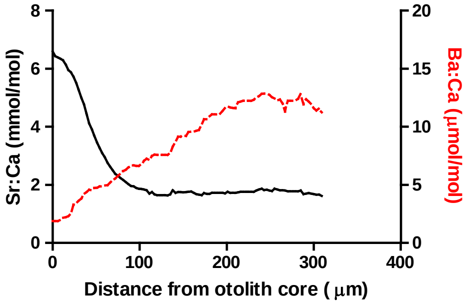
12


Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300

13


Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
400
500

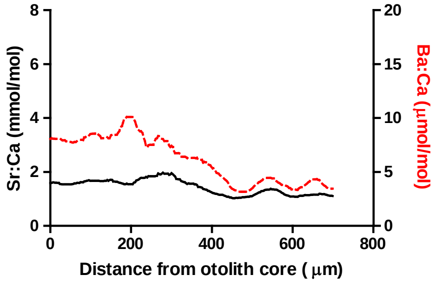
14


Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
Distance from otolith core (µm)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
400

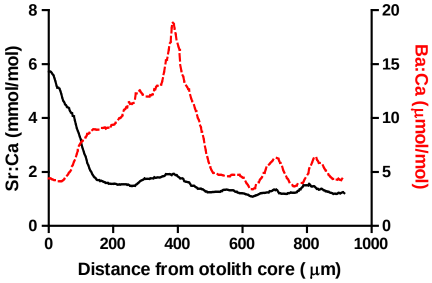
APPENDIX C

Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 7 fish sampled from the Below Reservoir site on Middle River. Plot 18 was assigned an Unclear morph. Plots 16 and 20 were assigned a Migrant morphs and the remaining 4 plots were assigned as Resident morphs.

