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**Fine-scale variability in elemental composition of estuarine water and otoliths:
developing environmental markers for determining larval fish dispersal histories
within estuaries**

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Abstract

Otolith chemistry has been widely used as a natural tag for determining environmental histories of fish that migrate across large distances and environmental gradients. However, it is not as well established as to whether otolith chemistry can be a useful tool for determining fine-scale movement and dispersal patterns of fish, particularly larvae, within estuaries. In this study we collected water samples and otoliths from larvae of black bream, *Acanthopagrus butcheri*, an estuarine resident fish, and analysed each for a suite of trace elements to determine the degree of spatio-temporal variability in elemental composition and which elements were incorporated into otoliths in relation to their associated water chemistry. We found that there were three distinct water layer signatures based on their $\text{Me}:\text{Ca}_{\text{water}}$ (Me = each element): a freshwater signature, a halocline signature, and a marine signature. There were also significant spatio-temporal patterns in $\text{Me}:\text{Ca}_{\text{otolith}}$, most notably for $\text{Li}:\text{Ca}$, $\text{K}:\text{Ca}$ and $\text{Mn}:\text{Ca}$. This study provides encouraging evidence for the utility of intra-estuary otolith chemistry to reconstruct environmental histories during larval development. This application will enable a better understanding of the abundance, timing and duration of residency in particular water masses and locations that are important larval nursery habitats for species that develop within estuaries.

Estuaries are often referred to as nursery habitats as they have high abundances of juvenile fish and are often dominated by young stages of marine species (Potter et al. 1990; Beck et al. 2001). Some species of fish have a pelagic larval stage that disperses passively or actively into or through an estuary from either fresh or marine waters (Islam et al. 2006; North and Houde 2006; Shoji and Tanaka 2006). The biophysical conditions within an estuary are thought to be favorable for larval and juvenile growth and survival due to optimal salinities and temperatures, rates of prey production and levels of turbidity (Beck et al. 2001; North and Houde 2001). Estuaries are also highly variable and dynamic systems with steep gradients in salinity, temperature, dissolved oxygen and turbidity, which can vary over small to large temporal and spatial scales (Kurup et al. 1998). This variance can influence the temporal and spatial distribution of larvae and associated feeding, growth and survival (North and Houde 2003; North et al. 2005; Williams et al. 2013). Particular features within an estuary such as salinity stratification (halocline) and the estuarine turbidity maximum have been shown to influence larval fish distributions (Islam et al. 2006; North and Houde 2006; Williams et al. 2012). An ability to determine the environmental history experienced by fish during larval development would allow us to assess what impact these environmental gradients have on larval growth, condition, and survival, and ultimately how much they influence recruitment variability.

Determining movement patterns, connectivity, and environmental histories of larval fish is logistically difficult due to their small size and high mortality rates. Traditional methods using artificial physical (Thorrold et al. 2002) or chemical (Thorrold et al. 2006) tags, and mark recapture experiments (Warren and Pardew 1998), are not feasible given the constraints on animal size, tag reliability, and recovery rates. Over the past three decades there has been growing awareness of the utility of using the chemistry of otoliths (ear bones) as natural tags to

investigate movement and habitat usage in fish (Kalish 1989; Secor et al. 1995; Gillanders and Kingsford 1996; Campana 1999; Swearer and Caselle 1999; Vasconcelos et al. 2008; Walther and Thorrold 2008; Aschenbrenner et al. 2016; Fraile et al. 2016; Rooker et al. 2016).

Otoliths are calcium carbonate (aragonite) structures that grow continuously on a daily basis. During growth various trace elements are incorporated at rates that can depend on concentrations in the surrounding environment, as well as environmental variables such as salinity and temperature (reviewed by Campana 1999; Elsdon et al. 2008). Therefore, a predictable relationship can potentially be established between water chemistry and otolith chemistry, while accounting for other environmental variables (Dorval et al. 2007; Elsdon et al. 2008; Stanley et al. 2015). The great advantage of natural tags is that all individuals are marked. In systems where environmental conditions vary considerably, characterizing such relationships is essential for accurately reconstructing movement patterns, natal origins and habitat use based on otolith microchemistry (Swearer et al. 2003; Crook et al. 2006; Barbee and Swearer 2007).

Previous research has identified that otolith chemistry is a useful tool for fish that move across significant environment boundaries such as between river, estuarine and marine waters, (Gillanders and Kingsford 2000; Barbee and Swearer 2007; Koster and Crook 2008). However, there are many species of fish that have an obligate life stage that is completed within the intermediate salinities and water temperatures experienced in an estuary (Day et al. 1989; Potter et al. 1990; Dolbeth et al. 2008). Although few studies have attempted to utilise otolith chemistry to determine environmental histories within estuaries (but see Kraus and Secor 2004; Elsdon and Gillanders 2006a) it is of particular interest as estuaries are highly variable and dynamic habitats with shifts in habitat conditions that may influence the survival, growth and dispersal of larvae.

Much of this intra-estuary variability is influenced by variability in freshwater flows (Gillanders and Kingsford 2002).

The chemical signature of ambient water in estuaries, which influences the chemistry of otoliths, is likely to be system dependent, based on, tidal movement, precipitation and hydrogeology (see Table 1 in Elsdon et al. 2008 for a list published and unpublished data on the spatial variability of ambient water chemistry). Many studies have measured Sr and Ba, although sometimes with conflicting results, based on known gradients in Sr and Ba concentrations between marine and freshwater. This has led to a reasonable understanding of how these two elements are incorporated into otoliths (Bath et al. 2000; Kraus and Secor 2004; Macdonald and Crook 2010). A multi-element approach, however, can result in less ambiguous data that is able to resolve finer-scale movement patterns across salinity gradients than using Sr and Ba alone (Hicks et al. 2010; Tanner et al. 2013, Reis-Santos et al. 2015).

Many estuaries in temperate Australia are highly stratified salt-wedge estuaries that have a distinct freshwater, brackish and marine layer (Kurup et al. 1998). Salt-wedge estuaries are highly dynamic ecosystems that are constantly changing (Newton 1996, Kurup et al. 1998). Elevated prey concentrations combined with optimal salinities and temperatures that are associated with salt-wedge estuaries can enhance larval grow rates and survival, which in turn improves recruitment (Sirois and Dobson 2000; Williams et al. 2013). We currently do not have an easy means for detecting associations between salt-wedge structure and formation, and larval distributions. Traditional ichthyoplankton studies are very coarse and limited to snapshots of the spatial distribution of larvae over time. Otolith microchemistry may be a method with sufficient resolution to resolve the fine-scale spatial distribution and dispersal patterns of larvae by reconstructing their water mass associations within an estuary over time.

This study focused on the larvae of black bream, *Acanthopagrus butcheri* (Sparidae); a species that typically spends its entire life within its natal estuary (Newton 1996; Sakabe et al. 2011; Williams et al. 2012) but see Gillanders et al. (2015). It is also a highly iconic species that is targeted by both recreational and commercial fisheries in temperate Australian estuaries. Importantly, it has also been the subject of extensive research into the influences of water chemistry, temperature, and salinity on otolith chemistry (Elsdon and Gillanders 2005a; b; c). Laboratory experiments have demonstrated that ambient water concentrations, salinity and temperature have variable and interactive effects on the uptake of Mn, Sr, and Ba into juvenile black bream otoliths (Elsdon and Gillanders 2002; 2003; 2004). Furthermore, otolith Ba:Ca concentrations from wild caught black bream reflect changes in ambient Ba:Ca concentrations (Elsdon and Gillanders 2005a). These findings suggest that otolith chemistry may be capable of reconstructing environment histories of estuarine fish that move across fine salinity gradients, such as black bream. As otolith chemistry can vary over short spatio-temporal scales (Elsdon and Gillanders 2006b), it is important to gain a sound understanding of how the physicochemical characteristics of the study system vary over time and space (Elsdon and Gillanders 2006a; b).

This study set three objectives; 1) Collect water samples at one meter depth intervals and at regular intervals along two neighboring estuaries (Mitchell and Tambo Rivers) to establish fine-scale variability in ambient concentrations of the following elements, ^7Li , ^{11}B , ^{24}Mg , ^{31}P , ^{34}S , ^{39}K , ^{43}Ca , ^{55}Mn , ^{63}Cu , ^{68}Zn , ^{88}Sr , ^{138}Ba and then use a multivariate approach to determine what environmental factors explain the variability; 2) Analyse the otoliths from larvae collected in the Mitchell River during the study of Williams et al. (2013), that were also collected at the same time and location as the water samples from the Mitchell River (objective 1), to establish fine-scale variability in concentration of elements ^7Li , ^{11}B , ^{24}Mg , ^{31}P , ^{34}S , ^{39}K , ^{43}Ca , ^{55}Mn , ^{63}Cu ,

^{66}Zn , ^{88}Sr , ^{138}Ba , ^{208}Pb ; and 3) Using a multivariate/multi-element approach, establish if there is a link between otolith chemistry, environmental variables and ambient chemistry to establish if otolith chemistry can be used to construct environmental histories of larvae that utilise estuaries.

