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Population connectivity of two reef fish species northwestern Australia using otolith geochemistry: A pilot study

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WAMSI Kimberley Marine Research Program

Final Report

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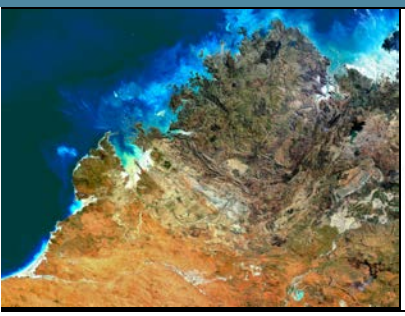


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WAMSI Kimberley Marine Research Program

Initiated with the support of the State Government as part of the Kimberley Science and Conservation Strategy, the Kimberley Marine Research Program is co-invested by the WAMSI partners to provide regional understanding and baseline knowledge about the Kimberley marine environment. The program has been created in response to the extraordinary, unspoilt wilderness value of the Kimberley and increasing pressure for development in this region. The purpose is to provide science based information to support decision making in relation to the Kimberley marine park network, other conservation activities and future development proposals.

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Image 1: Satellite image of the Kimberley coastline (Image: Landgate)

Image 2: Stripey snapper (*Lutjanus. carponotatus*) (Image: © R.Swainston/www.anima.net.au)

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Contents

Executive Summary

This report focuses on two species of fishes, the stripey snapper, *Lutjanus carponotatus*, and Miller's damselfish, *Pomacentrus milleri*, that are abundant on inshore reefs along the northwest coast of Australia including the Kimberley. These species were selected to complement the work already completed using genetic analyses to assess population structure across 51 sites from the Northern Territory south through the Kimberley, Pilbara and Gascoyne (Ningaloo and Shark Bay) fisheries management areas (Berry et al. 2017; DiBattista et al. 2017). They are both widespread and common along the northwestern Australian coast and *L. carponotatus* is an important recreational target species (Ryan et al. 2015). While genetic information provides evidence of gene flow, which is invaluable for understanding population structure (Ashford et al. 2006), it is limited in its ability to provide contemporary information about the movements of individual fish (Saenz-Agudelo et al. 2009). However, otolith geochemistry, which uses changes in trace elements and isotopes from the inner core to the outer margin of an otolith, to act as proxies for changes in habitat (environment), can provide individual life-histories by recording the chemical signatures of the environment at larval, juvenile and adult stages. Trace elements can provide evidence of movements between different marine habitats while changes in strontium and oxygen isotopes provide evidence of movement between marine and estuarine environments. The combinations of these measurements can be used to construct a detailed understanding of the population structure and movements of individual fish over the course of their lives and when integrated with genetic techniques can greatly strengthen inferences from genetic connectivity and stock structure studies (Welch et al. 2015). Consideration of the stock structure of exploited populations is a fundamental resource issue and the results from otolith microchemistry studies provide information on movements and spatial mixing of species which can be used to inform the complex issue of the appropriate spatial scales required for stock assessment.

A total of 127 otoliths from *L. carponotatus* and 39 otoliths from *P. milleri* were analysed. A suite of three different analytical techniques were used. All three techniques measured the target elemental composition of each otolith in a line from the core to the edge of the otoliths. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to measure trace elements; multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) was used to measure strontium, and secondary ion mass spectrometry (SIMS) was used to measure oxygen isotopes. Trace element data were analysed to determine if there were relationships between elemental composition (otolith core and margin) and either of the four bioregional classifications or whether differences in composition occurred at the site level. Strontium and oxygen results were graphed for visual analysis.

Six trace elements were found to be significant and above the limits of detection (LOD) for *L. carponotatus* (^{23}Na, ^{24}Mg, ^{60}Ni, ^{63}Cu, ^{88}Sr and ^{137}Ba), while seven trace elements were significant and above the LOD for *P. milleri* (^{23}Na, ^{29}Si, ^{31}P, ^{60}Ni, ^{63}Cu, ^{88}Sr and ^{137}Ba). A PCA on the *L. carponotatus* data showed that ^{137}Ba and ^{63}Cu were the cause of most of the variation in the core while ^{24}Mg and ^{137}Ba were most significant for the margin. The PCA results for *P. milleri* showed that for both the core and the margin ^{31}P was the main influence on the variation. The pelagic larval duration (PLD) of each fish species was determined and values were input into associated hydrodynamic models to inform the genetic results for these species.

For *L. carponotatus*, elemental composition at the margin differed significantly at all geographic scales with the highest significance at the site level. For otolith core composition of *L. carponotatus* there were significant differences only at the IMCRA bioregion and site levels. *P. milleri* had significant results for all geographical analyses of the margin, however core composition was only significant at the site level. These interim analyses for *P. milleri* are restricted to otoliths from sites south of the Kimberley. Strontium analysis found the results consistent with a fully marine condition. Oxygen isotopes showed variation outside the margin of error.

A key finding of this study was that the trace element data from the margins of the otoliths (older fish) in both *L. carponotatus* and *P. milleri* show population separation between the major bioregions examined. The results agree with the findings of the genetic companion studies of these two fish species (Chapters 1.1.3.4a,b), and add support

to their conclusions that there are genetically distinguishable populations of both species in the Kimberley, Pilbara and Gascoyne management bioregions. However, this result should be considered cautiously as the margin otolith microchemistry only tells part of the story and additional core samples will need to be analysed to allow interpretation of population connectivity. Differences in otolith chemistry between the Kimberley and other bioregions are likely to reflect variation in geology and climate. High summer rainfall brings terrigenous muds and gravels into the coastal waters of the Kimberley, while regions further south have far fewer rivers and estuaries, limiting terrestrial input. These differences will then be reflected in the types and abundances of trace elements input to the local marine waters. While data from the otolith cores (juvenile life stage) was much more equivocal, this disparity is most likely due to the margins all representing a single temporal period of otolith formation (the time immediately prior to capture), while the cores represent multiple periods, depending on the age of the fish. Furthermore, the varied core signatures (larval phase) may reflect a pre-settlement environment that differs from the area where it was collected as an adult. Additional analysis of the extent of differences between core, near core and marginal signatures within individual fish will be undertaken to elucidate the underlying importance of any differences in relation to connectivity.

Implications for management

Broad scale

The significant variation in margin otolith elemental composition for both fish species between bioregions indicates adult fish are not moving between bioregions. The lack of any significant differences in otolith core elemental composition between bioregions is equivocal in terms of determining whether adult fish were situated within the same area as their free swimming larval or juvenile stages. Further analysis of samples will be undertaken in order to answer this question. The elemental environment associated with otolith margins of *L. carponotatus* in the Kimberley fisheries management bioregion differed to those of the Pilbara and Gascoyne however there was no such difference between the Pilbara and Gascoyne suggesting?.

The identification of fish stocks or management units and thus the understanding of population structure is a critical element in sustainable fisheries management and also for the implementation and management of marine reserves and IPA's. The coastal associated pelagic spawning *L. carponotatus* is harvested by commercial, recreational, charter, and indigenous fishers at various locations throughout its range, while the non-harvested damselfish *P. milleri* is an obligate reef-dwelling egg-brooding fish endemic to northwestern Australia. Between them, they represent a large suite of tropical reef fish. Currently, spatial partitioning of *L. carponotatus* in terms of otolith microchemistry in the more northern bioregions largely conforms to existing management bioregions or boundaries. Knowledge of stock separation does not imply that population-specific stock assessments and populations-specific management arrangements are required *per se*, but the implications need to be understood and considered by fisheries, marine reserve and IPA managers, and need to be evaluated within assessment, monitoring and management frameworks depending on current pressures and the risk to sustainability.

Fine scale

The significant variations in core and margin otolith elemental composition for both fish species between sampling sites suggests finer scale population structure may be evident. Higher levels of variation and finer scale population structure during the juvenile phase are also possible, but currently unproven. Both trace element and oxygen isotope analysis show variation through the life of the fish, indicative of environmental variation, larval dispersal and less likely ontogenetic movement. Additional analyses of a larger number of otoliths throughout the study area will be undertaken in order to develop inferences of population connectivity at the site level .

Residual knowledge gaps

The work that has been described here is a portion of an ongoing PhD project. A number of additional research questions that are being followed as part of this PhD and ongoing research include:

- The water chemistry of the marine environments around NW Australia is poorly understood. Pairing water sampling with any future fish sampling is likely to significantly improve the robustness of geochemical interpretation (Amakawa et al. 2012; Brennan et al. 2015; Warner et al. 2005; Zimmerman et al. 2013). Due to the short term persistence and potential high level variability of trace elements in the marine area of the Kimberley, water sampling for this purpose separate (e.g. in a different season or year) from fish sampling, is likely to be of only limited use. However, hypotheses about the variability of oxygen isotopes at different geographic locales and with temperature and salinity can be tested independently of the fish, and a water sampling trip using Camden Sound as a model environment is being undertaken to provide this evidence.
- Each analysed otolith in this study will be individually aged to fully understand the juvenile phase trace element data, and patterns of individual change through time. ~20% of otoliths have been aged so far.

