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PRIMARY RESEARCH PAPER

Combining otolith chemistry and telemetry to assess diadromous migration in pinkeye mullet, *Trachystoma petardi* (Actinopterygii, Mugiliformes)

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Abstract This study examines the complex diadromous movements in pinkeye mullet (*Trachystoma petardi*) of south-eastern Australia. The techniques used to study these movements included LA-ICP-MS single line ablation transects and microchemical imaging as well as preliminary acoustic telemetry results which were used to aid in interpretation of chemical signatures related to complex movement patterns across salinity gradients. Ba:Ca and Sr:Ca from single ablation transects and microchemical images revealed differences between the otolith core and outer regions. Otolith Ba:Ca and Sr:Ca patterns were more easily distinguished in images compared to transects and these revealed that *T. petardi* spend their early life in saline waters. Movement patterns for adults varied, with a range of movements identified between fresh and saline waters. Telemetry data assisted in explaining the likely cause of the ambiguity in otolith microchemistry data, including identification of multiple rapid movements across salinity gradients. However, many movements through salinity gradients appear too brief to result in any clear Sr:Ca or Ba:Ca saline or mesohaline signature within the chemistry of the otolith. The combination of otolith chemistry and telemetry proved useful in providing information on this poorly understood species suggesting that *T. petardi* display a catadromous life history.

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Introduction

Migration is an integral component of the lifecycles of many riverine fishes (McDowall, 1988). Among these, the species that display diadromy (obligatory migrations between fresh and saltwater) have been described as one of the most threatened groups of vertebrates in the world (Jonsson et al., 1999; McDowall, 1999). Many diadromous species are difficult to study in the wild due to their complex migratory patterns, irregular distribution, small size and that many have migrating larvae and juveniles (McDowall, 1988; Closs et al., 2003; Keith, 2003; Pusey et al., 2004; Miles et al., 2014). Recent advances in technology have resulted in a range of new and improved research techniques to study the lifecycle and ecology of migratory fishes (Elsdon et al., 2008; Zeigler & Whitledge, 2011; Ueda, 2012; Zydlewski & Wilkie, 2013; Miles et al., 2014; Hussey et al., 2015). These include techniques aimed at better understanding migratory patterns and habitat use (e.g., through use of telemetry, otolith chemistry and stable isotope analyses), mechanisms behind migration (e.g., through physiological, telemetry and olfaction based studies) and connectivity between populations and dispersion (e.g., visual representation of complex spatial data through GIS and other software platforms, spatially explicit statistical modelling and molecular ecology) (Miles et al., 2014).

Recent advances in analytical chemistry techniques have allowed greater resolution and interpretation of chemical data in a variety of calcium structures (Hare et al., 2011; Zeigler & Whitledge, 2011; Austin et al., 2013). In particular, fine-scale quantitative images of trace elements based on laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) have recently been used to contribute to biological understanding in a range of situations (McGowan et al., 2014). LA-ICP-MS images have been used, for example, to examine the diet in primates through trace elements in teeth (Hare et al., 2011; Austin et al., 2013) and to better understand the settlement of reef fish larvae and juveniles using otoliths (McGowan et al., 2014). Fish otoliths are composed of a calcium carbonate in which certain trace elements are incorporated in proportion with ambient water concentrations and as a result, have increasingly been used in fisheries science to reconstruct environmental histories (Campana, 1999; Walther & Limburg, 2012). The key trace elements used for examining movements in diadromous fishes across the salinity gradients have been strontium (Sr) and barium (Ba), as these elements are found in different concentrations in salt water and freshwater (Kalish, 1990; Secor & Rooker, 2000; Gillanders, 2005; Crook et al., 2006). The interpretation of movement patterns is possible as Sr is generally more available for uptake in the otolith in salt water while Ba is more available for uptake in freshwater (Elsdon & Gillanders, 2005b; Crook et al., 2006; Walther & Limburg, 2012). Similarly, Sr isotopes in freshwater are measurably distinct from the globally homogenous marine values (Walther et al., 2011). Therefore, it is not surprising that both isotopic (e.g., ^{87}Sr/^{86}Sr) and elemental ratios (Sr:Ca, Ba:Ca and Sr:Ba) have successfully been used to interpret movement patterns in diadromous fishes (Walther & Limburg, 2012). However, the relationship between salinity and Sr:Ca, Ba:Ca and ^{87}Sr/^{86}Sr is not linear with some curvature away from a linear slope occurring at low salinities (typically < 10) (Walther & Limburg, 2012). As a result, interpretation of otolith chemistry transects for diadromous species can be challenging and the usefulness of each ratio can vary depending on the habitats/salinities utilised (Milton & Chenery, 2003; Walther & Limburg, 2012). For example, Milton & Chenery (2003) found that ^{87}Sr/^{86}Sr was valuable for interpreting movements at lower salinities but Sr:Ba became more useful in higher salinities. Furthermore, estuarine cycling of Ba and Sr can be highly complex and although there are similarities between estuaries, the details can be quite specific to each water body (Coffey et al., 1997; Kraus & Secor, 2004; Walther & Limburg, 2012). Overall, this can prove problematic when reconstructing environmental histories, especially when there is extensive use of estuarine waters with low to moderate salinities, however, this can partly be overcome by examining transects from a variety of ratios or isotopes (Walther & Limburg, 2012). For example, a common method of analysis for understanding diadromous movements has been the simultaneous presentation of line transects for Sr:Ca and Ba:Ca for interpreting patterns of movement (Crook et al., 2006; Miles et al., 2009;

Hamer et al., 2015) or Sr isotopes in conjunction with Sr:Ba (McCulloch et al., 2005). However, interpretation of diadromous movement patterns from analytical variations can be difficult in transects (e.g., Miles et al., 2009) regardless of what is used and the increased amount of data and visual representation with in otolith imaging techniques potentially offer opportunities for interpretation of smaller or less obvious changes in elemental ratios (McGowan et al., 2014).

Investigating movements of fishes in their natural environment has also improved due to technological advances (Robichaud & Rose, 2001; Sibert & Nielsen, 2001; Thorrold et al., 2001). This has included the use of smaller and longer lasting tags that not only provide location data, but also data on environmental conditions (Cooke et al., 2008; Heupel & Webber, 2012). The deployment of large acoustic receiver arrays has also proved a useful tool for examining the distribution, site fidelity and large-scale movements of many fish species (Crook et al., 2010; Walsh et al., 2012b; Koster et al., 2013), the results of which have generated long-term data sets providing a base for effective management of diadromous fishes in south-eastern Australia (Walsh et al., 2012a).

Individually, otolith chemistry and telemetry have aided in understanding migratory patterns and habitat use of previously unclear lifecycles of diadromous fishes (Miles et al., 2014). However, like all research tools, limitations have been identified for both techniques. Otolith chemistry is typically used to provide generalised information on migratory patterns over the lifetime of the fish, but is less suited to understanding more complex movements over short temporal scales (Honda et al., 2012; Miles et al., 2014). Contrastingly, telemetry studies can provide detailed information, but this is typically over a shorter time frame and is also limited to species or life stages capable of holding a tag (Miles et al., 2014). Despite the limitations of both the techniques, otolith chemistry and telemetry are highly suitable complimentary research tools, which when combined, can provide valuable information on the entire lifecycle of a species (Honda et al., 2012).