Methods

Study area

Water samples and black bream larvae were collected from two tributary estuaries of the Gippsland Lakes; the Mitchell and Tambo Rivers (Fig. 1). The Gippsland Lakes are one of the largest estuarine lagoon systems in Australia (Fig. 1). These two tributary estuaries are micro-tidal, where tidal movement has little effect on the physical structure of the estuary (Gee 2014). The Mitchell and Tambo Rivers flow into the estuarine Lake King in close proximity to each other (~4.5 km) (Fig. 1). Both estuaries have been previously identified as providing important spawning habitat for black bream (Williams et al. 2012). Sampling sites were established at ~3 km intervals starting at the estuary mouth, along the Mitchell River (n = 8; Fig. 1) and Tambo River (n = 5; Fig. 1).

Estuarine water sampling

Estuarine water samples were collected over two sampling dates (15 November and 3 December 2008), coinciding with the peak black bream spawning period in the austral spring. Water samples (n = 104) were collected from all sites with a range of salinities from 0.1 – 29.2. Estuarine water samples were collected at one-meter depth intervals from the surface to the nearest one meter depth increment above the bottom. A 6-m PVC spiral hose attached to a submersible bilge pump (with all metal surfaces coated with an epoxy resin) was used to collect

water from each depth interval. Before collecting a water sample, the pump was lowered to the desired depth and run for several minutes. Each water sample was collected in an acid leached 50 ml Millipore™ centrifuge tube attached to a Steriflip filter unit with vacuum port and 0.22 µm filter paper and attached to another acid leached 50 ml Millipore™ centrifuge tube. The water sample was passed through the filter unit using a hand vacuum pump. After filtration, the tube used for water collection was discarded. The water samples were acidified to pH 2 with double-distilled HNO₃ within 24-48 hours of collection and were stored in the dark at room temperature prior to analysis. Filtered water samples were sent to the Australian Government National Measurement Institute for analysis using inductively coupled plasma optical emission spectrometry (ICP-OES) for the following elements: ⁷Li, ²⁴Mg, ³⁴S, ³⁹K, ⁴³Ca; and inductively coupled plasma atomic emission spectroscopy (ICP-MS) for the following elements: ¹¹B, ³¹P, ⁵⁵Mn, ⁶³Cu, ⁶⁸Zn, ⁸⁸Sr, ¹³⁸Ba and ²⁰⁸Pb. Reporting limits for these elements were as follows: 50 µg/L for S; 20 µg/L for K; 5 µg/L for B, Ca, Mg; 2 µg/L for P; and 1 µg/L for Ba, Cu, Li, Mn, Pb, Sr, Zn. For quality assurance and quality control, with every batch of 20 samples or less, 2 blanks were analysed, 1 blank spike (water spiked with a known amount of the elements of interest) and 1 sample spike (a client sample spiked with a known amount of the elements of interest). The criteria for the spiked samples were that they were within ± 20 % of expected values. The majority of samples contained Cu, Zn, and Pb concentrations that were below reporting limits and therefore these elements were removed from the dataset.

At each site of water collection, water quality variables were also recorded using a Hydrolab DS4X data sonde fitted with salinity, temperature, dissolved oxygen and turbidity probes.

Larval fish collection and otolith preparation

Black bream larvae were opportunistically collected from ichthyoplankton sampling from another study in the Mitchell conducted at the same time as the collection of water samples in November and December 2008 (Fig. 1; Williams et al. 2013). In November, larvae were collected at five sites spanning 15 km of the estuary and in December larvae were collected from 2 sites spanning 6 km of the estuary. Overall, 22 larvae came from the halocline water layer and 31 larvae were collected in the marine water layer (refer to Water Data Analysis section below for classification of 'water layer'. Also, refer to Williams et al. (2013) for more information on sampling methodology). Larval samples were washed from the 333 μm mesh ichthyoplankton net using estuarine water and preserved in 75% ethanol. Due to low numbers of larvae from ethanol preserved samples ($n = 33$), otoliths from formalin-preserved larvae ($n = 20$) collected during the same sampling trips were also used. Analysis of $\text{Me}:\text{Ca}_{\text{otolith}}$ (Me refers to each individual element) data from both ethanol and formalin preserved larvae that were collected from the same site and sampling dates revealed no significant differences between the two preservation methods in chemistry (MANOVA, $p > 0.05$). Therefore, we combined both sets of samples in all subsequent analyses ($n = 53$).

In the laboratory, black bream larvae were identified based on morphology and removed from the sample. Otoliths were removed by placing the larvae in a droplet of MilliQ water and one sagittal otolith was removed using an electro-chemically sharpened tungsten needle and fine tungsten forceps. The otoliths were placed in a cleaning solution (H_2O_2 buffered with NaOH) for 10-15 minutes before being rinsed three times in MilliQ water. After the third water rinse, each otolith was transferred to a gridded slide where it was mounted within a thin coat of Buehler EpoThin resin.

The elemental composition of the outer surface of the otolith (the most recently deposited material) was determined by Laser Ablation Inductively-Coupled Plasma Mass Spectrometry (LA-ICPMS). We used an Agilent 7700x ICPMS fitted with a HelEx laser ablation system located in the Department of Earth Sciences, University of Melbourne. Samples were ablated using a 19 μm spot, with a repetition rate of 5 Hz and a laser output energy of ~ 60 mJ, which results in a drill rate of 0.5 $\mu\text{m}/\text{sec}$. Samples were run in blocks of 16 with NIST 610, 612, and 614 calibration standards run before and after each block. Thirty-second blanks were acquired before ablating each standard and sample. The first 10 scans during otolith ablation were averaged and used as the measure of otolith edge chemistry. 10 scans takes 3 seconds, which corresponds to 1.5 microns of ablated otolith material, or ~ 1 -2 days of growth. Otolith chemical signatures are likely to be reflective of environmental conditions at the time of capture due to the larvae being pre-flexion stage and not able to swim against currents (Gee 2014) and that delays in elemental incorporation into otoliths typically occurs at later life stages due to greater biomass and physiological regulation (e.g., Elsdon and Gillanders 2005c). This is assuming the physico-chemical properties of the estuarine water containing and transporting larval bream are stable over time scales of a few days.

Single point calibrations were used to estimate concentrations for each element using either the NIST 610 or 612 standard, depending on which one most closely matched the count rates in the samples. Concentrations were expressed as a ratio to calcium to correct for variable sample yield during ablation. Based on this method, the limit of detection for each elemental ratio was (in $\mu\text{mol element/mol } ^{43}\text{Ca}$): ^7Li - 30.61, ^{11}B - 146.2, ^{24}Mg - 12.63, ^{31}P - 340.5, ^{34}S - 524.0, ^{39}K - 82.97, ^{55}Mn - 4.203, ^{63}Cu - 0.834, ^{66}Zn - 7.100, ^{88}Sr - 0.004, ^{138}Ba - 0.031, ^{208}Pb - 0.153. External precision (i.e. analytical reproducibility) for each elemental ratio based on repeated

analyses on the NIST standards not used for calibration was (in %RSD): ^7Li - 9.0, ^{11}B - 3.7, ^{24}Mg - 8.4, ^{31}P - 34.7, ^{34}S - 38.2, ^{39}K - 10.3, ^{55}Mn - 9.0, ^{63}Cu - 13.5, ^{66}Zn - 9.2, ^{88}Sr - 7.6, ^{138}Ba - 10.1, ^{208}Pb - 7.5. External precision was poor for ^{31}P and ^{34}S because count rates for these elements in the NIST 612 standard were close to the limit of detection for this method (this was not the case for samples). Inspection of the post-processed data revealed that 4 samples had extreme elemental ratios, suggesting contamination, and these were therefore excluded from further analysis.

Water data analysis

A factor 'water layer' (3 levels) was created based on salinity, with each sample classified as being from either the freshwater (salinity <2), halocline (salinity 2-10) or marine (salinity >10) layer. These categories were determined *a priori* based on the findings from Williams et al. (2012 and 2013) where a significant salinity gradient was observed at salinities between approximately <2 and >10. To incorporate the location of the sample along the estuary, distance from estuary mouth (DEM) was calculated as a proportion of the estuary length (i.e., 0 = estuary mouth, 1 = most upstream site) due to the differences in estuary length and the variable number of sites along each estuary. Spatio-temporal patterns in water chemistry were analysed using Multivariate Analysis of Covariance (MANCOVA). A full factorial model was used, with all $\text{Me:Ca}_{\text{water}}$ as the dependent variables, river, sampling date and water layer as independent (fixed) factors, and distance from estuary mouth (DEM) as the covariate. The full factorial model revealed significant three-way interactions with all independent factors and thus the model was re-run separately for each estuary (sampling date and water layer as fixed factors and DEM as the covariate) and separately for each sampling date (estuary and water layer as fixed factors and DEM as the covariate).