Future research directions that will improve our ability to interpret otolith geochemical signals in this context include:

- Additional geochemical analyses of *P. milleri* otoliths at sites in the Kimberley bioregion would enable an improved understanding of both broad and fine-scale connectivity.
- Each individual geochemical proxy will be subject to a number of complicating factors in its interpretation, due to the complexity of the natural environment. To obtain the most robust interpretation, it is therefore advisable to conduct multi-proxy investigations, combining two or more approaches. To further understand the ontogenetic movement of these fish, we suggest that $\delta^{18}O$ and trace element analyses should be more extensively paired, and that additional consideration is given to including other proxies, such as compound specific stable isotope analysis of carbon, which can reflect the organic and trophic system in which a fish exists. By combining multiple parameters in this way, distinct geochemical fingerprints for each population and sub-population can be developed.

1 Introduction

1.1 Marine Environment of northwestern Australia

Coastal ecosystems of northwestern Australia (NWA) between the Northern Territory and Shark Bay in Western Australia support extraordinary marine biodiversity, commercial and socioeconomic value and contain two UNESCO world heritage listed areas (*i.e*. the Ningaloo Coast and Shark Bay). The Kimberley marine region of Western Australia is the most under-researched area along the NWA coast due to its remoteness, inaccessibility and high costs associated with operating in this isolated and under populated region (Wilson 2014). Until recently it has been considered one of the few marine regions to have been relatively unaffected by human impacts (Halpern et al. 2008) but the growth of oil and gas extraction (Petroleum Division & Geological Survey of Western Australia 2014) and tourism (Collins 2008) in the area are increasing the need to understand the ecology of the region.

The coastal waters of the Kimberley are dominated by tides, which can range up to ~12 m in King Sound (Wilson 2014), leading to high levels of water turbidity (Wilson 2013). Salinity and nutrient levels are generally high in these coastal waters but are reduced in open water (Wilson 2013). Strong, multidirectional local currents are created by the wind and tides and override the larger-scale currents that exhibit seasonal reversal in flow (Wilson 2013).

1.2 Population Connectivity

Knowledge of the spatial structure of populations of exploited fish species is essential for best practice fishery management. Commercial fishing in the north coast bioregion, of which the Kimberley is a key part, is the most valuable finfish sector in the state (Fletcher & Santoro 2009) and information on the stock structure of key species is a requirement for ongoing assessment and management of exploited species. The majority of boat based recreational fishing activity in the North Coast and Gascoyne bioregions occurs within inshore and nearshore waters (Ryan et al. 2015). Many fish species are found over wide geographical areas and are often managed as a single unit yet this may not always be appropriate (Kritzer & Liu 2014). The inherently patchy environments of reefs imposes fine-scale structure on many of the fauna inhabiting them (Kritzer & Liu 2014) and are thus best considered in terms of metapopulation theory (Kritzer & Sale 2004). It is therefore both economically and ecologically important to understand the structure of the region's fish populations.

Population genetic analysis using a genotype-by-sequencing approach has been recently undertaken for two common fish species to the NWA coast, and the species considered in this study: the stripey snapper *Lutjanus carponotatus* (DiBattista et al. 2017) and Miller's damselfish *Pomacentrus milleri* (Berry et al. 2017). These studies demonstrated that both species were genetically differentiated between the Kimberley, Pilbara and Shark Bay bioregions with the Kimberley being the most distinct in the case of *P. milleri*, while Shark Bay was the most distinct in the case of *L. carponotatus*. *L. carponotatus* showed a unique and distinctive 'transition zone' of larval retention in the Buccaneer Archipelago and adjacent waters which was not apparent for *P. milleri* (Berry et al. 2017; DiBattista et al. 2017). The genetic results presented in these two studies provide managers with the identification of bioregion-specific 'stocks' that can be considered within assessment, monitoring and management frameworks. Results for *L. carponotatus* indicated that the management boundaries of stocks require re-evaluation or alternatively the barriers to connectivity need to be considered within management arrangements.

While genetic analyses provide information based on long-term effects over generations (Cowen & Sponaugle 2009), other techniques, such as otolith structure and/or microchemistry, and demographic characteristics, provide information on a shorter, intra-generational, time scale (Begg et al. 1999; Welch et al. 2015). Integrated approaches using multiple techniques are becoming more common for the identification of fish stocks to support the spatial management of fisheries (Begg et al. 1999; Izzo et al. 2017). Such integrated approaches provide a historical perspective on population movement that is invaluable for understanding population structure for fisheries management, and allow for information on stocks to be synthesised across a

range of temporal and spatial scales (Saenz-Agudelo et al. 2009). Ontogenetic movement of individual fish cannot be determined through genetics, and when such information is utilised, such as through otolith geochemistry techniques, the ability to identify stocks is enhanced (Welch et al. 2009; Welch et al. 2015; Izzo et al. 2017).

1.3 Otolith Geochemistry

Otoliths are calcium carbonate structures, typically in the form of aragonite, found within the inner ear of teleost fishes. They are paired and consist of a saggita, lapillus and asteriscus (Popper & Lu 2000, **Figure 1**). The sagitta is often the largest component and as such is, unless explicitly stated otherwise, the otolith used in geochemical studies (Campana 1999). Otoliths grow continuously through life, depositing calcium carbonate in fine layers (Campana 1999). Chemical signatures from the environment, mediated by biological processes, are incorporated into the otolith matrix, and due to the incremental growth of the structure, can provide a time series record of environmental conditions (Campana 1999; Elsdon et al. 2008). There are a wide range of chemical signatures within otoliths that are used for understanding population structure and movement. Those chosen as the basis of this study are trace elements, strontium isotopes and oxygen isotopes.

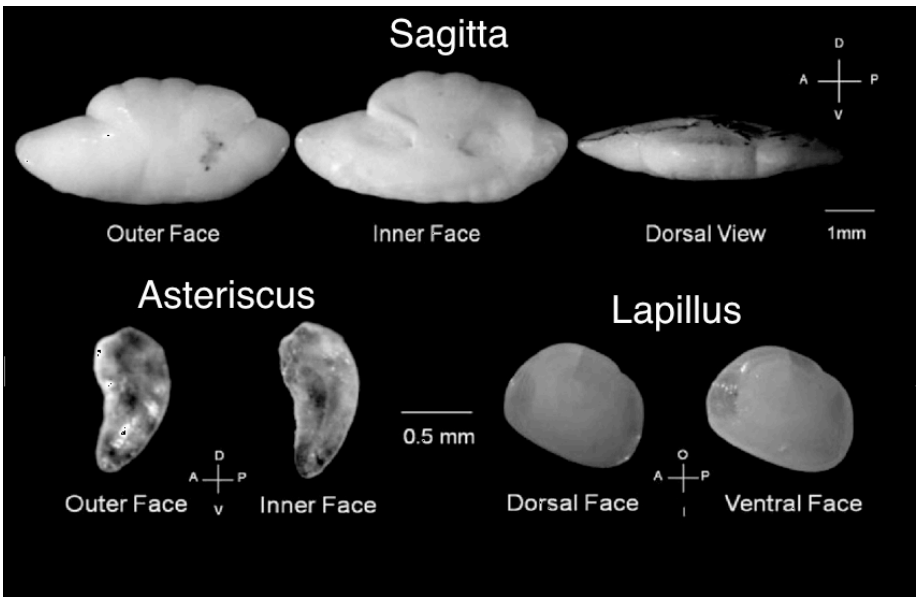

Figure 1: An example of sagitta, asteriscus and lapillus of *Lepidonotothen larseni* (modified from(Curcio et al. 2014)).

Trace elements are elements that occur in naturally low concentrations in the environment and do not significantly bio-accumulate (Pais & Jones Jr 1997). They derive from the lithosphere and enter water bodies after being washed out from bedrock and soils (Pais & Jones Jr 1997). Open ocean trace elements are largely uniformly distributed due to their long residence times (McMahon et al. 2013) but closer to the coasts they are influenced by riverine and estuarine inputs (King et al. 2001). These variable inputs mean that it is possible to identify fish from different regions based on their otolith geochemistry (Thorrold et al. 2001; Brazner et al. 2004; Correia et al. 2012). By measuring trace elements over the lifespan of a fish it is possible to identify changes in trace elements that correspond to changes in their environment and thus track ontogenetic movement as individuals increase in size (Sturrock et al. 2012).