Pinkeye or freshwater mullet *Trachystoma petardi* (known previously as *Myxus petardi*), of south-eastern Australia is one of the few mullets thought to be truly diadromous rather than highly euryhaline (McDowall, 1988). There are, however, reports of both amphidromy and catadromy in this species, highlighting the complexity of its lifecycle (Humphrey, 1979; Miles et al., 2009, 2014). Overall, there is a substantial gap in available information for *T. petardi*, and indeed diadromous mugilids, with crucial information missing on probable spawning location as well as habitat usage by juvenile fish (Miles et al., 2014). Given the knowledge gaps in the lifecycle of this species, a range of methods is, therefore, required to fully understand their migratory and habitat requirements (Miles et al., 2009, 2014).

In light of these knowledge gaps, the aims of this paper are the following:

1. Compare Sr:Ca and Ba:Ca data obtained from microchemical imaging to data obtained using single ablation transects. Single ablation transects are a common method of otolith analysis used to infer diadromous fish movements whereas microchemical imaging is an emerging technique.
2. Compare interpretations of *T. petardi* movements across salinity gradients derived from otolith microchemistry with recorded movements determined from acoustic telemetry.

Methods

Study sites

This study included fish from two catchments (Shoalhaven and Clarence) in New South Wales, Australia, which covers a substantial portion of the range of *T. petardi* and helps to account for any geographical variation in the lifecycle of this species (Thomson, 1996). The Shoalhaven River (− 34°53′S, 150°45′E) is approximately 327 km long and has a total catchment area of 7300 km^2 (Roy et al., 2001) (Fig. 1). This study examined *T. petardi* movements across 25 km of freshwater and 50 km of estuarine habitats in the Shoalhaven River below Tallowa Dam (Fig. 1). The Clarence River system (− 29°25′S, 153°21′E) is located on the north-coast of NSW and has a total catchment area of ~22,700 km^2. The Clarence is the third largest river system in eastern Australia and it considered to be relatively unregulated, with few dams or barriers to fish movement.

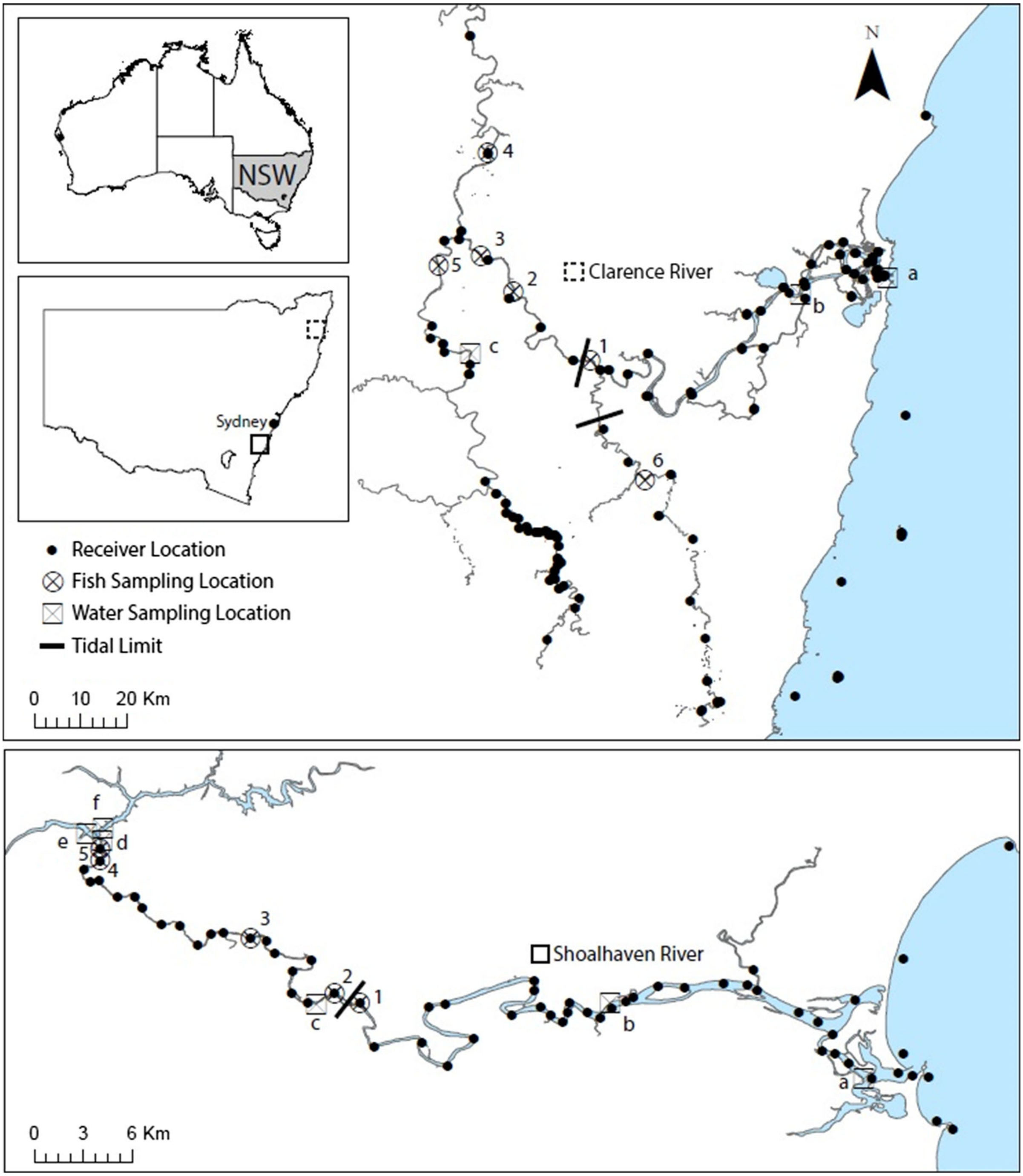

Fig. 1 Study area showing the location of the Shoalhaven and Clarence River catchments in NSW, Australia and the location of sampling sites in each catchment. Fish sampling sites were common for both otolith chemistry and telemetry. Letters associated to water sampling sites correspond to those in Table 1

Table 1 Salinity, Sr:Ca and Ba:Ca of water samples from freshwater, brackish and estuarine reaches of the Shoalhaven and Clarence Rivers

afdxrf	Distance from ocean (km)	Date	Salinity (PSU)	Sr:Ca mmol mol^{-1} (± SE)	Ba:Ca µmol mol^{-1} (± SE)
Shoalhaven River					
Wharf[a]	1	28/5/2013	33.5	10.2 (0.10)	19.3 (0.1)
Nowra[a]	15	28/5/2013	18.9	8.8 (0.11)	45.4 (0.6)
Burrier	47	28/5/2013	0.094	3.0 (0.03)	757.6 (4.7)
Tallowa Dam (below)	74	26/5/2013	0.102	2.3 (0.01)	635.3 (5.0)
Tallowa Dam (above)	75	27/5/2013	0.102	2.3 (0.01)	654.4 (34.1)
Kangaroo River	80	26/5/2013	0.100	3.1 (035)	830.1 (159.2)
Clarence River					
Yamba[a]	2	15/12/13	22.8	9.6 (0.23)	12.7 (0.4)
McClean[a]	24	15/12/13	14.8	8.8 (0.16)	201.6 (1.8)
Mann	195	15/12/13	0.100	3.4 (0.10)	449.2 (6.2)