Multiple regressions were used with combined data from the Mitchell and Tambo Rivers to determine which environmental variables (salinity, temperature, dissolved oxygen and turbidity) influenced each $\text{Me:Ca}_{\text{water}}$. All variables were checked for collinearity using Pearson's correlation coefficients. For any relationship where Pearson's r was >0.7 , one of the variables was removed to avoid over-influencing any models. All possible models were run, with selection of the best model based on the lowest corrected Akaike Information Criterion (AICc) score and AICc weight. The AICc weights were used to establish that the lowest AICc was $>10\%$ different from the next best fitting model and more likely to describe the actual relationship.

Otolith data analysis

Spatio-temporal patterns in otolith chemistry were analysed using MANCOVA, with all $\text{Me:Ca}_{\text{otolith}}$ as the dependent variables, sampling date and water layer as independent (fixed) factors, and DEM as the covariate. Due to low replication across sites, samples were grouped into regions based on whether they came from the lower estuary (< 10 km from the estuary mouth; $n = 28$) or upper estuary (> 10 km from the estuary mouth; $n = 25$) and whether they were collected from the halocline or marine water layer (no larvae were caught in the freshwater layer).

As with water chemistry, multiple regression was used to determine which subset of environmental variables [salinity 1 (the nearest measurement from the upper edge of plankton net), salinity 2 (the nearest measurement from the lower edge of plankton net), temperature, dissolved oxygen, turbidity, and ambient $\text{Me:Ca}_{\text{water}}$], influenced each $\text{Me:Ca}_{\text{otolith}}$. A SIMPROF procedure was used to objectively allocate each otolith to a cluster based on $\text{Me:Ca}_{\text{otolith}}$. Chi-squared tests were then used to establish if otolith clusters were non-randomly distributed with

respect to each combination of estuary region and water layer (upper-marine, upper-halocline, lower-marine and lower-halocline), sampling date or distance from estuary mouth.

All data (water and otolith) were log, natural log or fourth root transformed to meet the assumptions of normality and homogeneity of variances. All multiple analyses of covariance (MANCOVA) were done using SPSS (Version 19.0). SIMPROF was done using Primer (Version 6). Multiple regressions were done using Systat (Version 12) with the best subsets feature to select the models of best fit.

Results

The saline influence penetrated at least as far as 21 km upstream in the Mitchell River and over 12 km upstream in the Tambo River over the study period (Fig. 2). The hydrology of each estuary was different over the sampling dates, providing a useful contrast of water chemistry changes between the two sampling dates (Fig. 2). On 15 November 2008 there was very little freshwater flowing into both the estuaries and only the most upstream sites were stratified (Fig. 2). Prior to the 3 December 2008 sampling, there had been a large rainfall event (>100 mm) that displaced the saline layer downstream (Fig. 2).

Variability in ambient water chemistry

MANCOVA tests revealed some significant spatial and temporal differences in water chemistry between and within estuaries and sampling date (Tables 1, 2, S1 and S2). Despite the large freshwater flow event that occurred between the two sampling dates, there was no significant difference in water chemistry between sampling dates for the Mitchell River (Table 1). However, there was a significant difference between sampling dates in the Tambo River (Table

1) and this was attributed to greater concentrations of Li:Ca and Mn:Ca on 3 December (Table S1). Tests for spatial variance revealed significant differences in water chemistry between the fresh (salinity <2), halocline (salinity 2-10) and marine (salinity >10) layers and this was attributed to differences in Li:Ca (highest in the halocline layer) and Mn:Ca and Ba:Ca (highest in the freshwater layer) (Table 2 and S2). The covariate distance from estuary mouth (DEM) was significantly different in both estuaries and this was most likely due to the salt-wedge effect (e.g., freshwater was more common in the upper estuary and marine water more common in the lower estuary) (Table 2). A comparison of estuaries within each sampling date revealed significant effects of estuary, water layer and DEM (Table S2). These effects were most likely driven by differences in estuary lengths and influences of freshwater flows and the salt-wedge structure. The main elements contributing to these differences were Li:Ca, Mg:Ca and Mn:Ca (Table S2).

Interpolating data for each $\text{Me:Ca}_{\text{water}}$ and plotting as longitudinal profiles visually displays the spatial and temporal variability within the estuaries on an individual elemental basis (Fig. 3,4). The vertical and horizontal structure of $\text{Me:Ca}_{\text{water}}$ (Figs. 3,4) closely reflects the salt-wedge structure of the estuaries (Fig. 2). Multiple regressions with combined data from the Mitchell and Tambo Rivers revealed that the ‘best’ fitting models used salinity and turbidity as the predictive variables for $\text{Me:Ca}_{\text{water}}$ (Table 3). In addition to salinity and turbidity, dissolved oxygen and often water temperature were included in the ‘best model’ selection, highlighting the complexity of the relationship between water chemistry and environmental variables (Table 3).

Variability in larval fish otolith chemistry

MANCOVA tests revealed significant spatial and temporal patterns in otolith chemistry (Fig. 5; Tables 4, 5). Significant spatial variability was detected with differences in otoliths

collected from the marine water layer compared to the halocline layer driven by difference in P:Ca and the heavy metals Cu:Ca, Zn:Ca and Pb:Ca (Tables 4, 5). Significant differences between the two sampling dates were driven by the heavy metals Cu:Ca and Zn:Ca (Fig. 5; Tables 4, 5). Distance from estuary mouth (DEM) showed significant differences, with otoliths in the lower estuary having higher concentrations of Li:Ca while otoliths in the upper estuary had higher concentration of Cu:Ca (Fig. 5; Tables 4,5).

Linking otolith chemistry with environmental variables and water chemistry

Multiple regression models revealed which subset of environmental variables, including ambient Me:Ca_{water}, best explained variability in otolith chemistry (Table 6). The majority of models included salinity, dissolved oxygen and temperature. When salinity was included, the higher salinity, salinity 2, was more often used in model selection than salinity 1. Turbidity was also selected in the models for Li:Ca_{otolith}, B:Ca_{otolith}, S:Ca_{otolith} and K:Ca_{otolith} (Table 6). Only ambient Li:Ca_{water}, K:Ca_{water} and Mn:Ca_{water} were selected for their corresponding Me:Ca_{otolith} models (Table 6).

The SIMPROF procedure objectively allocated individual otoliths into nine clusters based on their Me:Ca_{otolith}, four clusters contained single otoliths (outliers), and three clusters contained >10 otoliths (Fig. S1). Chi-square tests used to compare otolith clusters with site characteristics of each otolith revealed that clusters were non-randomly distributed with respect to regional water layers ($df = 8, p = 0.001$), sampling date ($df = 1, p = 0.039$), and distance from the estuary mouth ($df = 4, p = 0.001$).

Discussion

The influence of freshwater flow into the estuary and the corresponding salinity structure was reflected in the elemental composition of the Mitchell and Tambo River estuaries. Using a multi-elemental/multivariate approach we were able to identify three unique water layers that were defined by salinity. The freshwater, halocline and marine water layers, which were decided *a priori*, all had a statistically unique chemical signature that has the potential to be reflected in the otoliths of fish. This study was limited by the fact larval black bream otoliths were only available from the Mitchell River. While the otolith chemistry was not as clearly resolved as for water chemistry, we were able to apply multivariate analyses to identify clusters or groups of otoliths that could be assigned to the upper or lower estuary, water layer and sampling date. These findings highlight the importance of using a multivariate approach for characterising estuarine water masses and fish otolith chemical signatures, and suggest such an approach could be a useful tool for identifying natal origins and tracking dispersal and habitat use by larval fish within estuaries (Hicks et al. 2010; Tanner et al. 2013; Reis-Santos 2015).

The ability to distinguish between otoliths collected from different regions within an estuary relies on there being variation in physicochemical properties of the water (Campana 1999; Schaffler et al. 2014; Mohan and Walther 2015). Estuarine water chemistry is highly variable and closely linked to freshwater flows and tidal movement (Aston 1978; Walther and Nims 2015). The variable nature of estuaries was observed during this study, with a distinct shift in the position of the salt-wedge due to a large increase in freshwater flows between the two sampling periods. As expected, this was also reflected in the $\text{Me}:\text{Ca}_{\text{water}}$ longitudinal profiles between sampling dates. These processes influence the water chemistry through mixing of water masses and ion exchange between sediments and organic matter with the water (Wilson 1975; Coffey et al. 1997; Walther and Nims 2015). The processes resulting in the unique halocline

signature is unknown and is beyond the scope of this study, but, it is likely a function of complex chemical reactions occurring at the interface between the freshwater and marine layers (Aston 1978; Millward 1995). Physical factors such as salinity, temperature, turbidity and dissolved oxygen all influence water chemistry (Aston 1978; Millward 1995). It is through these complex processes that unique water chemistry signatures are established for individual estuaries.