Traditionally trace elements within otoliths were measured by bulk analysis, where otoliths were ground to a homogenous powder that was then analysed to provide trace element values averaged over the entire life of the fish (e.g. Edmonds et al. 1989; Swan et al. 2003; Humphreys Jr et al. 2005). This resulted in a loss of ontogenetic information. However, modern analytical techniques such as laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) have become increasingly popular and informative (e.g. Thorrold & Shuttleworth 2000; Arai & Hirata 2006; Fairclough et al. 2011; Bailey et al. 2015; Cuif et al. 2015; Fraile et al.

2016; Stanley et al. 2016). These modern methods produce maps and transects of otoliths, enabling the entire life-history to be acquired and a more detailed picture of their movements to be deduced (Chittaro et al. 2006; Ben-Tzvi et al. 2008; Di Franco et al. 2012).

Strontium isotopes are used as a proxy for measuring salinity. ^{87}Sr is the product of the radioactive decay of ^{87}Rb while ^{86}Sr is stable so the ratio $^{87}Sr/^{86}Sr$ at a locality depends on the underlying geology of the area (Kennedy et al. 2000). While salinity in oceans is constant at 0.709, closer to land it will vary due to run-off from streams and rivers (Palmer & Edmond 1989). Strontium does not undergo fractionation through trophic levels (Kennedy et al. 2000) and it does not undergo fractionation when incorporated into otoliths (Kennedy et al. 2000). Thus, by measuring Sr isotopes it is possible to determine whether fish have an estuarine phase of their life. Additionally, because the ratio varies due to local geology, it is possible to ground-truth measurements and identify specific localities where the fish have undergone an estuarine phase (Kennedy et al. 2000; Hobbs et al. 2010; Brennan et al. 2015). Strontium isotopes can be measured using multicollector inductively coupled plasma mass spectrometry (MC-ICPMS). This method works in a very similar way to LA-ICPMS but is set up specifically to measure strontium isotopes.

Salinity can also be measured indirectly through the use of oxygen isotopes due to the fractionation between isotopic species in ocean and freshwater. ^{16}O is the most common isotope of oxygen, accounting for 99.76% of all naturally occurring oxygen isotopes. However, oxygen also occurs naturally as ^{17}O and ^{18}O. ^{17}O occurs very rarely (natural abundance is 0.04%) while ^{18}O is slightly more common, accounting for 0.2% of all naturally occurring oxygen isotopes. ^{16}O is lighter than ^{18}O which means that it evaporates more readily, so that seawater has more ^{18}O than freshwater. The ratio of ^{18}O to ^{16}O ($\delta^{18}O$) thus provides a measure of salinity, although complicating signals may arise from changes in temperature, which controls the evaporation rate, and depth, due to an isotopic gradient from the evaporation surface through to deeper waters (Epstein & Mayeda 1953). The interplay of these factors is potentially complex to interpret, although, at least on large scales, the isotopic composition of a water mass is considered to be conservative, i.e. a mass may retain the isotopic signature of a source area at a considerable distance from the sampling point (Rohling 2013). In an environment with large sudden freshwater flushes, it is therefore potentially possible that the $\delta^{18}O$ of the water and thus the otoliths may preserve some of this freshwater isotopic signature even after the water mass has obtained marine salinity. This means that although both the strontium isotopic ratios and the $\delta^{18}O$ may be considered as reflecting salinity, they do not necessarily have the same sensitivity to freshwater input, and potentially may provide complimentary information, for example distinguishing between estuarine fish (where a fresh or brackish water signal would be expected in both proxies), and fully marine fish but in an area prone to large freshwater influxes.

Traditionally oxygen isotopes were measured using acid-digestion methods with samples being manually micromilled and then homogenised to powder for analysis (Matta et al. 2013). This technique is not practical to determine life histories in species with small otoliths as the amount of material required results in the homogenisation of the majority of the life-history into a single sample. In recent years a much more precise method of geochemical analysis has been sporadically used, that of secondary ion mass spectrometry (SIMS). SIMS uses a 5-15 µm ion beam, giving a much greater temporal resolution than previous methods. It enables changes in water chemistry over weeks or even days to be measured compared to the months that are measured by the other methods. This method has been used in fish previously, but only on those known to exhibit strong ontogenetic movement and only using a single fish in their analyses (Matta et al. 2013; Shiao et al. 2014). In this study multiple fish from multiple sites will be analysed to provide a more robust test of the utility of this technique.

The combination of these analytical techniques will provide a range of geochemical proxies for location, thus enabling the contemporary connectivity of populations to be determined.

1.4 Research Objectives

The aims of this project were to assess the population connectivity of coastal reef fishes along the NWA coast using geochemical methods to inform spatial management of biodiversity and fisheries resources. The key

objectives were to:

- Determine whether population connectivity can be demonstrated through the spatial analysis of otolith geochemistry;
- Determine whether ontogenetic movement can be identified through the analysis of otolith geochemistry;
- Determine whether the results derived from the geochemistry analyses informs the spatial analysis obtained from genetic techniques for these two fish species.

2 Materials and Methods

2.1 General approach

Otoliths were analysed using a trio of geochemical analytical methods that measured trace elements, strontium isotopes and oxygen isotopes in order to assess the connectivity and ontogenetic movement of key fish populations along the NWA coastline at a range of spatial scales.

2.2 Site selection

The focal area for sampling was the Kimberley region, with sampling occurring along both the North and West Kimberley coast. Additional sites were sampled along the coast down to the Gascoyne Coast with particular focus along the Pilbara Coast (**Figure 2**).

In order to permit comparison with the genetic results the sites were categorised according to each of the two different biogeographic regimes, *i.e.* the meso-scale bioregions of the Integrated Marine and Coastal Regionalisation of Australia (IMCRA) *sensu* Commonweath of Australia (2006) and the Marine Ecoregions of the World (MEOW) provincial and ecoregional boundaries *sensu* Spalding et al. (2007), and the fisheries management boundaries *sensu* (Fletcher et al. 2017) that closely align with IMCRA.

2.3 Species selection

Population connectivity of two species from the region, *Lutjanus carponotatus and Pomacentrus milleri*, has recently been analysed using population genetic analysis. Both species are widespread and abundant and have contrasting reproductive strategies: *L. carponotatus* produces pelagic eggs while *P. milleri* produces demersal eggs that are guarded by the males (Breder & Rosen 1966). *L. carponotatus* is a moderately-sized fish that inhabits coral reefs in both coastal and marine coral reefs in the tropics of Australia and South-East Asia (Allen 1985). It is a popular recreational target species, particularly in the Kimberley and Pilbara regions (Ryan et al. 2015). *P. milleri* is a small fish (maximum size 75mm standard length) that inhabits inshore reefs areas, mainly on dead coral, along the north and west coasts of Australia from Arnhem Land in the north to Rottnest Island in the southwest (Allen 1991). The contrasting reproductive strategies combined with the recent genetic data made these two species highly suitable for use in the current study.