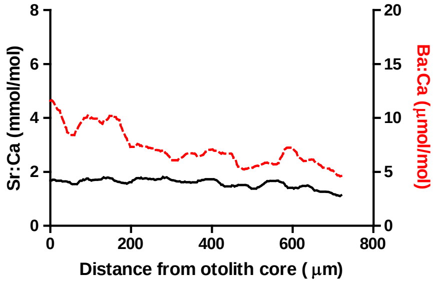
15



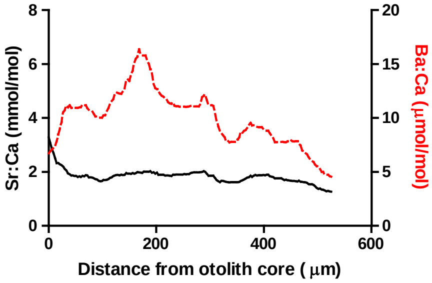
16



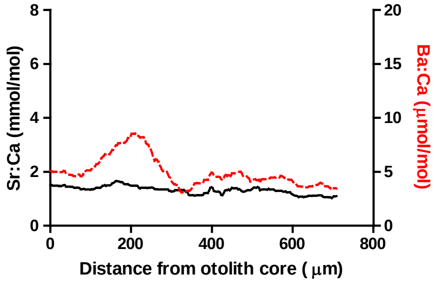
17



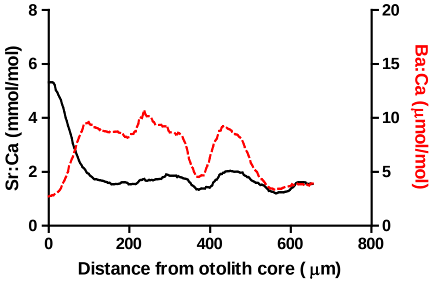
18



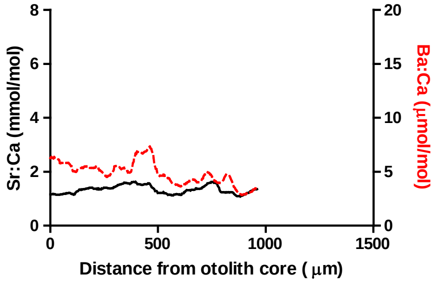
19



20