Between estuaries, factors such as geography, precipitation, tides and groundwater hydrology all combine to influence the ambient chemical signature of the water (Secor et al. 1995; Elsdon et al. 2008; Walther and Nims 2015). This study has clearly emphasized that two neighbouring estuaries can have different hydrology, with the Mitchell River and its larger catchment picking up more freshwater and flushing the salt wedge downstream during a freshwater flow event in early December 2008. While at the same time the Tambo River was less affected by the same freshwater flow event. This was clearly reflected in significant differences in water chemistry between these two neighbouring estuaries. Not only between the two estuaries, but, also when comparing each individual water layer (freshwater, halocline and marine) between estuaries. These facts highlight the importance of having a base understanding of the water chemistry of individual estuaries when seeking to understand variation in otolith chemistry.

In this study, the variability in otolith chemistry was less spatially structured than water chemistry. To be able to accurately establish fish environmental histories at fine spatial scales based on otoliths, there needs to be a predictable link between environmental conditions (salinity, temperature, ambient chemistry variability) and otolith chemistry (Elsdon et al. 2008; Tanner et al. 2013; Reis-Santos et al. 2015). There also needs to be some understanding of the potential for larval movement, either as a function or independently of estuarine circulation, which could lead to spatial decoupling between individuals and the water masses responsible for the resulting

otolith chemistry. In the present study, black bream larvae in early life are poor swimmers and the Gippsland Lakes is a microtidal system with weak circulation except during high rainfall (Gee 2014), suggesting that spatial decoupling is unlikely the explanation. Alternatively, two other possibilities included; 1) the degree of signal integration due to the LA-ICPMS methodology or; 2) physiological variability among individual larvae (Sturrock et al. 2014; 2015), could provide an explanation for why otolith chemistry patterns were less structured than water chemistry.

Regardless of the underlying reason, otoliths could still be assigned into groups based on salinity and region within estuary. The key elements in distinguishing between the marine layer and the halocline layer within an estuary were Li:Ca, K:Ca, Mn:Ca, and Ba:Ca. In particular, Li:Ca had a strong relationship between otolith and water chemistry across a range of salinities, consistent with other studies using a multi-element approach in estuaries (Hicks et al. 2010; Tanner et al. 2013; Reis-Santos et al. 2015). Hicks et al. (2010) suggested that a combination of Sr:Ca and Li:Ca could be used to identify movements over fine salinity scales and also to minimise false positives. In this study, $\text{Mn:Ca}_{\text{water}}$ was also an important factor in discriminating between the halocline and marine layers and was correspondingly related to $\text{Mn:Ca}_{\text{otolith}}$. Manganese (Mn) varies greatly in estuaries and this is likely due to microbial activity and linked to hypoxia/anoxia (Mohan et al. 2014; Ramsay et al. 2015; Turner and Limberg 2015). It is these characteristics that make Mn an element of interest for distinguishing between water layers as the bottom marine layers tend to be hypoxic, even anoxic, in stratified estuaries, while the halocline and upper layers are either well mixed or are an area of high productivity.

Unlike in other studies (Thorrold et al. 1998; Bradbury et al. 2008; Mohan and Walther 2015), Sr:Ca did not prove to be a useful element, with no relationship between otolith

concentrations and ambient concentrations or salinity. Laboratory experiments using black bream otoliths have shown that otolith concentrations of Sr and Ba are explained by complex interactions between ambient concentrations, salinity and temperature (Elsdon and Gillanders 2002; 2003; 2004). $\text{Sr}:\text{Ca}_{\text{otolith}}$ and $\text{Ba}:\text{Ca}_{\text{otolith}}$ have been widely used to establish environmental histories due to the presumed positive correlation for Sr and the negative correlation for Ba with salinity (Kimura et al. 2000; Secor and Rooker 2000; Gillanders 2005). However, neither of these elements contributed to the variability in otolith chemistry in the current study. Instead, concentrations of Li, Mn and the heavy metals Cu, Zn and Pb showed the greatest variability. This finding further supports the need for a multi-element approach to investigate fish distributions and movement in estuaries and the need for future validation studies on the mechanisms controlling incorporation of these other elements into otoliths (Macdonald and Crook 2010; Tanner et al. 2013).

Further investigation, over a greater temporal period, is needed to further elucidate if fine scale environmental chronologies can be established from otoliths in estuarine environments. Although there is evidence for temporal variability in otolith chemistry for estuarine species (Thorrold et al. 1998; Gillanders 2002; Schaffler et al. 2014), the degree of temporal variability is likely dependent on the element of interest, with Ca, Ba and Mn varying over short temporal scales of days to weeks compared to Sr that varies over seasonal time scales (Elsdon and Gillanders 2006a). Elsdon and Gillanders (2006a) went on to suggest that Sr would be an ideal tracer for determining differences between environments as changes in Sr concentrations would constitute large spatial and temporal shifts. In contrast, Ca, Ba and Mn would be better suited to determining fine-scale shifts if these elements are combined and correlated with environmental variables such as salinity and temperature (Macdonald and Crook 2010; Tanner et al. 2013;

Ramsay et al. 2015). This suggests that it will be important to confirm which elements vary at appropriate spatio-temporal scales for the particular question being addressed, if otolith chemistry is to be a useful method to employ.

It is also important to have some prior knowledge on the movements of the species of interest. Fish larvae may pass through different water layers either by convergence, retention, active migration or diel vertical migration at different life stages, and the ability to distinguish or map this movement may prove a useful tool for fisheries and environmental scientists and managers. For example, the establishment of the halocline layer and/or estuarine turbidity maximum is believed to be an important process for spawning and larval survival (North and Houde 2001; Islam et al. 2006; Williams et al. 2013). Numerous studies have been able to correctly assign juvenile fish, based on otolith chemistry, to the estuary of origin (Thorrold et al. 1998; Gillanders and Kingsford 2000; Walther et al. 2008) or to the site or location of origin within an estuary (Dorval et al. 2005; Schaffler et al. 2014; Reis-Santos et al. 2015). In the present study, we have shown that it is also possible to distinguish broad capture locations for larval fish within an estuary, and to distinguish fine-scale differences in association between water layers defined by differences in salinity. In future, this technique could also be applied to generate multi-elemental chronologies of the larval period, with the potential to determine the timing and age of larval residency within each region and water mass within an estuary, and the consequences this might have for larval growth and survival. This knowledge would lead to a better understanding of the larval ecology of estuarine fishes and, in particular, the important environmental drivers of variability in year class strength.

References

- Aschenbrenner, A., Ferreira, B. P., and J. R. Rooker. 2016. Spatial and temporal variability in the otolith chemistry of the Brazilian snapper *Lutjanus alexandrei* from estuarine and coastal environments. *J. Fish Biol.* **89**: 753-769.
- Aston, S. R. 1978. Estuarine Chemistry, p. 361-440. In J. P. Riley and R. Chester [eds.], *Chemical Oceanography*. Academic Press.
- Barbee, N. C., and S. E. Swearer. 2007. Characterizing natal source population signatures in the diadromous fish *Galaxias maculatus*, using embryonic otolith chemistry. *Mar. Ecol. Prog. Ser.* **343**: 273-282.
- Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta.* **64**: 1705-1714.
- Beck, M.W., J. K. L. Heck, K. W. Able, and others. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* **51**: 633.
- Bradbury, I. R., S. E. Campana, and P. Bentzen. 2008. Estimating contemporary early life-history dispersal in an estuarine fish: integrating molecular and otolith elemental approaches. *Mol. Ecol.* **17**: 1438-1450.
- Campana, S.E. 1999. Review: Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser.* **188**: 263-297.
- Coffey M., F. Dehairs, O. Collette, G. Luther, T. Church, and T. Jickells. 1997. The behaviour of dissolved barium in estuaries. *Estuarine, Coastal Shelf Sci.* **45**: 113-121.
- Crook, D.A., J. I. MacDonald, J. P. O'Conner, and B. Barry. 2006. Use of otolith chemistry to examine patterns of diadromy in the threatened Australian grayling *Prototroctes maraena*. *J. Fish Biol.* **69**: 1330-1344.