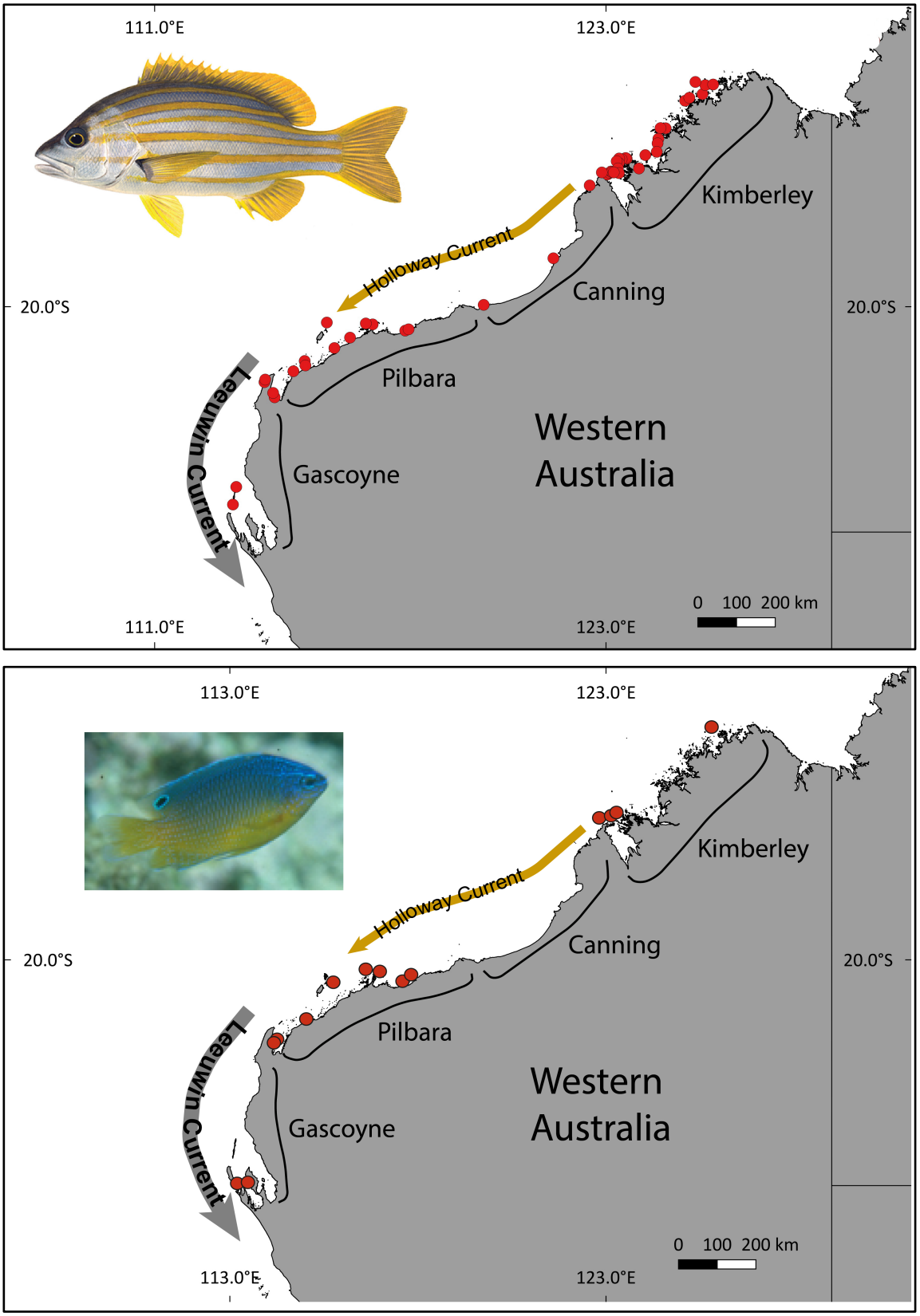

Figure 2: Map of northern Western Australia showing the sample sites for *L. carponotatus* (top) and *P. milleri* (bottom) and the extent of the four IMCRA Bioregions. Illustration of *L. carponotatus* © R.Swainston/www.anima.net.au.

Table 1: Sample sites classified by biogeographic regime with the number of fish sampled (N) from each.

Site	MEOW Province	MEOW Ecoregion	IMCRA Bioregion	Fisheries Management Zone	*Lutjanus carponotatus*	*Pomacentrus milleri*
					N	N
Adieu Point	Sahul	Bonaparte	Kimberley	Kimberley	1	
Bigge Island	Sahul	Bonaparte	Kimberley	Kimberley	11	
Cape Voltaire	Sahul	Bonaparte	Kimberley	Kimberley	2	
Hall Point	Sahul	Bonaparte	Kimberley	Kimberley	4	
Heywood Island	Sahul	Bonaparte	Kimberley	Kimberley	3	
Jamieson Reef	Sahul	Bonaparte	Kimberley	Kimberley	4	
Long Reef	Sahul	Bonaparte	Kimberley	Kimberley	4	
Longitude Island	Sahul	Bonaparte	Kimberley	Kimberley	3	
Montgomery Reef	Sahul	Bonaparte	Kimberley	Kimberley	4	
Ngalanguru Island	Sahul	Bonaparte	Kimberley	Kimberley	4	
Pascal Island	Sahul	Bonaparte	Kimberley	Kimberley	4	
Raft Point	Sahul	Bonaparte	Kimberley	Kimberley	2	
Sale River	Sahul	Bonaparte	Kimberley	Kimberley	1	
Samson Inlet	Sahul	Bonaparte	Kimberley	Kimberley	3	
Shenton Bluff	Sahul	Bonaparte	Kimberley	Kimberley	4	
Tallon Island	Sahul	Bonaparte	Kimberley	Kimberley	4	
Traverse Island	Sahul	Bonaparte	Kimberley	Kimberley	1	
Emeriau Point	NWA	EX to BRM	Canning	Kimberley	3	
Ngamakoon	NWA	EX to BRM	Canning	Kimberley	2	
Cape Keraudren	NWA	EX to BRM	Canning	Pilbara	4	
Cape Preston	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	
Central Montebello	NWA	EX to BRM	Pilbara (offshore)	Pilbara		2
Decipien Island	NWA	EX to BRM	Pilbara (offshore)	Pilbara	4	4
Dendelion Island	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	
Gidley Island	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	2
Paroo Shoal	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	4
Passage Island	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	
Rosemary Island	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	4

Thevenard Island	NWA	EX to BRM	Pilbara (offshore)	Pilbara	3	4
West Moore	NWA	EX to BRM	Pilbara (Nearshore)	Pilbara	4	8
Bay of Rest	NWA	EX to BRM	Pilbara (Nearshore)	Gascoyne	4	
Exmouth Gulf	NWA	EX to BRM	Pilbara (Nearshore)	Gascoyne		3
Locker Point	NWA	EX to BRM	Pilbara (Nearshore)	Gascoyne	4	
Roberts Island	NWA	EX to BRM	Pilbara (Nearshore)	Gascoyne	4	
Milyering	NWA	Ningaloo	Ningaloo	Gascoyne	4	
Bernier Island	WCAS	Shark Bay	Shark Bay	Gascoyne	4	
Dorre Island	WCAS	Shark Bay	Shark Bay	Gascoyne	4	
Barflats	WCAS	Shark Bay	Shark Bay	Gascoyne		4
Homestead	WCAS	Shark Bay	Shark Bay	Gascoyne		4

2.4 Sample Collection

Sampling occurred across the three management regions, *i.e*. the Kimberley, Pilbara and Gascoyne, in order to examine the geographical connectivity of the population(s). Sites were sampled through a combination of routine collections by the Department of Fisheries (DoF) and the Department of Parks and Wildlife (DPAW) and WAMSI-funded sampling which was conducted in August and October 2014 and March and May 2015 (Berry et al. 2015).

Adult and juvenile fish were caught using a combination of baited fish traps, line fishing, spear fishing and clove oil/rotenone (used under permit). Once caught, the fish were euthanised by being placed in an ice bath. Use of the fish in this project was under Curtin University Animal Ethics approval (AEC_2016_24).

The total length, standard length (to the nearest mm) and weight (to the nearest g) of the fish were recorded prior to dissection. Sex and maturity were recorded through visual inspection of the gonads for 86% of the fish. Tissue samples and gut contents were also collected from these fish for separate studies. The otoliths were then dissected out by opening the otic bulla from under the operculum and stored in paper envelopes for otoliths larger than approximately 5mm and plastic microtubes for otoliths smaller than approximately 5mm.

2.5 Sampling Strategy

Due to the high numbers of otoliths available (895 .*L. carponotatus* and 247 *P. milleri*) sub-sampling was necessary. Fish were deemed eligible for sampling if they met the following criteria: they had been sampled from fish used in the genetics study, they had two otoliths available; they were of known sex; they were from sites where both males and females were available. These criteria were designed to provide the ability to replicate an analysis if necessary via the second otolith, and to enable any differences in ontogenetic movement of the sexes to be identified. Fish that met these criteria were then mapped using QGIS (QGIS Development Team 2016) and the otoliths selected for analysis were chosen to give a broad geographical distribution. In the early stages of the sampling design geographical coverage was emphasised over the presence of both sexes at a site and so there are two sites where a single *L. carponotatus* was sampled.

The sampling strategy for *P. milleri* was modified to take account of the lack of adult otoliths available, as the six sites in the Kimberly only yielded juvenile otoliths, which were too small for standard preparation. The analyses here therefore focus on adult sexed fish from the Pilbara sites. Kimberley samples will be revisited at a later date following development of a preparation technique for juvenile otoliths.

2.6 Otolith Preparation

A total of 127 *L. carponotatus* and 39 *P. milleri* otoliths were prepared for analysis. Otoliths were embedded in Stuers EpoFix epoxy resin and sectioned using a Buehler Isomet low speed saw with diamond-tipped blade (Reis-Santos, Tanner, Elsdon, et al. 2013; Steer et al. 2009). Sections were cut to approximately 450 µm encompassing the core. The sections were polished using 15 µm and 5 µm lapping film lubricated with deionised water (Steer et al. 2009; Reis-Santos, Tanner, Elsdon, et al. 2013) and the polished side identified using a mark made by a diamond-tipped scribe. The otolith sections were then mounted to undergo three geochemical analyses: trace element analysis, strontium isotope analysis, and oxygen isotope analysis.