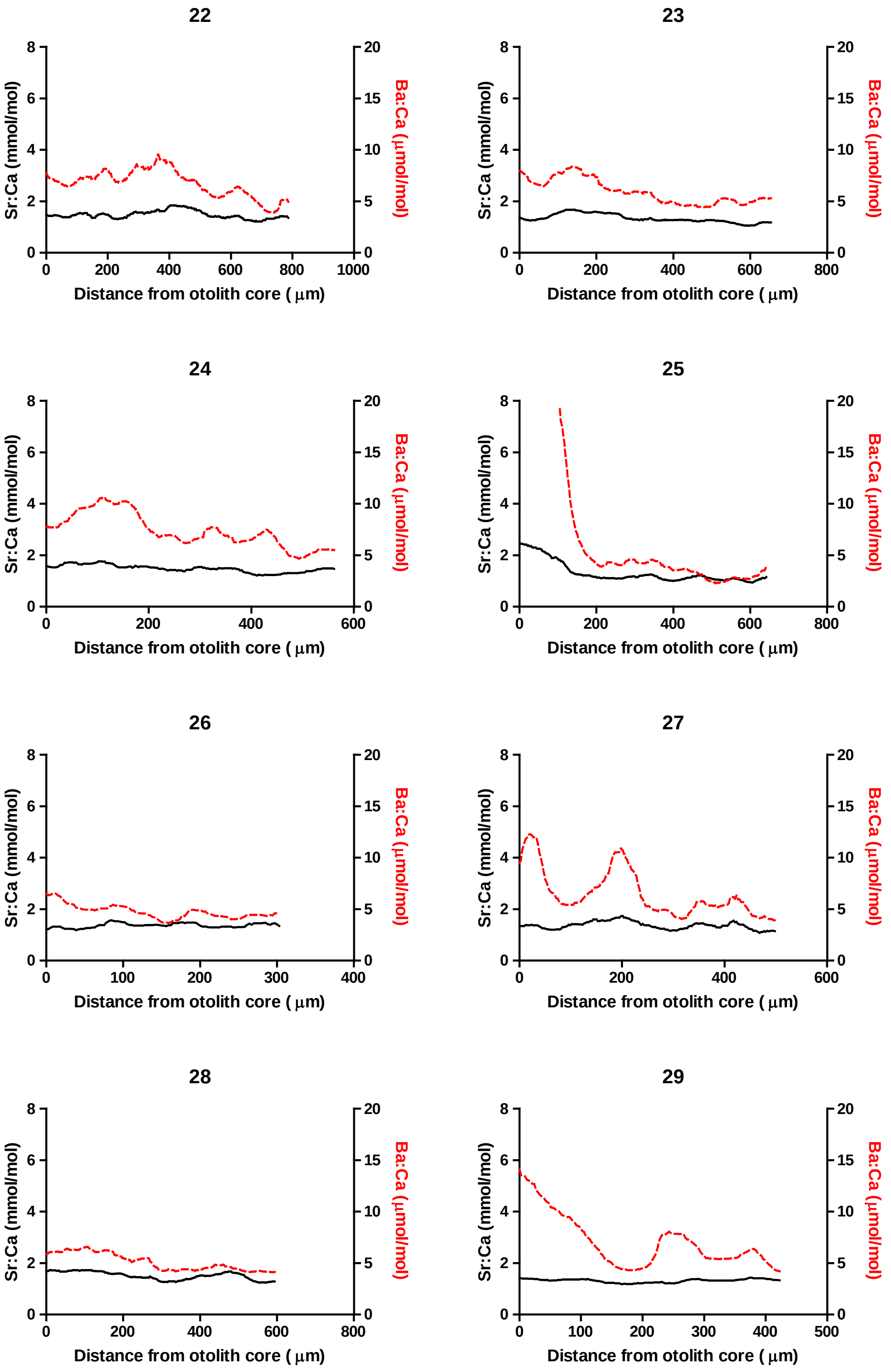


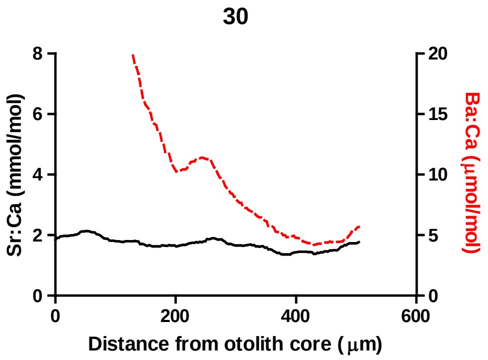
21



APPENDIX D

Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 9 fish sampled from the Above Reservoir site on Middle River. All plots were assigned a Resident morph.