[a] Denotes tidal waters

Water chemistry

Replicate water samples were collected from six sites in the Shoalhaven River catchment and three sites in the Clarence River catchment in May 2013 (see Table 1 and Fig. 1). Samples were collected in acid washed bottles, filtered through a 0.45 µm filter, acidified with re-distilled nitric acid and then analysed with an Agilent 7500cx inductively-coupled plasma mass spectrometer (ICP-MS) at the University of Technology, Sydney, Australia. Following Milton & Chenery (2003) and Macdonald & Crook (2010), simple mixing models and curves were developed for each river to predict the relationship between Sr:Ca and salinity and for Ba:Ca to salinity and to test these predictions against the empirical data from each river.

Otolith chemistry

Otolith removal and preparation

Forty five *T. petardi* were collected ad hoc in the Shoalhaven and Clarence Rivers between 2010 and 2012 (Table 2). Sagittal otoliths were removed, cleaned, rinsed and allowed to dry before being stored in micro-centrifuge tubes for later preparation. All samples were cleaned using ultra-pure water. Plasticware and glassware were washed in 10% HNO_3 and rinsed several times prior to use. Otoliths were embedded in epoxy resin (EpoFix, Struers, Denmark) spiked with 50 ppm indium and sectioned transversely through the core using a low speed Isomet diamond saw lubricated with water (Elsdon & Gillanders, 2002; Crook et al., 2006). Sections were then polished using 9-µm lapping film until they were approximately 300 µm thick. The sectioned otoliths were then

Table 2 Summary of biological information for *Trachystoma petardi* used in otolith chemistry and telemetry studies

	Otolith chemistry			Telemetry	
	No.	Fork length (mm)	Age (years)	No.	Fork length (mm)
Shoalhaven River					
Male	3	357–366	14–15	3	335–374
Female	27	337–461	4–17	5	368–430
Clarence River					
Male	3	391–427	6–12		
Female	12	356–459	4–12	8	338–446

examined under a compound microscope using reflected light with a 2× objective lens. Although, no age validation experiments have been conducted on *T. petardi* otoliths, ages were estimated by examining otolith opaque zones which were counted radially from the primordium to the outer edge of the ventral lobe following the outer margin of the sulcus acusticus. A camera attached to the compound microscope was used to photograph an image of each otolith and counts of opaque zones were recorded using the digital image analysis programme, Image J (http://rsb.info.nih.gov/ij/). To determine the precision of the otolith age estimates, a 10% re-read of randomly chosen otoliths was used to calculate a coefficient of variation (Campana, 2001). Following ageing, sections were mounted on a glass slide, sonicated for 5 min, rinsed in ultra-pure water and dried in a laminar-flow cabinet before being stored in clean sealable plastic bags.

Microchemical analyses

Microchemical analyses of otoliths was undertaken using a New Wave Research UP213 laser ablation unit (Kenelec Technologies, Victoria, Australia) coupled to an Agilent Technologies 7500 cx inductively-coupled plasma mass spectrometer (Agilent Technologies Australia, Victoria, Australia). The UP213 laser unit employed a Nd: YAG solid-state laser source emitting a 213 nm laser pulse in the fifth harmonic. A Large Format Cell (LFC) was fitted to the UP213 unit, which was connected to the ICP-MS via polyvinylchloride tubing. The 7500 cx ICP-MS was fitted with a ‘cs’ lens system for enhanced sensitivity. The LA-ICP-MS system was tuned for sensitivity prior to each experiment using NIST (National Institute of Standards and Technology) 612 Trace Elements in Glass. The instrument measures a number of masses sequentially and Ca^{43}, Sr^{88} and Ba^{137} were chosen for the current study.

Laser ablation was conducted using a continuous scan across the otoliths from one edge to the other along the longest plane (from the dorsal to ventral margins) using an automated stage. Two different settings were used; (1) for otolith transects a single line was used to pass from the edge of the otolith through the core and to the other edge using a 10-Hz pulse rate, a 30 µm diameter spot and a scan rate of 5 µm per second (Miles et al., 2009), and (2) to produce otolith images a series of transects were used to ensure coverage of the otolith (e.g. a scan every 30 µm down the otolith) using a 20 Hz pulse, 30 µm diameter spot and due to the large area and number of scans required, the speed was increased to 61 µm second. A pre ablation pass was undertaken before all scans to ensure the surface was free from any contamination.

Reference standards (NIST 612 glass) were measured regularly to calibrate the relative sensitivity of each analyte mass and to correct for drift (Norman et al., 1996; Miles et al., 2009). The standard was measured each time the sealed sample stage was opened, and again mid-way through each sample set (approximately every 5–7 samples). 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.

Data analyses

Each analysis was normalised to the CaO 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) and Miles et al. (2009). Elemental concentrations were normalised to Ca^{43} and data were presented as concentration ratios in units of mmol mol^{-1} (Sr:Ca), and µmol mol^{-1} (Ba:Ca).

Analysis of transect data first included smoothing the data points (Crook et al., 2006) using a six-point running mean for Sr:Ca and Ba:Ca. Data was then presented as line graphs developed in Microsoft Excel and interpreted by examining Sr:Ca and Ba:Ca profiles to identify areas where an inverse relationship between the two elements was evident. Particular focus was placed on the inner regions of the otoliths, relating to larval and juvenile growth, as well as the outer regions relating to adult growth.

To produce otolith images, each line of ablation produced a single data file in comma separated value (.csv) format (see Hare et al., 2011). Images were produced by reducing each data file into a single exportable format for imaging in a number of software suites. Interactive Spectral Imaging Data Analysis Software (ISIDAS) was used for image reduction, an in-house software package at the University of

Technology Sydney written in the Python programming language (https://www.enthought.com/products/epd/). ISIDAS reduced all.csv files into a single, exportable visualisation toolkit (.vtk) file format. The processed data was then exported as a vtk file readable by MayaVi2 (Enthought, Inc., TX, USA), an open source data visualisation application (see Hare et al., 2011; Austin et al., 2013). The colour indicates the strength of the signal which is received following transport of an ablated otolith section to the ICP-MS, and is directly related to the concentration of the elements. The same method of data transformation was used for images as it was for transects except that the calculations were performed in ISIDAS using the expression function. Colour scales were applied using the linear blue–red LUT. Image backgrounds were converted to black (absent from the colour intensity scale) to clarify sample boundaries from the substrate (see Austin et al., 2013).

Comparison between line transects and images of otolith microchemistry was made by running both analyses on a subset of otoliths ($n = 14$). This included running a single line transect, followed by a pre ablation pass and then full scans of the otolith in order to produce images. As images are constructed from a series of line transects, the initial investigations focused around a comparison of transects produced by the two techniques. This was then followed by visually comparing line transect graphs and elemental image data.