- Day, J.W., C. A. S. Hall, W. M. Kemp, W.M. and A. Yanez-Arancibia. 1989. Estuarine Ecology. A Wiley-Interscience Publication.
- Dolbeth, M., F. Martinho, I. Viegas, H. Cabral, and M. A. Pardal. 2008. Estuarine production of resident and nursery fish species: Conditioning by drought events? Estuarine, Coastal Shelf Sci. **78**: 51-60.
- Dorval, E., C. M. Jones, R. Hannigan, and J. Van Montfrans. 2005. Can otolith chemistry be used for identifying essential seagrass habitats for juvenile spotted seatrout, *Cynoscion nebulosus*, in Chesapeake Bay? Mar. Freshwater Res. **56**: 645-653.
- Dorval, E. C., C. M. Jones, R. Hannigan, and J. Van Montfrans. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Can. J. Fish. Aquat. Sci. **64**: 411-424.
- Elsdon, T. S., and B. M. Gillanders. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining fish life histories. Can. J. Fish. Aquat. Sci. **59**: 1796-1808.
- Elsdon, T. S., and B. M. Gillanders. 2003. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. Mar. Ecol. Prog. Ser. **260**: 263-272.
- Elsdon, T. S., and B. M. Gillanders. 2004. Fish otolith chemistry influenced by exposure to multiple environmental variables. J. Exp. Mar. Biol. Ecol. **313**: 269-284.
- Elsdon, T. S., and B. M. Gillanders. 2005a. Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. Can. J. Fish. Aquat. Sci. **62**: 1143-1152.
- Elsdon, T. S., and B. M. Gillanders. 2005b. Consistency of patterns between laboratory experiments and field collected fish in otolith chemistry: an example and applications for salinity reconstructions. Mar. Freshwater Res. **56**: 609-617.

- Elsdon, T. S., and B. M. Gillanders. 2005c. Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. *Mar. Ecol. Prog. Ser.* **285**: 233-243.
- Elsdon, T. S., and B. M. Gillanders. 2006a. Temporal variability in strontium, calcium, barium, and manganese in estuaries: Implications for reconstructing environmental histories of fish from chemicals in calcified structures. *Estuarine, Coastal Shelf Sci.* **66**: 147-156.
- Elsdon, T. S., and B. M. Gillanders. 2006b. Identifying migratory contingents of fish by combining otolith Sr:Ca with temporal collections of ambient Sr:Ca concentrations. *J. Fish Biol.* **69**: 643-657.
- Elsdon, T. S., B. K. Wells, S. E. Campana, and others. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypothesis, assumptions, limitations and inferences. *Oceanogr. Mar. Biol.* **46**: 297-330.
- Fraille, I., H. Arrizabalaga, J. Santiago, N. Goñi, I. Arregi, S. Madinabeitia, R. J. D. Wells, and J. R. Rooker. 2016. Otolith chemistry as an indicator of movements of albacore (*Thunnus alalunga*) in the North Atlantic Ocean. *Mar. Freshwater Res.* **67**: 1002–1013.
- Gee, E. M. 2014. Linking freshwater flows, salt wedge dynamics and black bream dispersal in the Mitchell River sub-estuary. Ph.D. thesis. Univ. of Melbourne.
- Gillanders, B. M. 2002. Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? *Mar. Ecol. Prog. Ser.* **240**: 215-223.
- Gillanders, B. M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. *Estuarine, Coastal Shelf Sci.* **64**: 47-57.

- Gillanders, B. M., and M. J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Mar. Ecol. Prog. Ser.* **141**: 13-20.
- Gillanders, B. M., and M. J. Kingsford. 2000. Elemental fingerprints of otoliths of fish may distinguish nursery habitats. *Mar. Ecol. Prog. Ser.* **201**: 273-286.
- Gillanders, B. M., and M. J. Kingsford. 2002. Impact of changes in flow of freshwater on estuarine and open coastal habitats and the associated organisms. *Oceanogr. Mar. Biol.* **40**: 233-309.
- Gillanders, B. M., C. Izzo, Z. A. Doubleday and Q. Ye. 2015. Partial Migration: growth varies between resident and migratory fish. *Biol. Lett.* **11**: 1-5.
- Hicks, A., J. P. Closs, and S. E. Swearer. 2010. Otolith microchemistry of two amphidromous galaxiids across an experimental salinity gradient: A multi-elemental approach for tracking diadromous migrations. *J. Exp. Mar. Biol. Ecol.* **394**: 86-97.
- Islam, M. S., M. Hibino, and M. Tanaka. 2006. Distribution and diets of larval and juvenile fishes: Influence of salinity gradient and turbidity maximum in a temperate estuary in upper Ariake Bay, Japan. *Estuarine, Coastal Shelf Sci.* **68**: 62-74.
- Kalish, J. M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *J. Exp. Mar. Biol. Ecol.* **132**: 151-178.
- Kimura, R., D. H. Secor, E. D. Houde, and P. M. Piccoli. 2000. Up-estuary dispersal of young-of-the-year bay anchovy *Anchoa mitchilli* in the Chesapeake Bay: inferences from microprobe analysis of strontium in otoliths. *Mar. Ecol. Prog.* **208**: 217-227.

- Koster, W., and D. Crook. 2008. Diurnal and nocturnal movements of river blackfish (*Gadopsis marmoratus*) in a south-eastern Australian upland stream. *Ecology of freshwater fish* **17**: 146-154.
- Kraus, R. T., and D. H. Secor. 2004. Incorporation of strontium into otoliths of an estuarine fish. *J. Exp. Mar. Biol. Ecol.* **302**: 85-106.
- Kurup, G. R., D. P. Hamilton, and J. C. Patterson. 1998. Modelling the effect of seasonal flow variations on the position of salt wedge in a microtidal estuary. *Estuarine, Coastal Shelf Sci.* **47**: 191-208.
- Macdonald, J. I., and D. A. Crook. 2010. Variability in Sr:Ca and Ba:Ca ratios in water and fish otoliths across an estuarine salinity gradient. *Mar. Ecol. Prog.* **413**: 147-161.
- Millward, G. E. 1995. Processes affecting trace element speciation in estuaries. A review. *Analyst.* **120**: 609-614.
- Mohan, J., M. S. Rahman, P. Thomas, and B. Walther. 2014. Influence of constant and periodic experimental hypoxic stress on Atlantic croaker otolith chemistry. *Mar. Ecol. Prog.* **20**: 1-11.
- Mohan, J., and B. Walther. 2015. Spatiotemporal Variation of Trace Elements and Stable Isotopes in Subtropical Estuaries: II. Regional, Local, and Seasonal Salinity-Element Relationships. *Estuaries Coasts* **38**: 769-781.
- Newton, G. M. 1996. Estuarine ichthyoplankton ecology in relation to hydrology and zooplankton dynamics in a salt wedge estuary. *Mar. Freshwater Res.* **47**: 99-111.
- North, E. W., R. R. Hood, S. -Y. Chao, and L. P. Sanford. 2005. The influence of episodic events on transport of striped bass eggs to the estuarine turbidity maximum nursery area. *Estuaries* **28**: 108-123.

- North, E. W., and E. D. Houde. 2001. Retention of white perch and striped bass larvae: Biological-physical interactions in Chesapeake Bay Estuarine Turbidity Maximum. *Estuaries* **24**: 756-769.
- North, E. W., and E. D. Houde. 2003. Linking ETM physics, zooplankton prey, and fish early life histories to striped bass *Morone saxatilis* and white perch *M. americana* recruitment. *Mar. Ecol. Prog.* **260**: 219-236.
- North, E. W., and E. D. Houde. 2006. Retention mechanisms of white perch (*Morone americana*) and striped bass (*Morone saxatilis*) early-life stages in an estuarine turbidity maximum: an integrative fixed-location and mapping approach. *Fisheries Oceanography*. **15**: 429-450.
- Potter, I. C., L. E. Beckley, A. K. Whitfield, and R. C. J. Lenanton. 1990. Comparisons between the Roles Played by Estuaries in the Life-Cycles of Fishes in Temperate Western Australia and Southern Africa. *Environ. Biol. Fishes*. **28**: 143-178.
- Ramsay, A. L., R. N. Hughes, S. R. Chenery, and I. D. McCarthy. 2015. Biogeochemical tags in fish: predicting spatial variations in strontium and manganese in *Salmo trutta* scales using stream water geochemistry. *Can. J. Fish. Aquat. Sci.* **72(3)**: 422-433.
- Reis-Santos, P., S. E. Tanner, S. França, R. P. Vasconcelos, B. M. Gillanders, and H. N. Cabral. 2015. Connectivity within estuaries: An otolith chemistry and muscle stable isotope approach. *Ocean & Coastal Management*. **118**: 51-59.
- Rooker, J. R., D. R. J. Wells, D. G. Itano, S. R. Thorrold, and J. M. Lee. (2016). Natal origin and population connectivity of bigeye and yellowfin tuna in the Pacific Ocean. *Fish. Oceanogr.* **25**: 277-201.
- Sakabe, R., J. M. Lyle, and C. M. Crawford. 2011. The influence of freshwater inflows on spawning success and early growth of an estuarine resident fish species, *Acanthopagrus butcheri*. *J. Fish Biol.* **78**: 1529-1544.