2.7 Age determination

The pelagic larval duration (PLD) of 19 *L. carponotatus* and 15 *P. milleri* were determined following standard protocols (Wilson & McCormick 1997). Only young of year (YOY) fish were used in this analysis. Mounted otoliths (with thermo- labile resin Cristal Bond 590TM in glass slides) were polished by hand, using wet lapping films (1000 to 0.3 µm), successively, until the core and the micro- increments could be observed clearly. Thin, transverse sections through the nucleus of each otolith were obtained and examined with transmitted light and images captured using a digital still camera and measurements taken on a computer. Otoliths displayed a prominent growth increment surrounding the primordium; the latter was used as the starting point in counting increments. Three blind counts of daily increments were counted on consecutive days by the same reader.

2.8 Trace Element Analysis

Trace element analysis was performed using laser ablation inductively coupled plasma mass spectrometry, LA-ICP-MS. The laser ablation spots were chosen using visual inspection of the otoliths. The first, core spot was positioned in the centre of the core at the base of the sulcal acusticus. This spot was used in the core analyses below. Subsequent spots were positioned in the translucent parts of the growth rings, where visible, with two spots placed where possible between the core and the first and second annual growth rings. If the growth rings were not visible then the spots were distributed as evenly as possible to provide even coverage using a spacing that approximated that of the otoliths where rings could be seen. The number of spots ranged from 4 to 20, mean 9, for *L. carponotatus* and from 5 to 9, mean 7, for *P. milleri*. A spot was positioned on the edge of the otolith to ensure that the environment at capture was sampled. This spot was used in the margin analyses below. The first 61 *L. carponotatus* and the 39 *P. milleri* otoliths were analysed at the John de Laeter Centre at Curtin University while the subsequent 66 *L. carponotatus* otoliths were analysed at the University of Adelaide. A spot size of 75 µm, laser energy of 100 mJ and ablation time of 30 s was used. A suite of isotopes were analysed: ^{7}Li, ^{23}Na, ^{24}Mg, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{87}Sr, ^{88}Sr, ^{137}Ba and ^{208}Pb using NIST612 as a standard. Data were processed relative to the standard using Iolite v3.32 (School of Earth Sciences, University of Melbourne; www.iolite.org.au) running on IgorPro v6.37 (WaveMetrics, Inc; www.wavemetrics.com) (Paton et al. 2011).

2.9 Strontium Isotope Analysis

Strontium isotope analysis was performed on all the sampled (39) *P. milleri* otoliths and 61 of the *L. carponotatus* otoliths at the University of Melbourne using multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). Transects from the core to the margin were made using a laser spot size of 72 µm, a translation rate of 5 µm/second and a laser fluence of 2J cm^{2}.

2.10 Oxygen Isotope Analysis

Six *P. milleri* otoliths from were selected to undergo preliminary oxygen isotope analysis from three sites in the Pilbara, representing the furthest north, furthest south and most offshore situated *P. milleri*. Oxygen isotope analysis was performed at the Centre for Microscopy, Characterisation and Analysis at the University of Western Australia. An ion beam size of 15µm was used to create transects from the core to the margin.

2.11 Statistical Analyses

Multivariate analyses of trace element data were performed in PRIMER v 7 (Clarke & Gorley 2015). PERMANOVA (Anderson et al. 2008) was used to test whether the core and margin isotopic data for each species at each of the bioregions as described in Table 1 differed between bioregions and or sites. Prior to PERMANOVA the isotopic variables were ln(x+1) transformed to meet the assumption of homogenous dispersion among *a priori* groups (Anderson 2001) and a Euclidean distance matrix was generated from the replicate data. In the event of a main effect, pairwise PERMANOVA tests were also conducted to determine what bioregions differed significantly from other bioregions. Principal Component Analysis (PCA) was also performed on the core and margin data using a Euclidean distance matrix derived from averaged isotopic values. Draftsmans plots of the replicate data were used to identify whether there were any highly correlated variables. The mean and standard deviation for each otolith were calculated for $^{87}Sr/^{86}Sr$ analysis. A qualitative analysis of $\delta^{18}O$ results was performed.

3 Results

3.1 Pelagic Larval Duration (PLD)

The mean estimated PLDs were 36.8 (0.48 SE) days for *L. carponotatus* and 20.1 (0.74 SE) days for *P. milleri*. These values were used to inform hydrodynamic modelling of potential larval distribution of these two species in the relevant genetic companion studies (Berry et al. 2017; DiBattista et al. 2017).

3.2 Trace Element Analysis

Of the trace elements that were measured nine were found to have sufficiently small error to be included in analyses: ^{23}Na, ^{29}Si, ^{24}Mg, ^{31}P, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{88}Sr and ^{137}Ba. For *L. carponotatus* six of these trace elements were above the limits of detection (LoD): ^{23}Na, ^{24}Mg, ^{60}Ni, ^{63}Cu, ^{88}Sr and ^{137}Ba. For *P. milleri* seven trace elements were above the LoD: ^{23}Na, ^{29}Si, ^{31}P, ^{60}Ni, ^{63}Cu, ^{88}Sr and ^{137}Ba.

The geochemistry at the core region of *L. carponotatus* differed significantly between IMCRA bioregions and individual sites with the IMCRA pairwise test demonstrating that the Kimberley in the north differed to Shark Bay in the south (**Table 2**). In contrast to the core, the geochemistry at the margin differed significantly for each of the four classification systems and also between sites (**Table 2**). Pairwise tests demonstrated that the otolith margin geochemistry of the most northern bioregion for each of the four classifications, *i.e.* Kimberley, Bonaparte or Sahul, differed from one of the next most southern bioregions (**Table 3**). For example, in the case of the Fisheries Management bioregions, the Kimberley in the north differed from both the Pilbara and Gascoyne bioregions, whereas for IMCRA bioregions, the Kimberley differed from the Pilbara (Nearshore) but not from the Pilbara (Offshore) or the more southern Ningaloo and Shark Bay bioregions (**Table 3**).

The PCA results for *L. carponotatus* showed ^{137}Ba and ^{63}Cu were the main sources of variation in the core, while the margin was most heavily influenced by ^{24}Mg, ^{137}Ba and ^{23}Na. The first two components of the core PCA represent the majority of the variation (92.4%) but the first three components are required to represent the majority of the variation in the margin (91.6%). PCA plots for the otolith margins (using averaged data) illustrate the significant regional differences demonstrated in pairwise PERMANOVA tests (**Figure 3**b,d,f,h). For example, in the case of the Fisheries management bioregions, the points representing samples from the Kimberley formed a group to the left and below those from the Pilbara bioregion (**Figure 3**b).

Table 2: Mean squares (MS), *F* values and significance levels (*P*) for PERMANOVAs of isotopic values derived from LA-ICPMS analysis of *Lutjanus carponotatus* otoliths (core and margin) along the north-western Australian coast. The four bioregional classifications are tested individually and also at the Site level. df, degrees of freedom. Significant *P* values in bold.

		Core			**Margin**		
	df	MS	*F*	*P*	MS	*F*	*P*
Bioregion (Fisheries Management)	2	1.16	1.8	0.110	2.746	3.8	**0.004**

Bioregion (IMCRA)	5	1.29	2.1	**0.023**	1.478	2.0	**0.029**
Ecoregion (MEOW)	3	1.25	1.9	0.056	2.233	3.1	**0.008**
Province (MEOW)	2	1.20	1.8	0.119	2.870	4.0	**0.005**
Site	34	<0.01	2.5	**0.001**	1.265	2.3	**<0.00**

Table 3: Results of pairwise PERMANOVA tests of the isotopic values derived from LA-ICPMS analysis of *Lutjanus carponotatus* otoliths (core and margin) along the north-western Australian coast. Only comparisons that differed significantly are shown, significant *P* values in bold, ns not significant.