Telemetry

The acoustic telemetry component of this study was based on the results of a study undertaken between 2009 and 2012. Linear arrays of 49 and 87 acoustic receivers (Vemco VR2 W) were deployed in the Shoalhaven River below Tallowa Dam and throughout the Clarence Basin, respectively (Fig. 1). The Clarence River acoustic telemetry array of 87 receivers covered approximately 600 km of the lower sections of system, all of which is inhabited by *T. petardi.* A more intensive acoustic telemetry array (due to the shorter river length covered) of 49 receivers similarly spanned the freshwater and estuarine habitats of the Shoalhaven River below Tallowa Dam.

Sixteen *T. petardi* (8 from the Shoalhaven River catchment and 8 from the Clarence River catchment) were tagged with either Vemco V13-1L/13TP (13 mm in diameter) or V9-2L/2LTP (9 mm in diameter) transmitters depending on the size of the fish (Table 2). Tagging procedures and post-operative care followed that described in Butler et al. (2009) and Walsh et al. (2012a). Data from receivers were downloaded bi-monthly and stored in a Microsoft Access database. Prior to analyses, 'false' detections and all single-hit data were discarded (Clements et al., 2005). The mean daily distance upstream of the sea for individual tagged fish was calculated and summarised graphically.

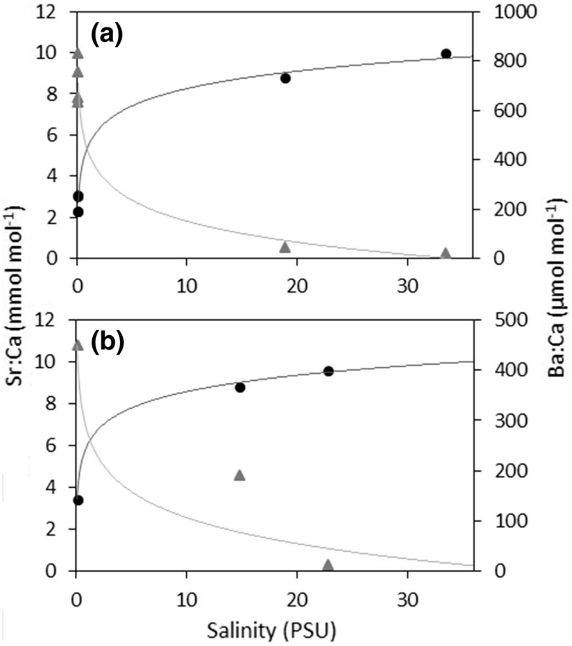

Fig. 2 Relationships between water Sr:Ca, Ba:Ca and salinity for **a** Shoalhaven and **b** Clarence Rivers. The estimated mixing curves for water Sr:Ca (black line), Ba:Ca (grey line) and salinity are shown overlain with the empirical values for Sr:Ca (black circles), Ba:Ca (grey triangles) measured in water samples collected from both rivers (see Table 1)

Results

Water chemistry

The analysis of water chemistry for the Shoalhaven and Clarence catchments showed there was a non-linear relationship between Sr:Ca and salinity and Ba:Ca and salinity (Table 1, Fig. 2). The mixing curve estimates generally provided a good fit to the empirical

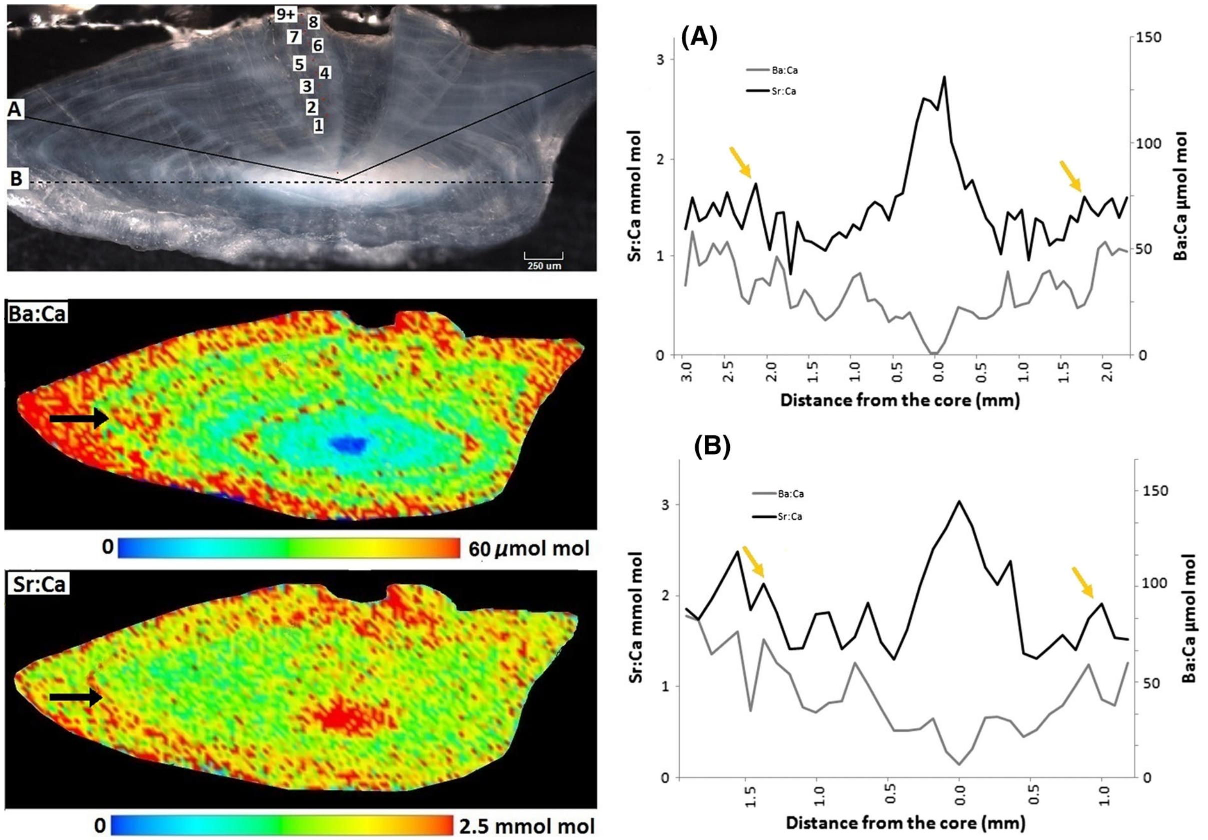

Fig. 3 An example of pinkeye mullet (fish Tp208) otolith micro chemistry transects and images for Sr:Ca and Ba:Ca showing two-dimensional structure in sagittal otoliths. Arrows indicate the same potential movement event. **A** Is the transect achieved using the single line settings and **B** is the transect achieved when using the image settings. The path of the transects for Figure **A** and **B** are shown on the microscope image

data collected in both rivers, with the exception of Ba:Ca from the estuarine McClean site in the Clarence River (Fig. 2). The mixing curves suggest that the greatest rate of change occurred at salinities of less than 5 (Fig. 2).