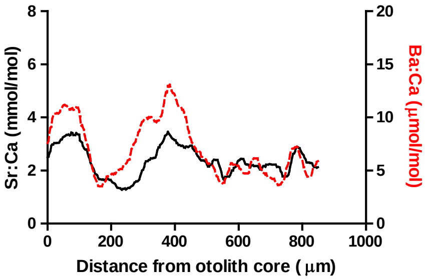



30
Sr:Ca (mmol/mol)
Ba:Ca (μmol/mol)
Distance from otolith core (μm)
0
2
4
6
8
0
5
10
15
20
0
200
400
600

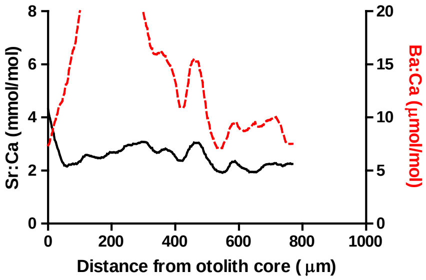
APPENDIX E

Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 9 fish sampled from the Lower Rocky River site. Plot c1 was assigned an unclear morph. c3, c5 and c9 were assigned resident morphs and the remaining 5 plots were assigned as migrant morphs.

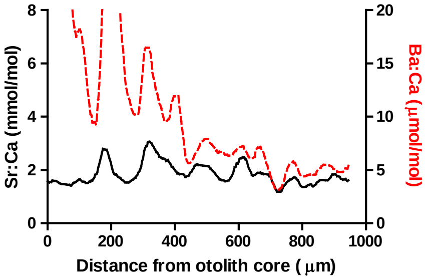
c1



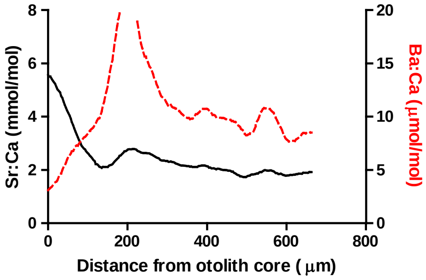
c2



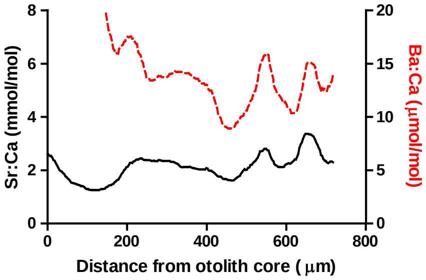
c3



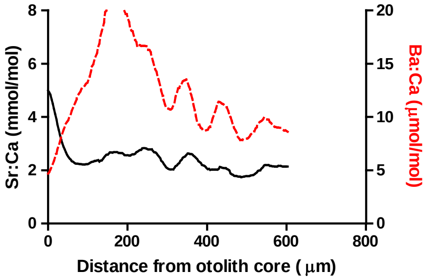
c4