- Schaffler, J. J., T. J. Miller, T.J., and C. M. Jones. 2014. Spatial and Temporal Variation in Otolith Chemistry of Juvenile Atlantic Menhaden in the Chesapeake Bay. *Trans. Am. Fish. Soc.* **143**: 1061-1071.
- Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *J. Exp. Mar. Biol. Ecol.* **192**: 15-33.
- Secor, D. H., and J. R. Rooker. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fisheries Research* **46**: 359-371.
- Shoji, J., T. Ohta, and M. Tanaka. 2006. Effects of river flow on larval growth and survival of Japanese seaperch *Lateolabrax japonicus* (Pisces) in the Chikugo River estuary, upper Ariake Bay. *J. Fish Biol.* **69**: 1662-1674.
- Sirois, P., and J. J. Dobson. 2000. Influence of turbidity, food density and parasites on the ingestion and growth of larval rainbow smelt *Osmerus mordax* in an estuarine turbidity maximum. *Mar. Ecol. Prog.* **193**: 167-179.
- Stanley, R. R. E., I. R. Bradbury, C. DiBacco, P. V. R., Snelgrove, S. R. Thorrold and S. S. Killen. 2015. Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). *ICES J. Mar. Sci.* **72**: 2350-2363.
- Sturrock, A. M., C. N. Trueman, J. A. Milton, C. P. Waring, M. J. Cooper, and E. Hunter. 2014. Physiological influences can outweigh environmental signals in otolith microchemistry research. *Mar. Ecol. Prog.* **500**: 245-264.
- Sturrock, A. M., E. Hunter, J. A. Milton, R. C. Johnson, C. P. Waring, C. N. Trueman and E. Leder. 2015. Quantifying physiological influences on otolith microchemistry. *Methods Ecol. Evol.* **6**: 806-816.

- Swearer, S. E., and J. E. Caselle. 1999. Larval retention and recruitment in an island population of a coral-reef fish. *Nature* **402**: 799.
- Swearer, S. E., G. E. Forrester, M. A. Steele, A. J. Brooks, and D. W. Lea. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. *Estuarine, Coastal Shelf Sci.* **56**: 1111-1123.
- Tanner, S. E., P. Reis-Santos, R. P. Vasconcelos, H. Cabral, and S. R. Thorrold. 2013. Does otolith geochemistry record ambient environmental conditions in estuaries? *J. Exp. Mar. Biol. Ecol.* **441**: 7-15.
- Thorrold, S. R., C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 1998. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). *Limnol. Oceanogr.* **43**: 1826-1835.
- Thorrold, S. R., G. P. Jones, M. E. Hellberg, R. S. Burton, S. E. Swearer, J. E. Neigel, S. G. Morgan, and R. R. Warner. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bull. Mar. Sci.* **70**: 291-308.
- Thorrold, S. R., G. P. Jones, S. Planes, and J. A. Hare. 2006. Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. *Can. J. Fish. Aquat. Sci.* **63**: 1193-1197.
- Turner, S. M., and K. E. Limburg. 2015. Does Daily Growth Affect the Rate of Manganese Uptake in Juvenile River Herring Otoliths? *Trans. Am. Fish. Soc.* **144**: 873-881.
- Vasconcelos, R. P., P. Reis-Santos, S. Tanner, A. Maia, C. Latkoczy, D. Günther, M. J. Costa, and H. Cabral. 2008. Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. *Estuarine, Coastal Shelf Sci.* **79**: 317-327.

- Walther, B., and M. Nims. 2015. Spatiotemporal Variation of Trace Elements and Stable Isotopes in Subtropical Estuaries: I. Freshwater Endmembers and Mixing Curves. *Estuaries Coasts* **38**: 754-768.
- Walther, B. D., and S. R. Thorrold. 2008. Continental-scale variation in otolith geochemistry of juvenile American shad (*Alosa sapidissima*). *Can. J. Fish. Aquat. Sci.* **65**: 2623-2635.
- Walther, B. D., S. R. Thorrold, and J. E. Olney. 2008. Geochemical signatures in otoliths record natal origins of American Shad. *Trans. Am. Fish. Soc.* **137**: 57-69.
- Warren Jr, M. L., and M. G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. *Trans. Am. Fish. Soc.* **127**: 637-644.
- Williams, J., J. S. Hindell, G. P. Jenkins, and S. E. Swearer. 2012. Spatial and temporal variation in black bream, *Acanthopagrus butcheri* (Munro, 1959), egg and larval distribution in response to freshwater flows in a drought affected estuary. *J. Fish Biol.* **80**: 2281-2301.
- Williams, J., G. P. Jenkins, J. S. Hindell, and S. E. Swearer. 2013. Linking freshwater flows and salt-wedge dynamics with the distribution of black bream, *Acanthopagrus butcheri*, eggs larvae and potential prey in a drought affected estuary. *Mar. Ecol. Prog.* **483**: 273-287.
- Wilson, R. L. 1975. Salinity and the major elements of seawater, p. 365-413. In J. P. Riley and G. Skirrow [eds.], *Chemical Oceanography*. Academic Press.

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Figure Legends

Fig. 1. A map of the Gippsland Lakes with the sampling sites marked as $\circ$ for water only samples and $\bullet$ for water and otolith samples. Inset: Location of Gippsland Lakes in south-eastern Australia.

Fig. 2. Longitudinal salinity profiles for the Mitchell and Tambo Rivers during the two sampling periods.

Fig. 3. Longitudinal profiles of the interpolated data for each Me:Ca concentrations for the 15 November 2008 sampling period.

Fig. 4. Longitudinal profiles of the interpolated data for each Me:Ca concentrations for the 3 December 2008 sampling period.

Fig. 5. Mean Me:Ca_{otolith} ratios along distance from estuary mouth (DEM) for samples collected on the 15 November 2008 ($\circ$) and 3 December 2008 ($\bullet$). Note the different transformations for each plot (error bars are standard error).

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Table 1. Results from the MANCOVA testing for temporal effects in $\text{Me:Ca}_{\text{water}}$ between sampling dates and water layer with distance from estuary mouth (DEM) as the covariate for samples from the Mitchell River and Tambo River. SD = Sampling date; WL = Water layer; DEM = Distance from estuary mouth. Significant values have been bolded. Some terms are missing due to a lack of degrees of freedom (na).

	Mitchell			Tambo		
	df	F	P	df	F	P
Sampling date (SD)	9,58	0.964	0.487	9,23	30.854	<0.001
Water layer (WL)	18,116	7.627	<0.001	18,46	7.086	<0.001
DEM	9,58	3.062	0.005	9,23	105.438	<0.001
SD * WL	18,116	1.644	0.060	na	na	na
SD * DEM	9,58	2.065	0.048	9,23	2.688	0.027
WL * DEM	18,116	2.875	<0.001	18,46	25.420	<0.001
SD * WL * DEM	18,166	2.692	0.001	na	na	na

Table 2. Results from the MANCOVA testing for spatial effects in Me:Ca_{water} between estuary and water layer with distance from river estuary (DEM) as the covariate for samples from the Mitchell River and Tambo River and temporal effects in Me:Ca_{water} between estuary and water layer with distance from estuary mouth (DEM) as the covariate for samples from 15 November 2008 and 3 December 2008. E = Estuary; WL = Water layer; DEM = Distance from estuary mouth. Significant values have been bolded. Some terms are missing due to a lack of degrees of freedom (na).

	15 November 2008			3 December 2008		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Estuary (E)	9,41	3.784	0.002	9,40	2.878	0.010
Water layer (WL)	18,82	7.589	<0.001	18,80	16.479	<0.001
DEM	9,41	11.089	<0.001	9,40	9.208	<0.001
E * WL	9,41	3.711	0.002	9,40	1.108	0.380
E * DEM	9,41	2.551	0.020	9,40	6.625	<0.001
WL * DEM	18,82	6.691	<0.001	18,80	14.402	<0.001
E * WL * DEM	9,41	3.370	0.004	9,40	5.609	<0.001

Table 3. The best fitting models from a sub-set of all models, selected based on corrected Akaike information Criterion (AICc) scores and their corresponding weights for each Me:Ca water. The hydrological variable used were salinity (Sal), temperature (Temp), dissolved oxygen (DO), natural log turbidity (Ln(Turb)). * P < 0.05, ** P < 0.01.