Comparison of otolith chemistry techniques

All otolith chemistry techniques revealed similar patterns of high Sr:Ca and low Ba:Ca in the core compared to the outer regions of the otolith (Fig. 3). Furthermore, all methods revealed that the inverse relationship between Sr:Ca and Ba:Ca was strongest in the inner regions of the otolith with the data becoming more difficult to interpret outside of the core region (Figs. 3, 4). Despite differences in the transect paths, analysis settings and the amount of otolith material analysed, similar patterns were identifiable in single line transects and transects derived from image data (Fig. 3). However, values beyond the core were difficult to interpret in all transects due to large amounts of variation in the data and the lack of any relationship between Sr:Ca and Ba:Ca (Figs. 3, 4). For example, TP210 and TP271 had distinct increases in Ba:Ca in the outer region of the otolith, but this was not consistently contrasted by a decline in Sr:Ca (Fig. 4).

Otolith images provided a much clearer indication of the composition of elemental ratios as changes in chemistry could be checked in other parts of the sample. Otolith microchemical images provided visual representation of easily discernible rings of Ba:Ca (and Sr:Ca to a lesser extent) throughout the otolith (Fig. 3). For example, a clear indication of the

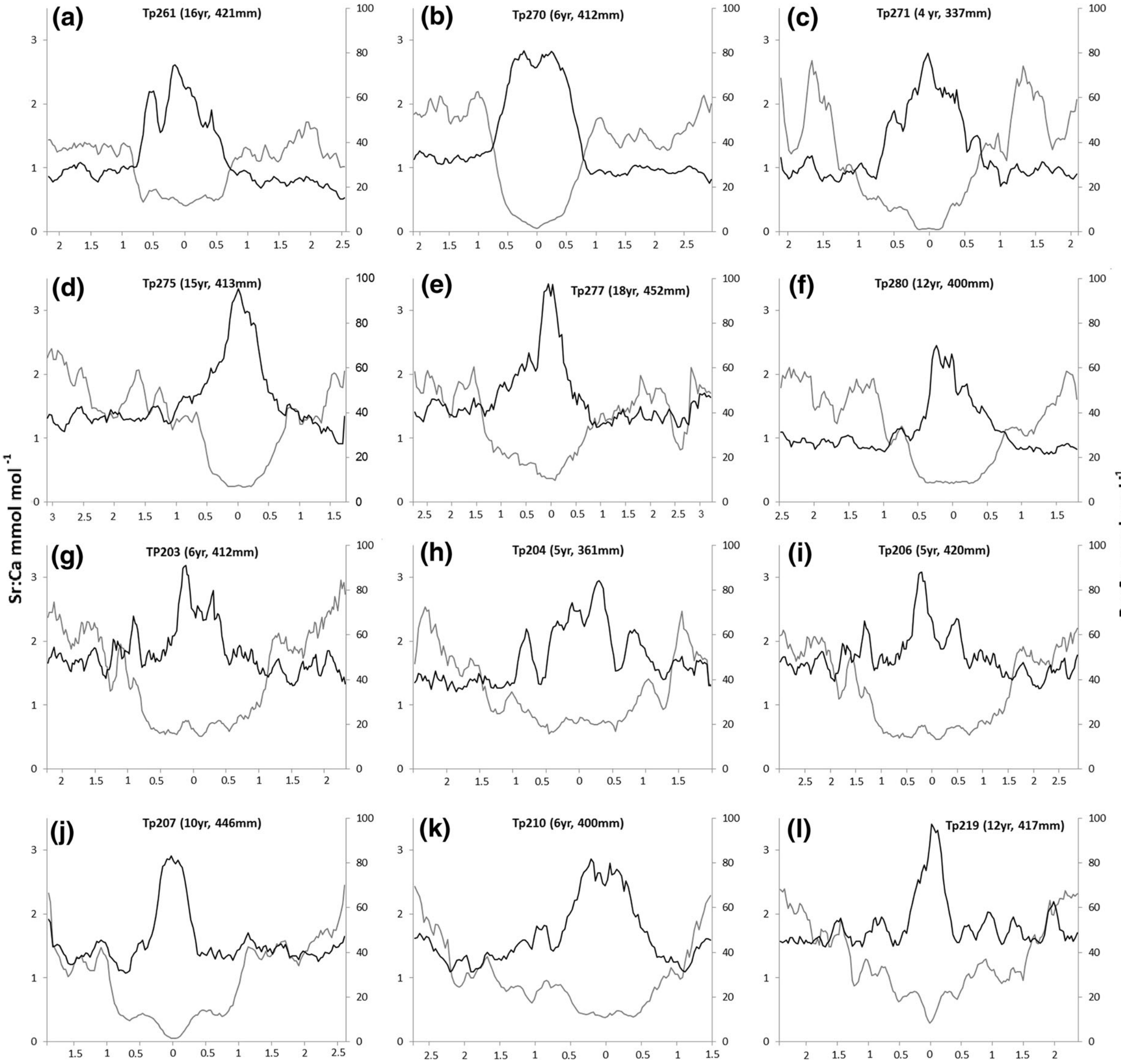

Fig. 4 Sr:Ca (mmol mol^{-1}, black line) and Ba:Ca (µmol mol^{-1}, grey line) values along transects across otoliths of representative samples of *T. petardi* from the Clarence and Shoalhaven catchments. Figures **a–f** are from the Shoalhaven River and figures **g–l** are from the Clarence River. Data is presented for transects achieved using the single line settings

change of Ba:Ca can be seen after year 1 on fish Tp208 in the image but this is less discernible in transects (Fig. 3). In Tp208, the change in Ba:Ca after year 1 is followed by a series of less clear Ba:Ca rings towards the edge. Faint contrasting rings of Sr:Ca can also be seen for some of these rings in the otolith for Tp208 (Fig. 3). In Tp208, the strongest inverse relationship between Sr:Ca and Ba:Ca outside of the core occurred at around 6–7 years (Fig. 3). Although the change in chemistry identified in images and transects at around 6–7 years is not as distinct as that identified near the core, the otolith images proved valuable in allowing this change to be checked and confirmed elsewhere in the sample (Fig. 3).