	Core	**Margin**
Bioregion (Fisheries Management)	ns	Kimb. *vs* Gasc. (**0.007**), Kimb. *vs* Pilb. (**0.014**)
Bioregion (IMCRA)	Kimb. *vs* SB (**0.027**)	Kimb. *vs* Pilb-Nearshore (**0.002**)
Ecoregion (MEOW)	ns	Bonaparte *vs* Ex to BRM **(<0.001**)
Province (MEOW)	ns	Sahul *vs* NW Shelf (**0.002**)

The geochemistry at the core region of *P. milleri* differed significantly between sites but not for any of the four bioregional classifications (**Table 4**). In stark contrast, the margin showed significant differences for each of the bioregional classifications tested as well as between sites (**Table 4**). The PCA results for *P. milleri* showed that for both the core and the margin ^{31}P was the main influence on the variation and the first two principal components represented over 90% of the variation overall. The PCA plots for the otolith margins (using averaged data) again illustrate the significant regional differences demonstrated in the pairwise PERMANOVA tests (**Table 5**). For example, in the case of fisheries management bioregions, all of the points representing the Gascoyne lay above all those from the Pilbara and in the case of IMCRA bioregions, all of the points representing the Pilbara nearshore lay below all those from the Shark Bay and to the left of all those from the Pilbara offshore bioregion (**Figure 4** b,d). There are visible differences between the core and the margin in the PCA plots, even though the same isotopes are driving that variation.

3.3 Strontium Analysis

Neither *P. milleri* nor *L. carponotatus* showed any variation in strontium isotopes between or within sub-regions (**Figure 5**). The values were all within the margin of error for seawater (0.709).

3.4 Oxygen Isotope Analysis

$\delta^{18}O$ shows variation outside the margin of error in all six fish. In fish 5 and 6, and to a lesser extent in fish 4, there is a sequential increase in $\delta^{18}O$ towards the margin, while in fish 1, there is a spike at the fourth analytical spot, and then a relatively stable signal from the sixth spot onwards.

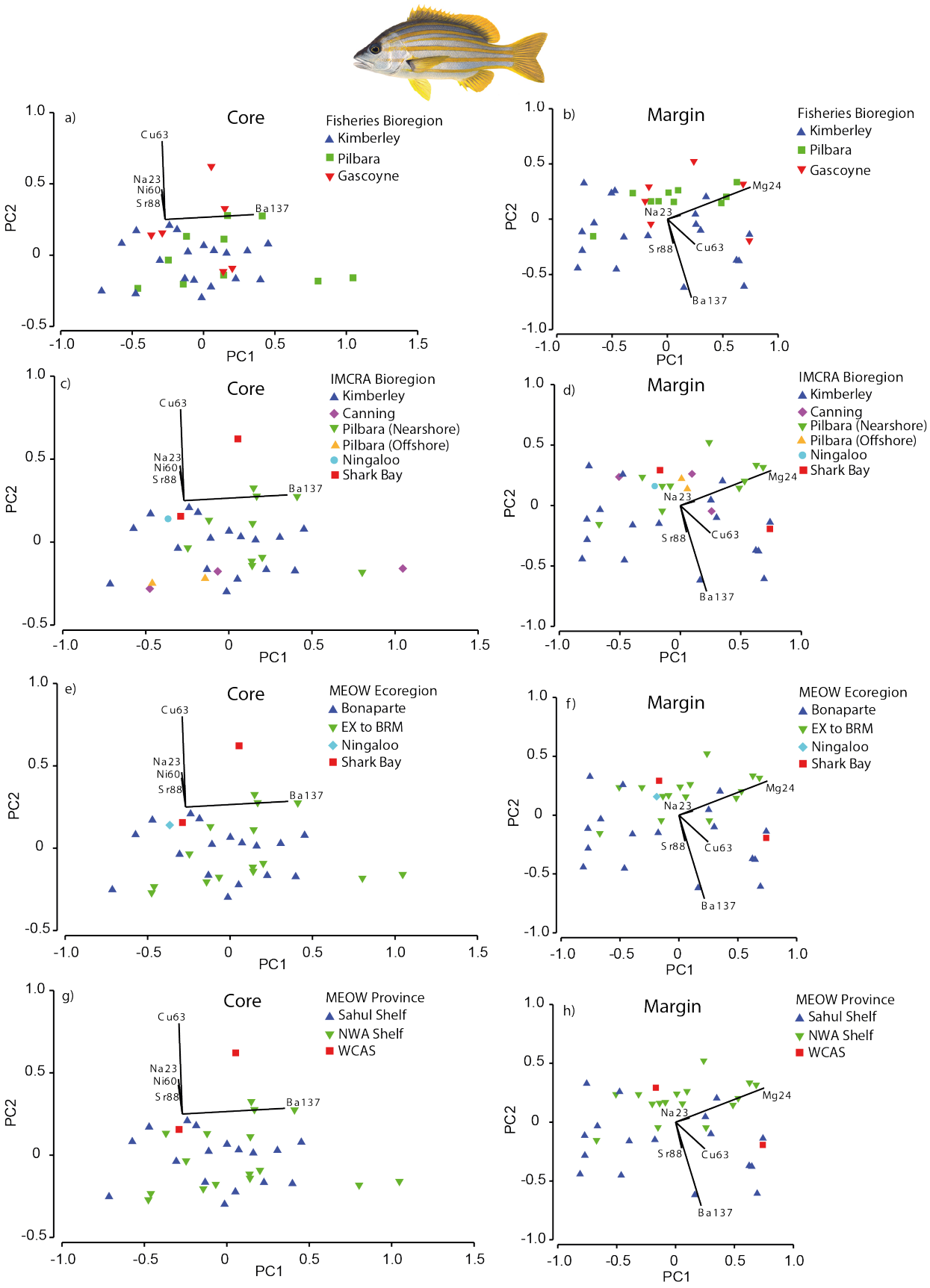

Figure 3: PCA analysis on the core (a,c,e,g) and margin (b,d,f,h) geochemistry (^{23}Na, ^{24}Mg, ^{60}Ni, ^{63}Cu, ^{88}Sr and ^{137}Ba) of *Lutjanus carponotatus* otoliths.

Table 4: Mean squares (MS), *F* values and significance levels (*P*) for PERMANOVAs of the isotopic values derived from LA-ICPMS analysis of *Pomacentrus milleri* otoliths (core and margin) along the north-western Australian coast. df, degrees of freedom. Significant *P* values in bold.

		Core			**Margin**		
	df	MS	*F*	*P*	MS	*F*	*P*
Bioregion (Fisheries Management)	1	0.57	0.6	0.486	5.238	4.5	**0.018**
Bioregion (IMCRA)	2	0.94	0.9	0.382	4.802	4.5	**0.004**
Ecoregion (MEOW)	1	<0.01	0.8	0.435	4.386	3.7	**0.029**
Province (MEOW)	1	1.34	1.4	0.232	4.386	3.7	**0.031**
Site	9	<0.01	1.9	**0.038**	2.090	2.1	**0.026**

Table 5: Results of pairwise PERMANOVA tests of the isotopic values derived from LA-ICPMS analysis of *Pomacentrus milleri* otoliths (core and margin) along the north-western Australian coast. Only comparisons that differed significantly are shown. *P* values in bold, ns not significant.

	Core	**Margin**
Bioregion (Fisheries Management)	ns	Pilb. *vs* Gascoyne (**0.016**)
Bioregion (IMCRA)	ns	SB *vs* Pilb(Off) (**0.007**), SB *vs* Pilb. (**0.078**), Pilb(Off) *vs* Pilb (**0.010**)
Ecoregion (MEOW)	ns	SB *vs* Ex to BRM **(0.03**)
Province (MEOW)	ns	WCAS *vs* NWA Shelf **(0.029)**