Ratio	Best sub-set	Adj. R^2	AIC_c	Δ_i	w_i
Li:Ca	Ln(Turb)	0.000 ^{NS}	-239.865	0	0.353
B:Ca	Ln(Turb)	0.003 ^{NS}	-121.381	0	0.343
Mg:Ca	Ln(Turb), Sal	0.559 ^{**}	22.207	0	0.372
P:Ca	Ln(Turb), DO, Temp, Sal	0.767 ^{**}	-137.645	0	0.488
S:Ca	Ln(Turb), DO, Temp, Sal	0.606 ^{**}	107.521	0	0.360
K:Ca	Ln(Turb), DO, Temp, Sal	0.695 ^{**}	-205.549	0	0.350
	Ln(Turb), Temp, Sal	0.597 ^{**}	-205.387	0.162	0.323
Mn:Ca	Sal, DO	0.916 ^{**}	-22.584	0	0.420
Sr:Ca	Ln(Turb), DO, Temp, Sal	0.521 ^{**}	-323.061	0	0.444
Ba:Ca	Sal, DO, Ln(Turb)	0.848 ^{**}	-6.826	0	0.331

Table 4. Results from MANCOVA testing for spatio-temporal effects in Me:Ca_{otolith} between sampling dates and water layer with distance from estuary mouth (DEM) as the covariate. SD = Sampling date; WL = Water layer; DEM = Distance from estuary mouth. Significant values have been bolded.

	df	F	P
Sampling dates (SD)	12,33	7.745	< 0.001
Water layer (WL)	12,33	4.565	< 0.001
DEM	12,33	5.035	< 0.001
SD * WL	12,33	3.587	< 0.001

Table 5. Results from the univariate tests of between-subject effects from the MANCOVA testing for spatio-temporal effects in each Me:Ca_{otolith} between sampling dates and water layer with distance from estuary mouth (DEM) as the covariate. SD = Sampling date; WL = Water layer; DEM = Distance from estuary mouth. Significant values have been bolded.

	Sampling Date		Water Layer		SD * WL		DEM	
	df 1, 44							
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Log(Li:Ca)	14.609	< 0.001	3.252	ns	1.090	ns	53.688	< 0.001
4 th Root(B:Ca)	2.715	ns	0.132	ns	1.029	ns	.825	ns
Log(Mg:Ca)	12.837	< 0.001	12.241	< 0.001	7.532	< 0.05	1.910	ns
4 th Root(P:Ca)	0.400	ns	3.877	ns	0.009	ns	.260	ns
Log(S:Ca)	0.067	ns	0.245	ns	1.105	ns	.126	ns
4 th Root(K:Ca)	0.547	ns	0.000	ns	0.683	ns	.194	ns
4 th Root(Mn:Ca)	0.092	ns	11.498	< 0.001	0.062	ns	3.218	ns
4 th Root(Cu:Ca)	9.210	< 0.01	14.546	< 0.001	6.515	< 0.05	.146	ns
4 th Root(Zn:Ca)	6.797	< 0.05	15.250	< 0.001	11.640	< 0.001	.004	ns
Sr:Ca	0.185	ns	.510	ns	0.106	ns	.225	ns
Ba:Ca	0.702	ns	8.634	< 0.01	0.729	ns	.010	ns
4 th Root(Pb:Ca)	4.967	< 0.05	18.425	< 0.001	0.468	ns	1.613	ns

Table 6. Best subset of variables that explain each Me:Ca_{otolith} ratio from otolith chemistry analysis. * P < 0.05, ** P < 0.01. Me:Ca_{water} has been bolded when included to highlight the significant link between water and otolith chemistry.

Ratio	Best Subset	Adj. R ²	AIC _c	Δ _i	w _i
Li:Ca	Ln(Turb), Temp, DO, Li:Ca_{water} , Sal 1	0.434**	82.157	0	0.475
B:Ca	DO, Sal 1, Ln(Turb)	0.258*	-21.383	0	0.297
Mg:Ca	Sal 2, DO, Temp	0.433**	-12.221	0	0.256
P:Ca	Sal 2, DO, Temp	0.115*	62.474	0	0.310
S:Ca	Ln(Turb), Sal 2	0.037 ^{ns}	-150.900	0	0.169
K:Ca	K:Ca_{water} , Ln(Turb), Sal 1	0.216*	-59.216	0	0.395
Mn:Ca	Sal 2, DO, Temp, Mn:Ca_{water}	0.262*	136.628	0	0.465
Cu:Ca	Sal 2, DO, Temp	0.318**	80.450	0	0.339
Zn:Ca	Sal 2, DO, Temp	0.277**	152.273	0	0.375
Sr:Ca	Temp, Sal 1, Sal 2, DO	0.199*	50.149	0	0.267
Ba:Ca	Sal 2, DO, Temp	0.201*	56.762	0	0.324
Pb:Ca	Sal 1, Temp	0.204*	27.584	0	0.259

Fig. 1. A map of the Gippsland Lakes with the sampling sites marked as ○ for water only samples and ● for water and otolith samples. Inset: Location of Gippsland Lakes in south-eastern Australia.

210x147mm (300 x 300 DPI)

Accep¹

Fig. 2. Longitudinal salinity profiles for the Mitchell and Tambo Rivers during the two sampling periods.

348x113mm (300 x 300 DPI)

Accepted A

Fig. 2 (b/w for print). Longitudinal salinity profiles for the Mitchell and Tambo Rivers during the two sampling periods.

348x113mm (300 x 300 DPI)

Accepted A

Fig. 3. Longitudinal profiles of the interpolated data for each Me:Ca concentrations for the 15 November 2008 sampling period.

357x471mm (300 x 300 DPI)

Fig. 3 (b/w for print). Longitudinal profiles of the interpolated data for each Me:Ca concentrations for the 15 November 2008 sampling period.

359x471mm (300 x 300 DPI)

Fig. 4. Longitudinal profiles of the interpolated data for each Me:Ca concentrations for the 3 December 2008 sampling period.

357x471mm (300 x 300 DPI)

Fig. 4 (b/w for print). Longitudinal profiles of the interpolated data for each Me:Ca concentrations for the 3 December 2008 sampling period.

359x471mm (300 x 300 DPI)

Fig. 5. Mean Me:Caotolith ratios along distance from estuary mouth (DEM) for samples collected on the 15 November 2008 (●) and 3 December 2008 (□). Note the different transformations for each plot (error bars are standard error).

394x475mm (300 x 300 DPI)

Table S1. Results from the univariate tests of between-subject effects from the MANCOVA testing for effects in $Me:Ca_{water}$ between sampling dates and water layer with distance from river mouth (DRM) as the covariate for samples from the Mitchell River and Tambo River. Significant values have been bolded. Some terms are missing due to a lack of degrees of freedom (na).

		Mitchell			Tambo		
		df	F	P	df	F	P
Sampling date (SD)	Li:Ca	1,66	3.163	0.080	1,31	181.867	.000
	B:Ca	1,66	0.307	0.581	1,31	.204	.655
	Mg:Ca	1,66	0.127	0.723	1,31	7.568	.010
	P:Ca	1,66	0.839	0.363	1,31	1.006	.324
	S:Ca	1,66	0.102	0.751	1,31	.947	.338
	K:Ca	1,66	0.225	0.637	1,31	.107	.745
	Mn:Ca	1,66	0.160	0.690	1,31	34.441	.000
	Sr:Ca	1,66	0.148	0.701	1,31	.470	.498
	Ba:Ca	1,66	0.243	0.624	1,31	1.800	.189
Water Layer (WL)	Li:Ca	2,66	27.721	0.000	2,31	4.935	.014
	B:Ca	2,66	0.113	0.894	2,31	6.988	.003
	Mg:Ca	2,66	0.270	0.764	2,31	20.314	.000
	P:Ca	2,66	1.556	0.219	2,31	47.764	.000
	S:Ca	2,66	0.116	0.891	2,31	14.605	.000
	K:Ca	2,66	1.201	0.307	2,31	.543	.586
	Mn:Ca	2,66	32.362	0.000	2,31	8.749	.001
	Sr:Ca	2,66	0.354	0.703	2,31	1.291	.289
	Ba:Ca	2,66	7.302	0.001	2,31	2.360	.111
DRM	Li:Ca	1,66	0.509	0.478	1,31	.738	.397
	B:Ca	1,66	0.184	0.670	1,31	9.314	.005
	Mg:Ca	1,66	4.632	0.035	1,31	422.903	.000
	P:Ca	1,66	3.063	0.085	1,31	17.748	.000
	S:Ca	1,66	3.314	0.073	1,31	312.343	.000
	K:Ca	1,66	5.722	0.020	1,31	5.916	.021
	Mn:Ca	1,66	14.129	0.000	1,31	7.236	.011
	Sr:Ca	1,66	1.203	0.277	1,31	13.427	.001
	Ba:Ca	1,66	1.999	0.162	1,31	4.164	.050
SD * WL	Li:Ca	2,66	8.800	0.000	na	na	na
	B:Ca	2,66	0.172	0.842	na	na	na
	Mg:Ca	2,66	0.081	0.923	na	na	na
	P:Ca	2,66	0.565	0.571	na	na	na
	S:Ca	2,66	0.047	0.954	na	na	na
	K:Ca	2,66	0.251	0.779	na	na	na
	Mn:Ca	2,66	0.011	0.989	na	na	na
	Sr:Ca	2,66	0.093	0.911	na	na	na
	Ba:Ca	2,66	0.208	0.813	na	na	na

Table S1 cont. Results from the univariate tests of between-subject effects from the MANCOVA testing for effects in Me:Cawater between sampling dates and water layer with distance from river mouth (DRM) as the covariate for samples from the Mitchell River and Tambo River. Significant values have been bolded. Some terms are missing due to a lack of degrees of freedom (na).