Movements as determined by otolith microchemical images

Otolith microchemical images for all fish displayed a clear core region that was high in Sr:Ca and low in Ba:Ca (Fig. 5). In contrast to transects (Fig. 4), a number of changes in Ba:Ca were identified in otolith

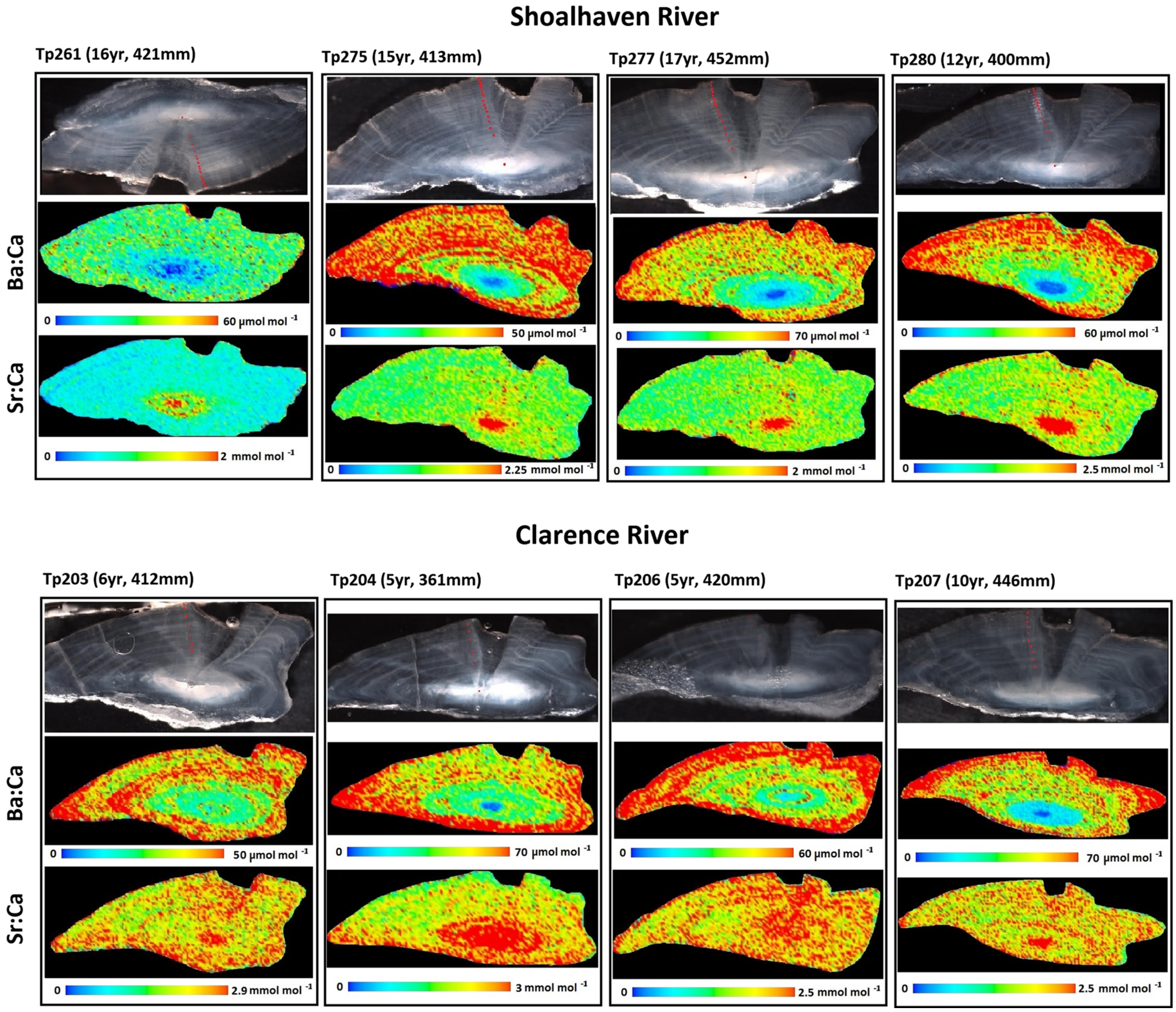

Fig. 5 A subset of eight pinkeye mullet otolith microchemistry images for Sr:Ca and Ba:Ca showing two-dimensional structure in sagittal otoliths. Included are examples from the Shoalhaven and Clarence Rivers. Data is expressed µmol mol for Sr:Ca in and mmol mol for Ba:Ca. Size of fish shown as FL

images with a series of rings present in most otolith. Rings of Sr:Ca in images were less distinct (Fig. 5) and as suggested previously, the inverse relationship for Sr:Ca and Ba:Ca was strongest in the core for all individuals. The changes in chemistries during adult stages were often difficult to interpret as regular movements, and were typically not as obvious as the change in chemistry experienced between juvenile and adult stages (Fig. 5). The patterns were reasonably consistent across all otoliths and both sexes with the only exceptions being (1) Sr:Ca displayed clearer more frequent changes in otoliths from the Clarence River compared to the Shoalhaven River and (2) ~10% of individuals displayed no discernible patterns beyond the core, such as Tp261 (Fig. 5). This would suggest these fish remained in the same habitat for all their adult life stage. As a result, otolith chemistry appeared to identify two lifecycle strategies which had a common saline larval or juvenile stage. The first strategy being fish that display freshwater residency as adults coupled with a range or irregular movements to higher saline waters and the second strategy being less common, with fish remaining in low saline waters for most of their adult life.