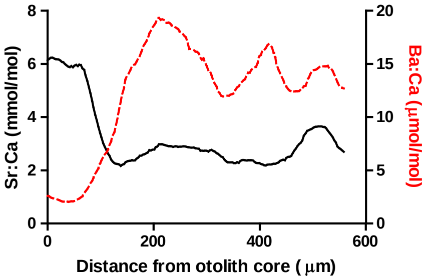
c5



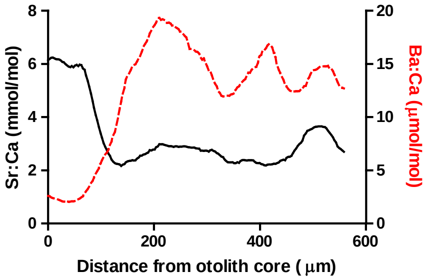
c5

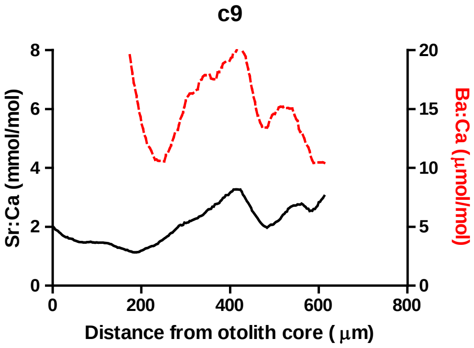


c7



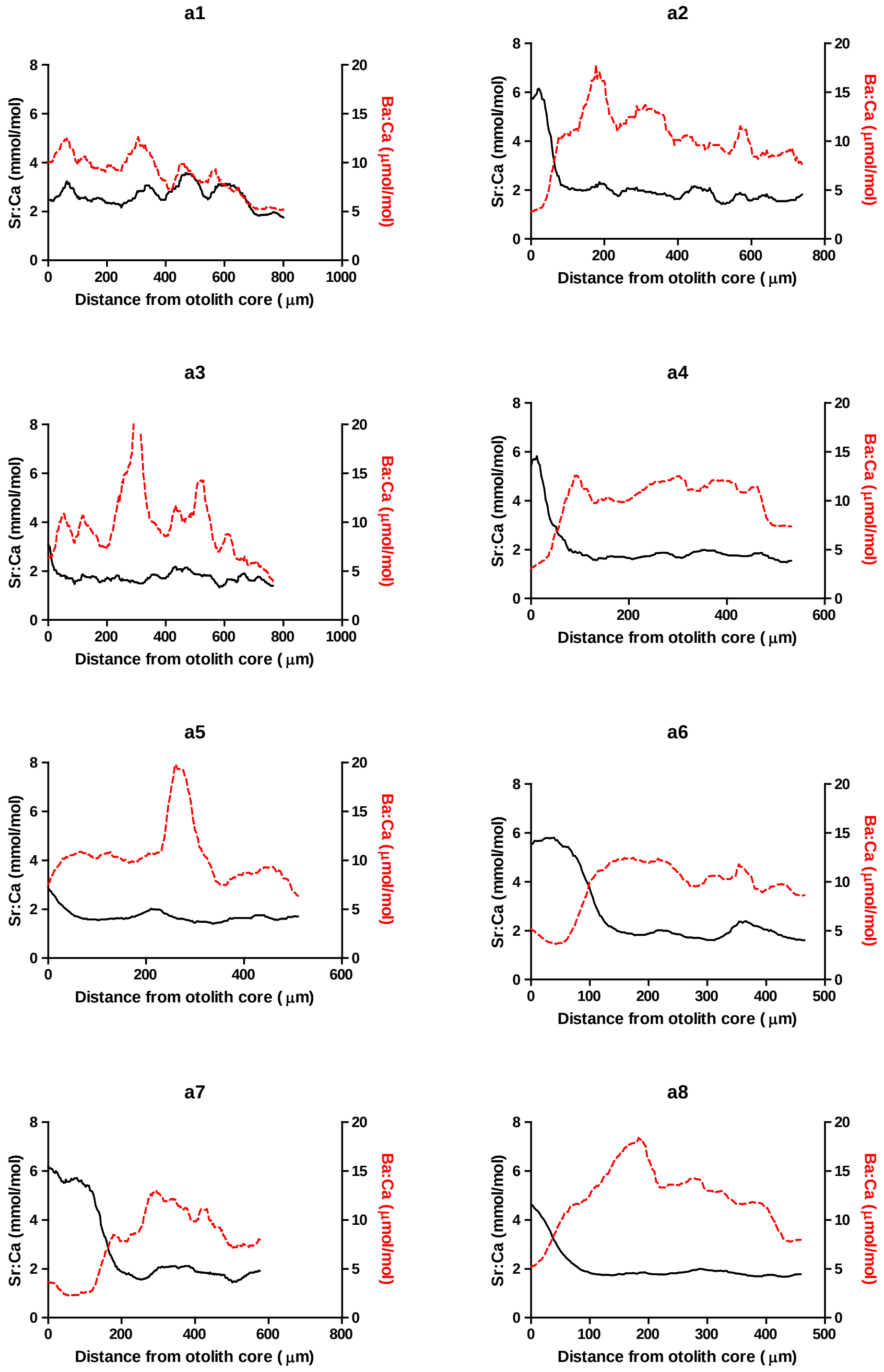
c8

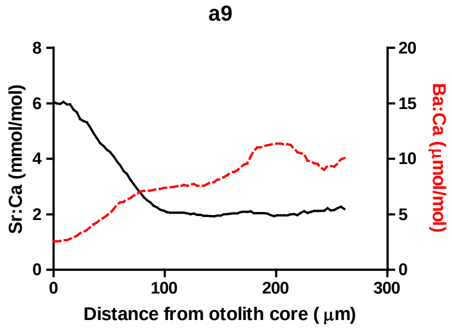


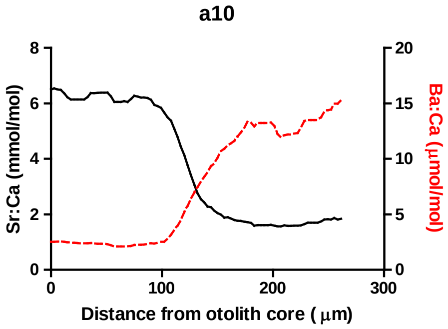

c9
Sr:Ca (mmol/mol)
0
2
4
6
8
Ba:Ca (µmol/mol)
0
5
10
15
20
0
200
400
600
800
Distance from otolith core (µm)

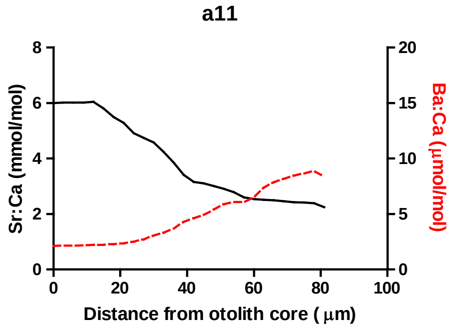
APPENDIX F

Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 11 fish sampled from Western River (South Branch) site. a3 and a5 were assigned unclear morphs, a1 was assigned a resident morph and the remaining 8 plots were assigned a migrant morph.