		Mitchell			Tambo		
		df	F	P	df	F	P
SD * DRM	Li:Ca	1,66	14.243	0.000	1,31	22.847	.000
	B:Ca	1,66	0.600	0.441	1,31	.109	.743
	Mg:Ca	1,66	0.627	0.431	1,31	.954	.336
	P:Ca	1,66	0.357	0.552	1,31	.003	.957
	S:Ca	1,66	1.865	0.177	1,31	.660	.423
	K:Ca	1,66	0.138	0.711	1,31	.046	.832
	Mn:Ca	1,66	0.008	0.928	1,31	2.434	.129
	Sr:Ca	1,66	0.133	0.717	1,31	.166	.687
	Ba:Ca	1,66	0.000	0.990	1,31	4.326	.046
WL * DRM	Li:Ca	2,66	0.773	0.466	2,31	13.736	.000
	B:Ca	2,66	0.069	0.934	2,31	14.165	.000
	Mg:Ca	2,66	2.824	0.067	2,31	536.393	.000
	P:Ca	2,66	0.851	0.432	2,31	3.067	.061
	S:Ca	2,66	2.364	0.102	2,31	369.249	.000
	K:Ca	2,66	3.759	0.028	2,31	6.161	.006
	Mn:Ca	2,66	7.163	0.002	2,31	2.203	.127
	Sr:Ca	2,66	2.191	0.120	2,31	28.483	.000
	Ba:Ca	2,66	0.574	0.566	2,31	.228	.798
SD * WL * DRM	Li:Ca	2,66	9.755	0.000	na	na	na
	B:Ca	2,66	0.363	0.697	na	na	na
	Mg:Ca	2,66	0.353	0.704	na	na	na
	P:Ca	2,66	0.055	0.947	na	na	na
	S:Ca	2,66	1.529	0.224	na	na	na
	K:Ca	2,66	0.104	0.901	na	na	na
	Mn:Ca	2,66	1.472	0.237	na	na	na
	Sr:Ca	2,66	0.693	0.504	na	na	na
	Ba:Ca	2,66	0.186	0.830	na	na	na

Table S2. Results from the univariate tests of between-subject effects from the MANCOVA testing for effects in Me:Ca_{water} between river and water layer with distance from river mouth (DRM) as the covariate for samples from 15 November and 3 December 2008. Significant values have been bolded.

		15 November 2008			3 December 2008		
		df	F	P	df	F	P
River (R)	Li:Ca	1,49	18.121	0.000	1,48	2.176	0.147
	B:Ca	1,49	2.612	0.112	1,48	0.628	0.432
	Mg:Ca	1,49	0.211	0.648	1,48	0.078	0.781
	P:Ca	1,49	4.076	0.049	1,48	0.001	0.972
	S:Ca	1,49	0.158	0.693	1,48	0.140	0.710
	K:Ca	1,49	2.295	0.136	1,48	0.120	0.730
	Mn:Ca	1,49	2.046	0.159	1,48	2.713	0.106
	Sr:Ca	1,49	3.591	0.064	1,48	0.103	0.750
Water Layer (WL)	Ba:Ca	1,49	1.505	0.226	1,48	0.125	0.725
	Li:Ca	2,49	9.445	0.000	2,48	6.742	0.003
	B:Ca	2,49	0.799	0.455	2,48	2.853	0.067
	Mg:Ca	2,49	7.443	0.002	2,48	1.008	0.373
	P:Ca	2,49	1.244	0.297	2,48	16.019	0.000
	S:Ca	2,49	0.882	0.420	2,48	2.227	0.119
	K:Ca	2,49	3.011	0.058	2,48	0.643	0.530
	Mn:Ca	2,49	6.483	0.003	2,48	89.568	0.000
DRM	Sr:Ca	2,49	0.932	0.400	2,48	1.237	0.299
	Ba:Ca	2,49	4.717	0.013	2,48	7.939	0.001
	Li:Ca	1,49	1.595	0.213	1,48	17.688	0.000
	B:Ca	1,49	2.878	0.096	1,48	1.032	0.315
	Mg:Ca	1,49	28.578	0.000	1,48	8.377	0.006
	P:Ca	1,49	4.817	0.033	1,48	3.066	0.086
	S:Ca	1,49	3.134	0.083	1,48	7.172	0.010
	K:Ca	1,49	10.290	0.002	1,48	3.729	0.059
R * WL	Mn:Ca	1,49	2.146	0.149	1,48	20.225	0.000
	Sr:Ca	1,49	0.689	0.410	1,48	1.877	0.177
	Ba:Ca	1,49	2.011	0.162	1,48	1.959	0.168
	Li:Ca	1,49	17.461	0.000	1,48	0.082	0.776
	B:Ca	1,49	2.736	0.104	1,48	0.693	0.409
	Mg:Ca	1,49	0.285	0.596	1,48	0.022	0.883
	P:Ca	1,49	1.922	0.172	1,48	0.040	0.843
	S:Ca	1,49	0.054	0.817	1,48	0.203	0.654
	K:Ca	1,49	0.925	0.341	1,48	0.117	0.734
	Mn:Ca	1,49	0.460	0.501	1,48	0.080	0.779
	Sr:Ca	1,49	1.095	0.301	1,48	0.116	0.735
	Ba:Ca	1,49	1.179	0.283	1,48	0.714	0.402

Table S2 cont. Results from the univariate tests of between-subject effects from the MANCOVA testing for effects in Me:Cawater between river and water layer with distance from river mouth (DRM) as the covariate for samples from 15 November and 3 December 2008. Significant values have been bolded.

		15 November 2008			3 December 2008		
		df	F	P	df	F	P
R * DRM	Li:Ca	1,49	16.070	0.000	1,48	7.203	0.010
	B:Ca	1,49	1.761	0.191	1,48	0.379	0.541
	Mg:Ca	1,49	0.396	0.532	1,48	0.654	0.423
	P:Ca	1,49	3.247	0.078	1,48	3.351	0.073
	S:Ca	1,49	0.002	0.961	1,48	4.819	0.033
	K:Ca	1,49	0.108	0.743	1,48	3.450	0.069
	Mn:Ca	1,49	0.667	0.418	1,48	2.137	0.150
	Sr:Ca	1,49	0.860	0.358	1,48	1.481	0.230
	Ba:Ca	1,49	0.013	0.909	1,48	0.007	0.933
WL * DRM	Li:Ca	2,49	9.496	0.000	2,48	17.887	0.000
	B:Ca	2,49	1.936	0.155	2,48	3.017	0.058
	Mg:Ca	2,49	14.459	0.000	2,48	29.661	0.000
	P:Ca	2,49	2.066	0.138	2,48	2.333	0.108
	S:Ca	2,49	1.307	0.280	2,48	33.557	0.000
	K:Ca	2,49	5.234	0.009	2,48	15.273	0.000
	Mn:Ca	2,49	1.287	0.285	2,48	27.617	0.000
	Sr:Ca	2,49	1.430	0.249	2,48	19.892	0.000
	Ba:Ca	2,49	0.263	0.770	2,48	0.367	0.695
R * WL * DRM	Li:Ca	1,49	19.307	0.000	1,48	0.531	0.470
	B:Ca	1,49	2.236	0.141	1,48	0.439	0.511
	Mg:Ca	1,49	0.733	0.396	1,48	0.626	0.433
	P:Ca	1,49	1.417	0.240	1,48	0.855	0.360
	S:Ca	1,49	0.026	0.872	1,48	4.536	0.038
	K:Ca	1,49	0.030	0.863	1,48	2.202	0.144
	Mn:Ca	1,49	0.013	0.909	1,48	1.076	0.305
	Sr:Ca	1,49	1.234	0.272	1,48	2.258	0.139
	Ba:Ca	1,49	0.788	0.379	1,48	0.089	0.767

Fig. S1. Dendrogram output from the SIMPROF analysis with each letter representing the allocated cluster. Closed orange symbols represent otoliths collected from the halocline layer and open blue symbols represent otoliths collected from the marine layer. Circle symbols are otoliths collected on the 15 November 2008 and square symbols are otoliths that were collected on 3 December.

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