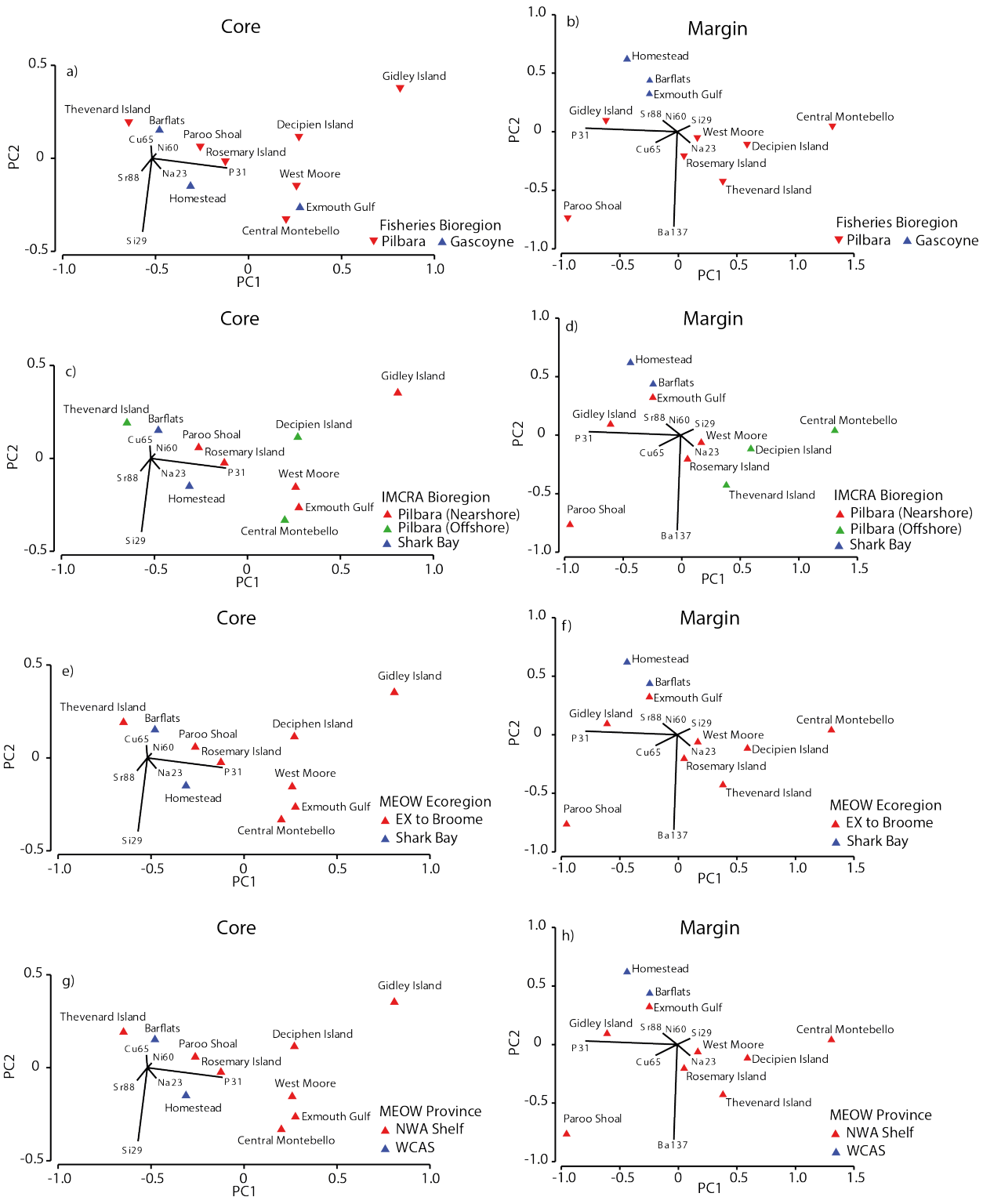

Figure 4: PCA analysis on the core (a,c,e,g) and margin (b,d,f,h) geochemistry (^{23}Na, ^{29}Si, ^{31}P, ^{60}Ni, ^{63}Cu, ^{88}Sr and ^{137}Ba) of *Pomacentrus millerii* otoliths.

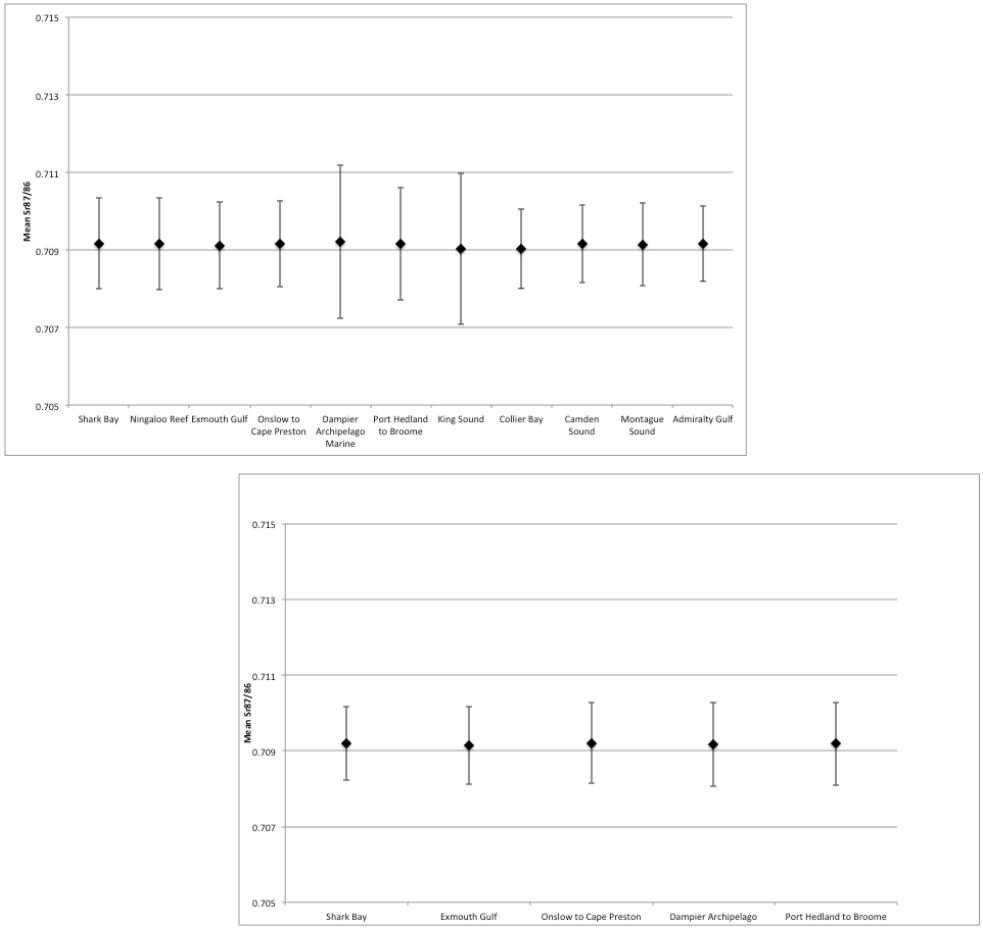

Figure 5: Mean Sr87/86 results for each sub-region for *L. carponotatus* (top) and *P. milleri* (bottom).

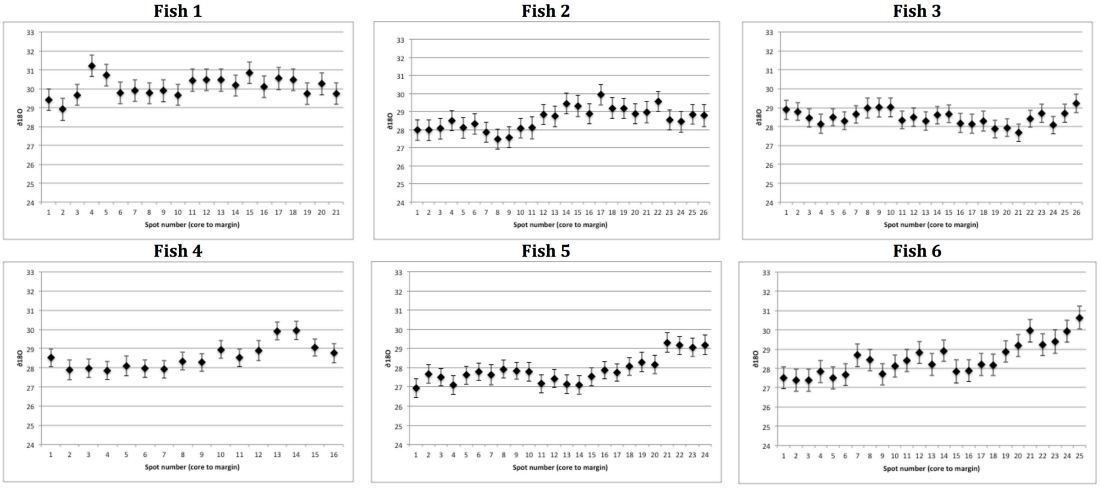

Figure 6: Oxygen isotope analysis results for *P. milleri* from the Pilbara region.

4 Discussion and Conclusions

4.1 Population Structure

The first aim of this study was to determine whether spatial population subdivision along the extensive NWA coast can be demonstrated through the analysis of otolith geochemistry, and in particular, trace elemental abundances.