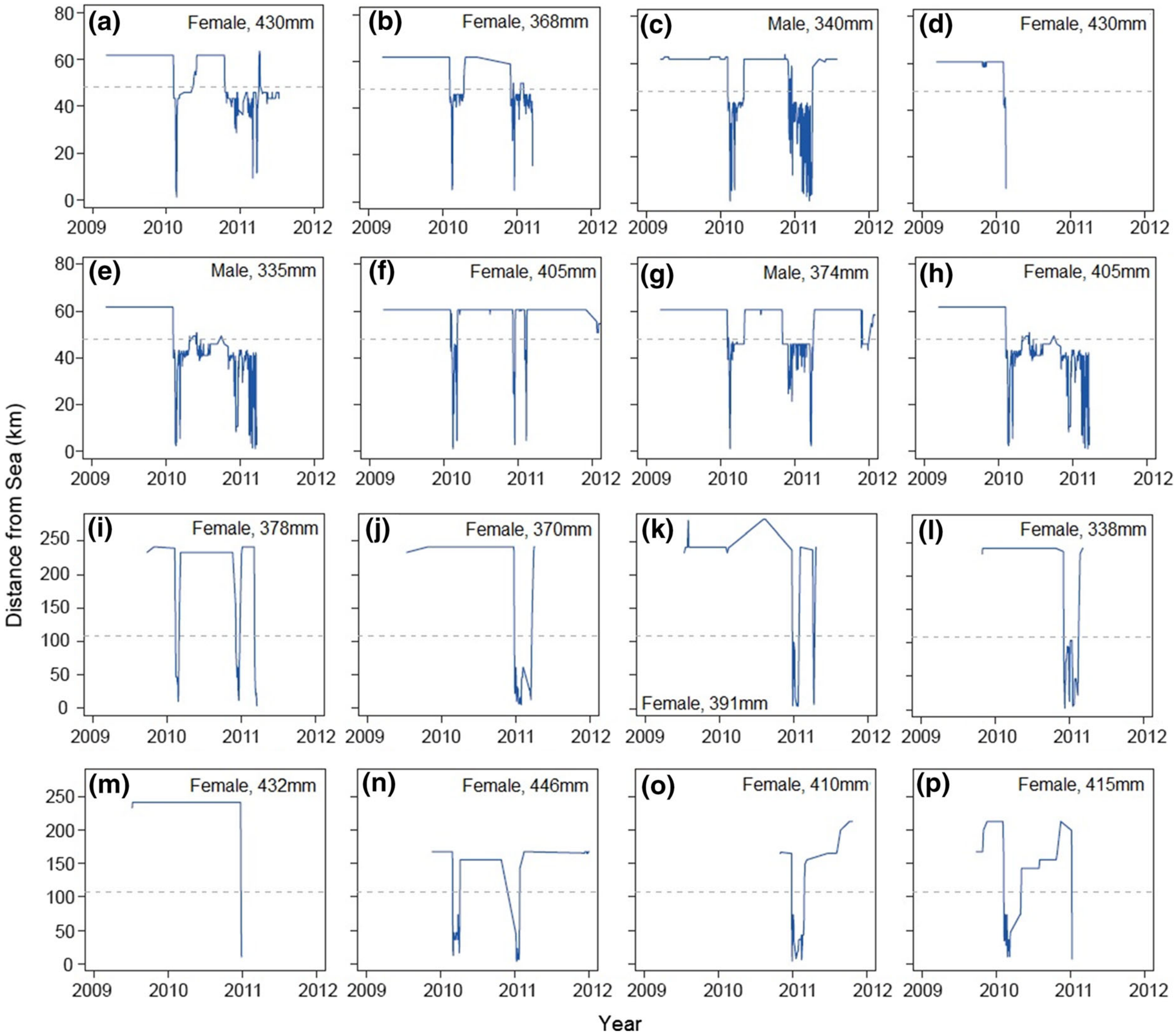

Fig. 6 Mean daily river location of tagged *T. petardi* individuals from the Shoalhaven River (**a**–**h**) and Clarence River (**i**–**p**) at liberty 2009 and 2012. Dashed grey line represents the tidal limit

Movements as determined by telemetry

Tagged adult *T. petardi* were monitored for up to 3 years in both catchments. During late autumn to early summer fish displayed high site fidelity, residing in the freshwater reaches close to their original capture location. In the summer through autumn months, the majority of *T. petardi* migrated from their freshwater home ranges to the lower estuarine reaches (Fig. 6). Dependent upon catchment, the frequency of these large-scale migrations ranged from singular to several within a season, with residency times in the lower reaches not lasting more than a few days. In particular, Shoalhaven River fish migrated to just downstream of the tidal limit for extensive periods (1–3 months) and made several repeated trips to the lower estuarine areas. In contrast, the Clarence River fish usually made only one long (> 200 km) migration to high salinity areas each year. The extent of these estuarine migrations was typically to within 5 km of estuary mouth in both catchments, although two fish (25%) from the Shoalhaven River were detected on offshore receivers during a flood event in March 2012.

Discussion

The current study highlighted the benefits of utilising complementary techniques to gain a better understanding of diadromous fish movements. As indicated by Honda et al. (2012), otolith chemistry and telemetry provide information on different spatio-temporal scales and provide complementary, independent information to strengthen understanding. Otolith chemistry revealed the movement history of individuals between fresh and saltwater across lifetimes, but failed to reveal the same level of detail of the rapid intra-annual and annual movements across salinity gradients revealed by acoustic telemetry. Otolith chemistry revealed *T. petardi* had a clear saline larval/juvenile stage and that there was some evidence of adult movements to saline habitats, but interpreting these data was difficult. In contrast, the telemetry data provided information on fish movement from freshwater through to estuarine and in some cases marine habitats in detail, but was limited in temporal scale and was restricted to adult fish (Honda et al., 2012; Miles et al., 2014).

The use of microchemical imaging and the increased level of detail it provided on the chemical composition of the otolith aided in interpreting potential movement events throughout the life of *T. petardi*. A key benefit of microchemical images is that the visualisation of the data in a two-dimensional image allows the homogeneity of changes in otolith chemistry to be examined throughout the structure, aiding in understanding more ambiguous patterns which may be more difficult to interpret in otolith microchemistry transects (McGowan et al., 2014). Although otolith images have been produced elsewhere using proton-induced X-ray emission PIXE scans (Elfman et al., 1999; Crook et al., 2006; Limburg et al., 2007), the technique described here (including the specialised software, ISIDAS) is reported to be more cost effective and does not require a high vacuum ion source, does not suffer from severe matrix effects experienced with other techniques, has a lower spatial resolution that is more suited to imaging whole structures (compared to PIXE) and allows easy selection of areas of interest from the image data for further more detailed analysis (Hare et al., 2011; McGowan et al., 2014).

A potential drawback associated with LA-ICP-MS otolith images is the time it takes to analyse the samples (when compared to single line transect), especially when being used on large-otoliths such as in the current study. Similarly to McGowan et al. (2014), ablation of *T. petardi* otoliths during microchemical imaging took at least 4 h per specimen to complete. This is despite the use of a larger spot size and faster scan speed then that used by McGowan et al. (2014). The processing time for otolith images outlined here is also considerably longer than for a single line transect analysis (McGowan et al., 2014). However, the time required to complete the analysis could be reduced in some cases by analysing less material and focusing the analysis on a particular region of the otolith (e.g., the core, a period of a few years of adult growth, or a smaller series of transects which focus around the core) or by analysing one of the smaller otoliths (asterisci or lapilli). Furthermore, information on early life stages could be obtained by undertaking more detailed analysis (reduced spot size and scan speeds) of juvenile rather than adult otoliths and this may be used to further narrow down potential larval and juvenile marine habitats (McGowan et al., 2014; Miles et al., 2014). Such analysis could be useful for providing information on more complex movements and may have presented clearer results for adult movements or provide data which could assist in understanding the conditions at the spawning locations for species such as *T. petardi*. Nevertheless, the application of this technique in the current study and that by McGowan et al. (2014) demonstrates that the method is adaptable and can be manipulated to better target the biology of particular species or to answer specific research questions.

Despite the increased sensitivity and improved interpretation of otolith images outlined above, the current study also demonstrated the limitation of otolith chemistry using Sr:Ca and Ba:Ca for highly mobile diadromous fishes. This same limitation was highlighted by Miles et al. (2014) who suggested that otolith chemistry may not be a suitable method for all species, life stages or research questions. Regarding *T. petardi*, telemetry was previously suggested as a core tool to fill gaps in our knowledge of adult movements of the species (Miles et al., 2014). This was confirmed in the current study where evidence of multiple adult movements from freshwater into estuarine, and in some cases marine habitats was indicated by telemetry. Many of these movements into saline waters were brief (< 7 days) and thus, little if any discernible differences would be detected in otolith chemistry with the techniques used in this study (Elsdon & Gillanders, 2003; Macdonald & Crook, 2010). In particular, research on other species has found that it may take 20–40 days for concentrations to stabilise (Elsdon & Gillanders, 2005c; Lowe et al., 2009; Macdonald & Crook, 2010). In addition to the short periods of time spent in the estuary, the tidal environments frequented by *T. petardi* would display typically low and fluctuating salinity conditions (see Table 2; Condie et al., 2012). Previous studies have suggested that these conditions are less favourable for detecting changes in otolith chemistry (Elsdon & Gillanders, 2002). These conditions may have also contributed to the difficulties in identifying the expected inverse relationship between Sr:Ca and Ba:Ca, especially in the outer regions of the otolith.