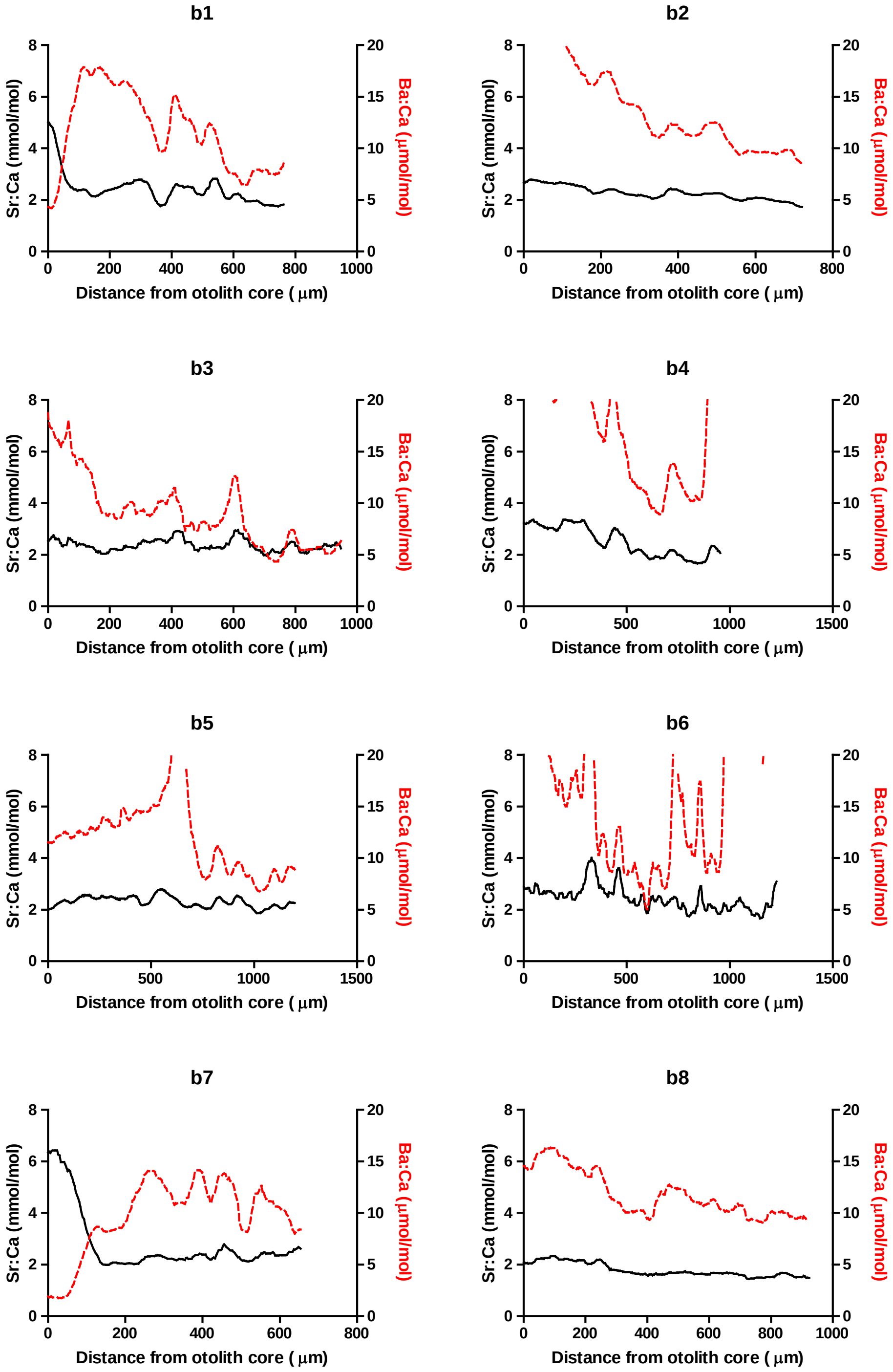

a9
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
Distance from otolith core (µm)

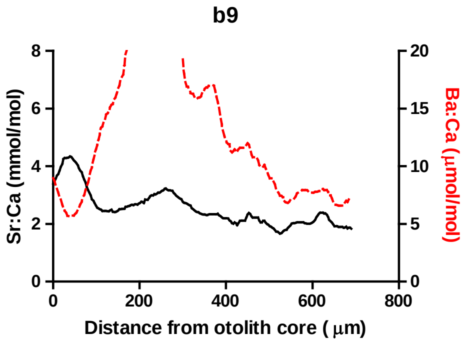

a10
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
0
2
4
6
8
0
5
10
15
20
0
100
200
300
Distance from otolith core (µm)

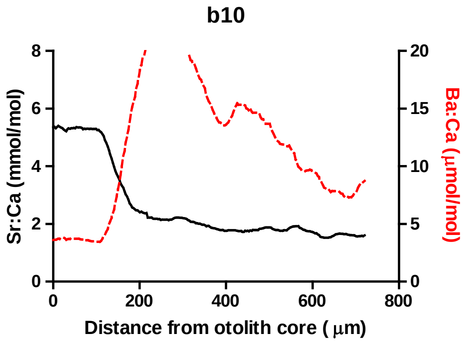

a11
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
0
2
4
6
8
0
5
10
15
20
0
20
40
60
80
100
Distance from otolith core (µm)

APPENDIX G

Otolith Ba:Ca (red) and Sr:Ca (black) profiles for the 10 fish sampled from the North West River site. Plot b9 was assigned an unclear morph. b1, b7 and b10 were assigned migrant morphs and the remaining 6 plots were assigned as resident morphs.




b9
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
0
2
4
6
8
0
5
10
15
20
0
200
400
600
800
Distance from otolith core (µm)


b10
Sr:Ca (mmol/mol)
Ba:Ca (µmol/mol)
0
2
4
6
8
0
5
10
15
20
0
200
400
600
800
Distance from otolith core (µm)