The analysis of the margin sections of both *L. carponotatus* and *P. milleri* otoliths show positive results for this, with significant bioregional differences demonstrated by PERMANOVA and illustrated when averages are compared via PCA. In the case of *L. carponotatus*, the Kimberley bioregion shows significant variation when compared to the more southern regions, regardless of the classification system used (**Figure 3**). Similarly, PCA plots and pairwise PERMANOVA tests for *P. milleri* margin data show evidence of spatial separation between respective bioregions in each of the four bioregional classifications tested (**Figure 4** b,d,f,h). While, the comparison of otolith microchemistry and genetic analyses (Berry et al. 2017; DiBattista et al. 2017) have broadly similar results, caution must be taken in the interpretation of these similarities. The genetic result is the consequence of decades of gamete exchange and larval dispersal, while the margin result proves that adult fish collected in each of the bioregions are exposed to similar chemistry and are therefore not moving between bioregions. In both species, the core sections of the otoliths show no consistency with the bioregional classification. There are two possible reasons underlying this. Firstly, if juvenile fish live in more inshore environments than adults, then they are likely to be more subject to short-scale chemical variations in the water resulting from terrestrial influxes. Secondly, whilst it is known that the margin of the otolith formed shortly before the time of collection, and so is synchronous in its deposition in all the fish sampled, the core (juvenile phase) analyses (from one site) will possibly represent a range of deposition sites and periods, subject to fish age. Further ageing of these samples will allow data from core analyses to be grouped for specific age cohorts within bioregions, and so test whether the geochemistry of juvenile phase fish also supports the current bioregion and management classifications or whether pelagic larvae are being transported through the changing elemental isoscapes of different bioregions.

The difference in otolith chemistry particularly between the Kimberley and other bioregions is likely to derive from the geology and climate. High summer rainfall brings terrigenous muds and gravels into the coastal waters of the Kimberley where strong tides create high turbidity, particularly at spring tides (Wilson 2014). In contrast, the southern part of the studied area has far fewer rivers and estuaries, limiting terrestrial input, and leaving the sedimentation dominated by carbonate sand (Wilson 2014). These differences will then be reflected in the types and abundances of trace elements input to the local marine waters. Trace elements can show distinct annual and inter-annual variation (Reis-Santos et al. 2012; Tanner et al. 2012), which may be reflected in the fish from the Kimberley, in response to the strong seasonality of the terrestrial input. For the *P. milleri*, where only fish from the Pilbara and Gascoyne were analysed, the importance of phosphorus as a controlling element in the variation indicates that there may be separation according to nutrient profiles.

4.2 Individual Movement

The second aim of this study was to determine whether ontogenetic movement can be identified through the analysis of otolith geochemistry, both using trace element data, and as a preliminary methodological study, $\delta^{18}O$ as measured via SIMS.

In the trace element data, very little consistency is seen between the core and the margin of each otolith, indicating that the fish has been living in differing water conditions through its life. However, the question here, is whether the fish has moved between different water masses, or whether the water chemistry of a single locale has changed through time. Given the short persistence times of trace elements in marine environments (Bruland 1983), and the heavily seasonal and varying input of terrestrial material the latter possibility cannot be ruled out, and nor can be a combination of the two factors, which seems the most likely real world explanation.

The six fish analysed for $\delta^{18}O$ via SIMS show variation through time which is outside the margin of error (**Figure**

6), indicating that a changing environmental signal is being recorded. The lack of variation in the strontium data suggests that this is not a straightforward measurement of moving between brackish and open water masses – all the otolith samples clearly formed in a marine environment. However, the relatively conservative nature of oxygen isotopic signatures in water masses (Rohling 2013) means that the influence of freshwater discharge into the locale may still be being seen. It is tempting, though highly speculative, to suggest that the increase in $\delta^{18}O$ seen through time in two of the samples is reflecting movement from inshore environments during the juvenile phase, to more open marine settings during the adult phase. To verify this hypothesis, a better understanding of $\delta^{18}O$ behaviour in the Kimberley and Pilbara waters is required in order to establish where the variations occur, and to what extent factors such as geography, proximity to river mouths, temperature, salinity and depth are influencing the signal in these areas.

4.3 Comparisons with Genetics

The third aim of this study was to determine whether the data derived from the geochemistry results is comparable to that obtained from genetically-derived population information. The otolith margin geochemical results broadly agree with those from the genetics studies (**Table 2**, **Table 4**, Berry et al. 2017; DiBattista et al. 2017), with separation between bioregions observed in both parameters. It is notable that the marginal trace element analysis for *L. carponotatus* showed consistent separation between the Kimberley and the more southern bioregions, irrespective of the classification system analysed, largely paralleling the genetic results for this species. A point of difference to the genetic results is provided with respect to the separation of the Shark Bay bioregion, which for both *L. carponotatus* and *P. milleri* were clearly genetically distinguishable from samples in all other bioregions. Thus, while the marginal elemental composition of *P. milleri* otoliths from Shark Bay differed significantly from all bioregions further north, thereby paralleling genetic results, there was no such difference for *L. carponotatus*. This may be a genuine environmental effect, reflecting the more offshore oceanic marine environment where *L. carponotatus* samples were collected at Bernier and Dorre Islands compared to the more enclosed and inshore marine environment where *P. milleri* samples were collected within the western Gulf of Shark Bay (**Figure 2**). More in-depth sampling to test this is warranted.

5 Conclusions

On the basis of trace elemental composition of otolith margins, both *L. carponotatus* and *P. milleri* show evidence of bioregional separation, broadly paralleling the genetics results, and showing a distinct separation between the Kimberley and the more southern bioregions in the case of *L. carponotatus*. For *P. milleri*, in which no Kimberley samples were able to be analysed, spatial separation was evident between each of the four bioregional classifications tested. Variations were also evident between sampling sites, suggesting finer scale population structure may be present. Both trace element and oxygen isotope data measured from the core to the margin in individual fish indicate changes in host water conditions through time, and potentially ontogenetic movement. Full aging of the otoliths will allow a better understanding of the data from the juvenile phase, whilst new analyses of the water chemistry in the region will help ground truth some of the techniques.

This study builds on the few studies that have used otolith geochemistry to explore population structure, connectivity and ontogenetic movement of fish from the Kimberley and broader NWA coast and the first such study on either *L. carponotatus* or *P. milleri*. Otolith geochemistry has been highly successful in understanding fish population dynamics in estuarine and diadromous fishes (Vasconcelos et al. 2008; Miller et al. 2011; Reis-Santos, Tanner, Vasconcelos, et al. 2013) but marine fish have proven harder to study due to the smaller chemical gradients (Ashford et al. 2006). These results add to the growing body of evidence (Labonne et al. 2008; Standish et al. 2008; Bailey et al. 2015) that otolith geochemistry can help elucidate population structure and connectivity in coastal fish populations.

5.1 Future work

The work that has been described here is a portion of an ongoing PhD project. A number of additional research questions that are being followed as part of this PhD and ongoing research include:

- The water chemistry of the marine environments around NW Australia is poorly understood. Pairing water sampling with any future fish sampling is likely to significantly improve the robustness of geochemical interpretation (Amakawa et al. 2012; Brennan et al. 2015; Warner et al. 2005; Zimmerman et al. 2013). Due to the short term persistence and potential high level variability of trace elements in the marine area of the Kimberley, water sampling for this purpose separate (e.g. in a different season or year) from fish sampling, is likely to be of only limited use. However, hypotheses about the variability of oxygen isotopes at different geographic locales and with temperature and salinity can be tested independently of the fish, and a water sampling trip using Camden Sound as a model environment is being undertaken to provide this evidence.
- Each analysed otolith in this study will be individually aged to fully understand the juvenile phase trace element data, and patterns of individual change through time.

Future research directions that will improve our ability to interpret otolith geochemical signals in this context include:

- Additional geochemical analyses of *P. milleri* otoliths at sites in the Kimberley bioregion would enable an improved understanding of both broad and fine-scale connectivity.
- Each individual geochemical proxy will be subject to a number of complicating factors in its interpretation, due to the complexity of the natural environment. To obtain the most robust interpretation, it is therefore advisable to conduct multi-proxy investigations, combining two or more approaches. To further understand the ontogenetic movement of these fish, we suggest that $\delta^{18}O$ and trace element analyses should be more extensively paired, and that additional consideration is given to including other proxies, such as compound specific stable isotope analysis of carbon, which can reflect the organic and trophic system in which a fish exists. By combining multiple parameters in this way, distinct geochemical fingerprints for each population and sub-population can be developed.

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8 Communication

8.1 Students supported

Sarah Hearne (PhD Student, Curtin University, commenced February 2016)

8.2 Submitted manuscripts

Manuscripts relating to this work will be produced as part of a PhD thesis

A manuscript with the proposed title "Comparison of otolith geochemistry from two reef fish from northwestern Australia" is currently being drafted.

8.3 Presentations

WAMSI Lunch & Learn 28 February 2017

A presentation titled "Connectivity of fishes from the Kimberley region, Western Australia, using otolith geochemistry" was given by Sarah Hearne at the Australian Society of Fish Biology annual conference to be held in Albany, WA, 21-23 July 2017.

8.4 Opportunities created as a result of this project

A water sampling trip with AIMS was completed between 29 May and 6 June 2017 in order to characterise the oxygen and strontium isotopes within Camden Sound, with the aim of improving our understanding of the relationship to temperature and salinity of these isotopes within the area.