Difficulties in interpreting the adult regions of otoliths has also been reported for other species from south-eastern Australia and this included a breakdown in the correlation between Sr:Ca and Ba:Ca (Crook et al., 2006; Miles et al., 2009; Hamer et al., 2015). There is some variation in the literature as to which ratio is more reliable and this can vary depending on environmental conditions (Secor & Rooker, 2000; Elsdon & Gillanders, 2005a, 2006; Macdonald & Crook, 2010). For example, Elsdon & Gillanders (2005a) suggested Ba:Ca is a more useful measure for discerning habitat usage than Sr:Ca in brackish water and Macdonald & Crook (2010) found that Ba:Ca uptake stabilised sooner then Sr:Ca. However, it is likely that difficulties in interpreting the chemistry of the outer region of *T. petardi* otoliths may result from the use of low salinity waters which is intersected by rapid movements in and out of more saline waters (Fig. 6). The use of other measures may aid in overcoming these interpretation issues, with $^{87}Sr/^{86}Sr$ proving useful in understanding movements in other diadromous fish (Kennedy et al., 2000; Walther et al., 2011). Key benefits of $^{87}Sr/^{86}Sr$ are that it is less affected by temperature and biological processes than elemental ratios and it provides a more unambiguous marker of diadromous movements that can be more useful than other ratios at lower salinities (Kennedy et al., 2000; Walther & Limburg, 2012). Milton & Chenery (2003) also suggest that otolith Sr isotopes can reflect those in the water within a week which would be an advantage for species such as *T. petardi* which displayed a range of rapid movements throughout salinity gradient in the current study (Fig. 6). Furthermore, $^{87}Sr/^{86}Sr$ has successfully been used to study movements on a range of temperate and tropical estuarine inhabitants such as Barramundi *Lates calcarifer* and the tropical shad hilsa *Tenualosa ilisha* (Milton & Chenery, 2003; McCulloch et al., 2005; Walther et al., 2011). However, Milton & Chenery, (2003) reported that both Sr isotopes and Sr:Ca were able to distinguish movements across the salinity gradient, but the isotopic ratios were not resolvable in higher salinities. Despite this limitation, the lack of significant biological fractionation and other characteristics of Sr isotopes can reduce ambiguity in transect data and as a result would appear to be suitable for future work on species such as T. *petardi* with complex movement patterns in lower salinity waters (McCulloch et al., 2005; Walther & Limburg, 2012). In order to gain further insight into the use of different salinities for *T. petardi* using otolith chemistry, a rigorous investigation of water chemistry in each catchment and otolith uptake rates would be required, with otolith microchemistry images based on Sr isotopes and Ba:Ca offering strong possibilities for future understanding of the movement and residency in low to moderate salinities for this species (Walther & Limburg, 2012).

Although otolith chemistry and telemetry were conducted on separate *T. petardi*, the analysis of remote, long-term telemetry data and historical data from otolith chemistry meant that movements were examined from the same time period (2009–2012). Overall, telemetry provided much needed information on the movement of adult *T. petardi* and the extensive array of receivers allowed complex movement patterns to be documented for most individuals. Despite the valuable movement information identified in telemetry data and the contribution it made to understanding otolith chemistry data, further telemetry studies are still required to provide precise information on the movements and ecology of *T. petardi*. In particular, future studies identifying spawning habitat, response of fish to freshwater flows and other environmental variables will provide much needed information on the life history strategy.

Lifecycle of *Trachystoma petardi*

In the current study, the combination of otolith chemistry and telemetry revealed that *T. petardi* undertakes a complex array of movements between fresh and saltwater throughout its lifecycle, and although complex and varied, the evidence supports the diadromous lifecycle hypothesised elsewhere (Miles et al., 2014). Otolith chemistry suggested that larval and juvenile *T. petardi* occupied estuarine or marine environments. These results support previous studies of *T. petardi* in the Shoalhaven and Clyde Rivers, where a clear marine signature was also identified in the core of all samples (Miles et al., 2009). Microchemical imaging provided a higher level of detail into the chemical composition of the otolith and the data suggested a range of complex post-larval movements. Despite the apparent movements into saline water indicated by telemetry, the lack of clear marine signature in the adult regions of the otolith (especially for Sr:Ca) and the lower Ba:Ca and higher Sr:Ca in the otolith core compared to the edge would suggest that *T. petardi* do not use saline waters for any extended period after the juvenile life stage. The use of lower salinity waters and the resulting lack of any clear patterns of marine residency as adults determined from otolith chemistry would also explain the previously hypothesised amphidromous lifecycle (Miles et al., 2009).

The hypothesised short residence time in saline waters identified by microchemical imaging was validated by telemetry data which showed adult fish often made movements across salinity gradients. Large-scale movements were concentrated around summer and autumn, and for many individuals, included several very rapid movements from freshwater into and back out of estuarine waters. Telemetry data, like otolith chemistry analyses, confirmed previous reports that freshwater reaches of rivers are the dominant habitat utilised throughout the year, with a lesser use of estuarine or brackish waters (Humphrey, 1979; Miles et al., 2009). Similar rapid movements have been reported in other diadromous fish, with Walsh et al. (2013) and Reinfelds et al. (2013) using telemetry to identify similar rapid spawning migrations in percichthyids in the Shoalhaven catchment. Other examples of rapid movements in Australian diadromous species can be found in Tupong (*Pseudaphritis urvilli*) and Australian grayling (*Prototroctes maraena*) (Crook et al., 2010; Koster et al., 2013). These movement patterns would subject spawning fish to a range of salinity conditions. Although rapid changes in salinity can stress many fish species, mugulids are well known for their ability to osmoregulate and can rapidly move between fresh and salt waters (Lasserre & Gallis, 1975). Furthermore, the eggs and newly hatched larvae of other mullet such as *Mugil cephalus* have been reported to have a broad salinity tolerance range of 17–36 ppt (Lee & Menu, 1981; Murashige et al., 1991; Walsh et al., 1991).

Combination of the two methods suggests that *T. petardi* uses estuarine process (river and tidal flow) to distribute their offspring within the marine environment and to provide access to suitable food sources, a strategy common in diadromous fish (Gross et al., 1988; Bilton et al., 2002). It is probable that the downstream movements observed from the telemetry study are related to spawning, with fish positioning themselves to release eggs when tides and currents are favourable for dispersal (van der Meulen et al., 2014). The saline signature at the core of the otolith is representative of a species with either a marine or estuarine larval and post-larval stage. More work is needed to determine the spawning and recruitment dynamics of this species and this could be aided by sperm vitality experiments at a range of salinities and sampling of eggs and larvae.

The movements by *T. petardi* identified in the current study highlight the complex lifecycle of this species and support the previously suggested diadromous lifecycle (Humphrey, 1979; Miles, 2007, 2009, 2014). However, it is evident that the species more likely displays a marginally catadromous lifecycle in both the Shoalhaven and Clarence Rivers, which is consistent with previous biological studies (Humphrey, 1979). Future investigations on diadromous fish could further test the otolith microchemistry methods outlined here and where ever possible this should be combined with telemetry and/or sampling of eggs and larvae to provide further insight into the interpretation of this data and the movement ecology of the species.

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